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Biochemical role of Tadalafil in experimental brain impairment activities in mice

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ABSTRACT

Tadalafil (TAD) is an FDA-approved selective long-acting phosphodiesterase 5 inhibitor (PDE5-I) for the treatment of male erectile dysfunction (ED). It also helps to improve cognitive function by lowering neuroinflammation and amyloid beta deposition. A biological, pharmacological, or physical toxicity that impairs the function of the central and/or peripheral nerve systems is referred to as brain impairment. Aluminum poisoning may affect the brain;, causing aberrant amyloid-beta (Aß) buildup, neuroinflammation, and neuronal death. This study included sixty mice divided into three groups. (20 each), control group: Mice were given no medication. aluminum chloride group: Mice were received $AlCl_3$daily at dose (8.5 mg/kg) for 4weeks .Tadalafil and $ALCL_3$ group: mice were received tadalafil (20 mg/kg/day) and $ALCL_3$ (8.5 mg/kg) for 4 weeks. Administration of $ALCL_3$ increases the level of brain Lactate, Pyruvate, acetylcholinesterase (AchE), Gamma-aminobutyric acid (GABA) and Brain Myeloperoxidase (MPO) activities. Whereas, tadalafil administration showing protective role as it causing significant decrease in all parameters. From the obtained results, it could be concluded that tadalafil has a neuroprotective effect against aluminum chloride induced brain impairment in mice.

1. INTRODUCTION

Tadalafil is a PDE-5 inhibitor with anti-inflammatory effect against various cognitive impairments by lowering neuro-inflammation and A deposition (França et al., 2019). Cyclic nucleotides, like cGMP, are essential for neuroplasticity and memory formation (Argyrousi et al., 2020). PDE5 regulates prostatitis (Okamoto et al., 2018), hepatic damage and induces cytoprotection and promotes cardio protection (Koka et al., 2020), pulmonary hypertension (Shen et al., 2020) and renal ischemia/reperfusion (Medeiros et al., 2017). Furthermore, PDE inhibitors was beneficial in the treatment of autoimmune illnesses including Rheumatoid arthritis (RA) (Mansour et al., 2018).

The rate extent of adsorption of tadalafil is not influenced by food like other PDE5-Is. Tadalafil is distributed in different tissues, plasma, as well as semen and volume of distribution following oral administration. The daily use of tadalafil is not recommended for men with severe renal insufficiency and sever hepatic impairment (Andersson, 2018). It demonstrated anti-inflammatory effects and improved cognitive function in patients with erectile dysfunction by reducing proinflammatory interleukins levels namely, IL-6, IL-7 and IL-8 (Urios et al., 2019). The administration of tadalafil restored spatial learning and memory deficits in thioacetamide induced hepatic encephalopathy in mice by attenuating hippocampal neuroinflammation and regulation the expression of glutamate receptor Franca et al. (2019).

Aluminum (Al) is an abundant metal on earth and a cumulatively detrimental heavy metal in body; it may also enter the body through drugs, heating food, and water, causing poisoning in a range of organs (Liaguat et al., 2019; Tietz et al., 2019). Aluminum poisoning affects the brain, resulting in abnormal amyloid-beta formation, neuro-inflammation, and death (Cheng et al.2019).

Brain impairment is a prevalent neurological disorder among the elderly, with a steady deterioration in cognitive function that has a considerable impact on quality of life (Rossini etal. 2020). Extracellular aggregation of A protein in the brain and hyperphosphorylation of tau protein inside neurons were signs of Alzheimer's disease (Ahmad et al. 2020).According to Maurer and Williams (2017) in early Alzheimer's disease, cholinergic neurons atrophy in the basal forebrain is thought to promote short-term memory loss. Our study will show the biochemical and protective role of tadalafil against the effect of administration of ammonium chloride in mice.

2. MATERIAL AND METHODS

2.1. Chemicals

Aluminum Chloride and Tadalafil was purchased from Sigma-Aldrich, St. Louis, Mo, USA.

2.2. Animals

Sixty male mice (20-30 gram) were provided from the animal house at Benha University's Faculty of Veterinary Medicine in Egypt. Before the trial, they were acclimatized for one week in a controlled setting. Fresh food and drink were provided on a regular basis. The Ethical animal Committee of Benha University accepted all experiment's protocol for the care of the laboratory animals (BUFVTM 05-12-23).

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2.3. Experimental protocol

Animals were divided into three equal groups and treated for 4 consecutive weeks. Control: mice no medication. $AlCl_3$ group: mice administered $AlCl_3$ (8.5 mg/kg, orally) daily for induction Neurotoxicity (Amjad et al., 2015). Tadalafil and $ALCL_3$ group: mice were daily curable by tadalafil (20 mg/kg/day/orally) (Socała et al., 2018) and $ALCL_3$ (8.5 mg/kg/orally).

2.4. Sampling

Blood and brain tissue samples were obtained from all groups twice over the experiment's length (2 and 4 weeks).

2.5. Blood samples

Blood samples were taken from retro-orbital venous plexus in clean dry tubes and to allow clot for separation of serum, then centrifuged at 3000 g for 15 minutes, serum aspirated by automatic micropipettes, in Eppendorf tubes, and stored at -20 C until used. Sera samples were analyzed for determination of the following parameters: Gamma amino butyric acid (GABA) The competitive ELISA uses the microtiter plate format according to Arrue et al. (2010), Lactate levels according to Hutchesson et al. (1997) and Pyruvate levels according to Gray et al. (2014).

2.6. Tissue samples

Ten mice from each group were rats were scarified by cervical decapitation and brain was excised and cleaned to remove any blood or clots, then placed between two filter papers and stored at -20 °C for analysis.

2.7. Preparation of brain homogenate

The homogenates (brain Segments were fixed in Bouin's solution and paraffin-embedded. Serial sections of 5 μm were obtained with a Leica microtome) were centrifuged for 5 minutes at 5000 g. to separate the supernatant after two freeze-thaw cycles were done to break the cell membranes. Which is used to determine Acetylcholine esterase activity (AChE) according to Henry (1974) and Myeloperoxidase (MPO) activity using mice Myeloperoxidase ELISA kit (Kamiya Biomedical Company, Cot No. KT-60345) according to the manufacturer's instruction.

2.7. Statistical Analysis

All data were presented as SEM. The data distribution was checked for normalcy using normality tests (SPSS version 25). ANOVA was used to determine statistical significance. Snedecor and Cochran (1994) defined statistical significance as a probability of P= 0.05.

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3. RESULTS

Table 1 and 2 showed that $AlCl_3$ administration to normal rats induced a significant increase in the level of brain lactate, pyruvate, acetylcholinesterase (AchE), gamma-aminobutyric acid (GABA) and brain myeloperoxidase (MPO) activities compared to control. Moreover, it was shown a significant decrease in their levels after treatment with tadalafil when compared to $AlCl_3$ induction group.

Table 1 Biochemical effect of tadalafil on $ALCL_3$ exposed mice after two weeks

Animals groups	Myeloperoxidase (ng/g)	Pyruvate (mg/dl)	Lactate (mmol/L)	Gamma-aminobutyric acid (ng/mL)	Acetylcholinesterase (U/L)
Control group	3.97 ± 0.19^{Ac}	2.29 ± 0.12^{Ab}	15.87 ± 0.23^{Ab}	6.95 ± 0.12^{Ac}	24.80 ± 0.75^{Ac}
$AlCl_3$ group	31.40 ± 2.02^{Ba}	2.87 ± 0.12^{Ba}	19.44 ± 0.70^{Ba}	41.27 ± 2.51^{Ba}	75.70 ± 1.57^{Ba}
AlCl3+tadalafil	21.53 ± 2.19^{Ab}	2.63 ± 0.07^{Aab}	17.40 ± 0.67^{Aab}	33.63 ± 2.58^{Ab}	62.80 ± 1.97^{Ab}

Data are presented as Mean ± S.E. Mean values in the same raw with different superscript Capital letters are significantly different at (P<0.05) in different times within the same group. While mean values in the same column with different superscript Small letters are significantly different at (P<0.05) between different groups at fixed time.

Table 2 Biochemical effect of tadalafil on $ALCL_3$ exposed mice after four weeks

Animals groups	Myeloperoxidase (ng/g)	Pyruvate (mg/dl)	Lactate (mmol/L)	Gamma-aminobutyric acid (ng/mL)	Acetylcholinesterase (U/L)
Control group	3.71 ± 0.58^{Ac}	2.28 ± 0.04^{Ab}	16.13 ± 0.26^{Ab}	7.05 ± 0.19^{Ac}	24.57 ± 0.78^{Ac}
$AlCl_3$ group	58.97 ± 3.03^{Aa}	5.70 ± 0.20^{Aa}	42.10 ± 0.76^{Aa}	55.47 ± 2.44^{Aa}	99.10 ± 1.72^{Aa}
AlCl3+tadalafil	16.40 ± 2.01^{Ab}	1.89 ± 0.12^{Bb}	16.74 ± 0.40^{Ab}	26.00 ± 2.06^{Ab}	46.20 ± 4.99^{Bb}

Data are presented as Mean ± S.E Mean values in the same raw with different superscript Capital letters are significantly different at (P<0.05) in different times within the same group. While mean values in the same column with different superscript Small letters are significantly different at (P<0.05) between different groups at fixed time.

4. DISCUSSION

Compared to control, AlCl3 administration of to normal rats resulted in a significant increase in brain Lactate, Pyruvate, acetylcholinesterase (AchE), gamma-aminobutyric acid (GABA), and brain myeloperoxidase (MPO) activities. The observed changes in AchE could be attributed to the fact that AchE catalyzes the hydrolysis of acetylcholine into choline and acetate, which control acetylcholine metabolism caused neuro-behavioral alterations, particularly memory and cognitive function, because it is a specific cholinergic marker protein that has received extensive attention in the study of AlCl3 neurotoxicity. These findings were essentially identical to those of Elhadidy et al. (2018), who found that daily treatment with AlCl3 induced a substantial rise in cortical and hippocampus AchE activity above control levels, potentially resulting in a reduction in cholinergic activity.

Similarly, Hussein et al., (2010) discovered a substantial rise in GABA levels in rats given AlCl3.The rise in GABA concentration might be due to an increase in glutamate levels. Which are decarboxylated by the glutamic acid decarboxylase enzyme (GAD) to create GABA, where GAD is the only enzyme responsible for glutamate decarboxylation. Also, the direct impact of aluminium in the cellular environment and the differential regional accumulation of glutamate or other alteration in glutamate-GABA system enzymes are thought to be one of the causes of aluminum-induced neurotoxicity (Nayak and Chatterjee, 2001).

Furthermore, Zahedi-Amiri et al. (2019) observed that rats given AlCl3 had a considerable increase in MPO activity. Increased MPO activity resulted in protein oxidation, which finally caused cell and tissue damage Kaur and Sodhi, (2015). Scavuzzo et al., 2020 discover that S/C injections of D-lactate impaired memory 15 minutes before the inhibitory avoidance (IA) test, but considerably improve rat memory 2 minutes later. This increase is due to high brain L-lactate synthesis due to lactate dehydrogenase A (LDH-A)/LDH-B ratio, but inhibition of L-lactate transport from glia to neurons, leads to L-lactate deficiency in neurons. Inadequate L-lactate utilization causes energy shortage in neurons, exacerbating the course of neuronal damage, including cognitive impairment, throughout the brain pathological changes in impairment patient or ageing Zhang et al., (2018).During an activation event, the accumulates lactate that in the brain is 5% of the amount of pyruvate generated from glucose. Lactate concentration in the typical resting brain is linearly connected to pyruvate concentration(LU et

al., (2015)and its increase during activity most likely associated with an increase in concentration of pyruvate.
Pretreatment with tadalafil, on the other hand, negated all of these findings as a protective treatment for brain damage. This finding is consistent with Thakur et al., (2019), who found that tadalafil pre-treatment greatly reduced liver damage, as seen by a decrease in MPO activity. In the rat model, tadalafil dramatically decreased leukocyte infiltration and combated liver damage induced by ischemia and reperfusion injury. In accordance with this, Haider et al.(2020) demonstrated that co-ingestion of vardenafil counteracted the significant increase in AChE activity caused by AlCl3/d-gal. Furthermore, (Hanchar et al., 2005 &Boix et al., 2010) shown that sildenafil reduced extracellular GABA and astrocyte activation in PCS rats. Furthermore, these findings are in accordance with Finsterer et al. (2012), who demonstrated that swimming exercise increased blood lactate and pyruvate concentrations and that 20-mg sildenafil citrate lessened increase in blood lactate and pyruvate concentrations caused by swimming exercise . Lactate is a glycolytic pathway product formed by the activity of lactate dehydrogenase on pyruvate, which is aided by the proton-linked MCT. Sun and colleagues (2017).MCTs are monocarboxylate transporters, play an important role in lactate metabolism. MCTs are a 14-member transporter family, with the first four members (MCT1-MCT4) transporting single-carboxylate molecules across biological membranes (Halestrap et al., 2013). MCT1 and MCT4 are lactate transporters that play a key role in the control of lactate flux across the plasma membrane in skeletal muscle (Bonen et al., 2001).in oxidative fibers, MCT1 is a high-affinity lactate transporter found largely whereas in glycolytic fibers MCT4 is primarily located (Benton et al., 2004).

5. CONCLUSIONS

It was concluded that tadalafil has a neuroprotective effect against aluminium chloride induced brain impairment in mice.

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