

Determination of Central Nervous Effect and Antidotal Effect Against Chlorpyrifos Toxicity of Alpha-Lipoic Acid Through Pharmacological and Toxicological Challenges

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Abstract

Pharmacological and toxicological challenges are among the most important studies in revealing the latent properties of drugs. The goal of this study was to evaluate the effects of alpha-lipoic acid on the central nervous system and chlorpyrifos toxicity in a chick's model. One-day-old chicks were used, the pharmacological challenge was done through propofol anesthesia and calculate the onset, duration, and recovery time, and while chlorpyrifos was used in the toxicological challenge for the median lethal doses LD_{50s} were determined using the up-and-down method. Alpha-lipoic acid at 20, 40, and 80 mg/kg caused an inhibition effect on the central nervous system, represented by a significant increase in the anesthesia period when chicks were anesthetized by propofol at 20 mg/kg intraperitoneally. Alpha-lipoic acid caused a significant decrease in the recovery period compared to the control group. Alpha-lipoic acid at 20 and 40 mg/kg increased the value of the median lethal dose of chlorpyrifos by 31 and 62%, respectively. The use of alpha-lipoic acid as preanesthetic has benefits such as decreased recovery time, furthermore Alpha-lipoic acid has a depressant effect in the central nervous system. Alpha-lipoic acid revealed an antidotal effect against chlorpyrifos toxicity.

Keywords:

Alpha-lipoic acid, Anesthesia, Chlorpyrifos, Pharmacological challenge, Toxicological challenge.

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Introduction

Alpha-Lipoic acid (ALA) is an antioxidant obtained from caprylic acid. It is formed in the mitochondria and roles as a cofactor in the enzymatic nutritional breakup (Packer and Cadenas, 2010). ALA comprises of a dithiol efficient group that removes free radicals by reducing the oxidized forms of other antioxidants (Laher, 2011). Lipoic acid was observed in 1937 while researchers discovered a strain of bacteria that reproduces by using potato juice (Pirlich et al., 2002). ALA is a highly potent antioxidant that neutralizes reactive oxygen species (ROS), such as superoxide radicals and hydroxyl radicals. ALA has been shown to be beneficial in several oxidative-stress-associated conditions such as ischemia-reperfusion or radiation injury (Packer et al., 1995). Furthermore, numerous studies show ALA to be effective in the treatment of diabetic neuropathy. It accomplishes this by increasing nitric oxide-mediated endothelium-dependent vasodilation, thereby getting better microcirculation in diabetic polyneuropathy patients (Vallianou et al., 2009). ALA is revealed to significantly inhibit inflammatory prostaglandin production, a key cytokine in the pathogenesis of the inflammatory process (Fronzoza et al., 2018; Abdul-Ghani and Naser 2022). ALA has a potent metal-chelation property and ALA's thiol groups are responsible for metal chelation by increasing the glutathione levels inside the cells, Lipoic acid preferentially binds to Pb, Zn, and Cu. On the other hand, reduced forms of ALA complexes with Hg, Pb, Fe, Zn, and Cu (Shay et al., 2009) we use in our study chicks as a model which has been used extensively as a research model throughout the history of biology, chicks were used as a model for analgesia (Albadrany et al., 2021), anesthesia (Alatrushi and Naser, 2021), and toxicity research (Al-Baggou et al., 2011). There are many pharmacological and therapeutic properties of alpha-lipoic acid that have not been discussed in detail.

Therefore, our study aimed to reveal some of the pharmacological properties of alpha-lipoic acid using a pharmacological challenge to determine the effect on the central nervous system and a toxicological challenge to show the effectiveness of alpha-lipoic acid in reducing toxic effects for the insecticide chlorpyrifos.

Materials and methods

Ethical approval:

The study was approved by the Scientific Council of the Department of Physiology, Biochemistry and Pharmacology at the College of Veterinary Medicine, University of Mosul. This study is part of a master's thesis.

Animals:

Forty-one days-old chicks were brought from local hatcheries in the city of Mosul and bred under standard conditions until they reach the seventh day of life, which is the time for the start of experiments. The bodyweight of chicks was between 80-110 g with unstopable light and kept at 30-33 °C. The food and water were provided *Ad libitum*.

Drugs:

1. Alpha-lipoic acid (capsule 600 mg) produced by America medic and science, USA.
2. Propylene glycol (99.5 %) produced by Thomas Baker, India.
3. Propofol (1% ampoule) produced by Fresenius kabi company, Austria.
4. Chlorpyrifos (45% concentration) produced by BHARAT INSECTICIDES LTD, India.

Drug preparation:

Alpha-lipoic acid dissolve in a propylene glycol (Abdulghani and Naser 2022) while Chlorpyrifos has been diluted

with distilled water (Mohammad et al., 2014).

Experiments:

1- Pharmacological challenge: The effect of alpha-lipoic acid on the anesthesia induced by propofol in chicks:

Twenty-four chicks were used and randomly divided into 4 equal groups, at a rate of 6 chicks for each group. The chicks were treated as follows: **Group I: control** (propylene glycol) at 5ml/kg/IP, **Group II: Alpha-lipoic acid** at 20 mg/kg/IP, **Group III: Alpha-lipoic acid** at 40 mg/kg/IP, and **Group IV: Alpha-lipoic acid** at 80 mg/kg/IP.

One hour after the treatment, the chicks were injected with an anesthetic dose of propofol at 20 mg/kg/IP, then the onset of anesthesia was recorded, which is the time between propofol injection, until the loss of Righting Reflex for each of the chicks separately, and the duration of anesthesia was calculated, which is the period From the loss of the Righting Reflex until they return and correct their body to its normal position, in addition recording the period of recovery from anesthesia, which is the period between the time of the return to normal body position until the chicks return to movement (Naser and Mohammad 2014).

2-Toxicological challenge: the effect of alpha-lipoic acid on the median lethal dose of chlorpyrifos:

Chicks were divided into 3 groups as follows: **Group I: control** (propylene glycol), **Group II: Alpha-lipoic acid** at 20 mg/kg/IP, and **Group III: Alpha-lipoic acid** at 40 mg/kg/IP.

One hour after the injection, the lethal dose of chlorpyrifos was calculated using the up and down method described by Dixon (Dixon, 1980) for all groups. This method is summarized by oral administration of the chick with a dose of chlorpyrifos and then

observing the survival or death 24 after the treatment. If death occurs, the chick is known an X mark and if it does not happen, the chick is known the mark O, and by repeating this method up and down the dose by a fixed amount (2mg/kg) after the change in effect was occurring enabling us to calculate the median lethal dose (LD_{50}) of chlorpyrifos based on the table mentioned and Using the following equation: $LD_{50} = Xf + Kd$. Whereas; Xf = the last dose used in the experiment, K = a tabular value extracted from the table mentioned by Dixon, d = the amount of constant increase or decrease in the administered dose.

Statistical analysis:

We used the SPSS program to analyze the parametric data, where we used the (One-way analysis of variance ANOVA) test, and then the least significant difference LSD test was applied to it. The difference level for all tests was at a probability level of less than 0.05. The data were presented as the mean \pm standard error.

Results

Pharmacological challenge: The effect of alpha-lipoic acid on the anesthesia induced by propofol in chicks:

The alpha-lipoic acid at 20, 40, and 80 mg/kg/IP had a depressant effect on the central nervous system revealed by significantly increasing the duration of anesthesia with propofol at 20 mg/kg/IP after one hour of lipoic acid administration in comparing with the control group in a dose-dependent manner, while there were no significant differences between groups with respect to the time of sleep onset. Furthermore, Alpha-acid at 20, 40, and 80 mg/kg/IP led to a significant decrease in the recovery period compared to the control group in a dose-dependent manner Table 1.

Table 1: Pharmacological challenge: The effect of alpha-lipoic acid on the anesthesia induced by propofol in chicks.

Groups	Onset (min)	Duration (min)	Recovery (min)
Vehicle (propylene glycol)	4.0 ± 0.51	47.5±1.62	51.5±12.70
ALA 20 mg/kg	4.5 ±1.25	63.1±14.61	45.5±3.66 *
ALA 40 mg/kg	6.5 ± 1.40	83.3 ±13.72*	41.3±5.42 *
ALA 80 mg/kg	3.3±0.66	100.7 ±16.18* a	30.5±4.52 ab*

n = 6, the observations are mean ± SEM, *P<0.05, as compared to control, a P<0.05, as compared to ALA at 20 mg/kg, and b P<0.05, as compared to ALA at 40 mg/kg.

Toxicological challenge: the effect of alpha-lipoic acid on the median lethal dose of chlorpyrifos:

Alpha-lipoic acid had a protective effect on the poisoning of the insecticide chlorpyrifos for chicks, and this effect was represented by determining the medium

lethal dose of oral chlorpyrifos using the Up and Down methods. The LD₅₀ of chlorpyrifos was 6.6, 8.6, and 10.6 mg/kg body weight, respectively (Table 2) to chicks previously injected with alpha-lipoic acid before one hour at 0 (control group) 20 and 40 mg/kg/IP. The percentage of increment in LD₅₀ was 31% and 62%.

Table 2: Toxicological challenge: the effect of alpha-lipoic acid on the median lethal dose of chlorpyrifos

Groups	LD ₅₀	Range	1st dose	Last dose	+or-dose	No. of chicks	Dose sequence	%
vehicle	6.6	10-6 = 4	10	8	2	XXOXOX	10-8-6-8-6-8	-
ALA 20 mg/kg	8.6	10-8 = 2	10	10	2	XOXOX	10-8-10-8-10	31%
ALA 40 mg/kg	10.6	12-8 = 4	10	10	2	XOOXO	10-8-10-12-10	62%

*X=death; O=survival The LD₅₀ was determined by the up-and-down method (Dixon 1980).

Discussion

The pharmacological challenge is one of the methods used in the research and detection of the latent or hidden effects of drugs and toxins on the neurological and behavioral functions in laboratory animals (De Kloet et al., 2006; Schlotz et al., 2008). The pharmacological challenge highlights the dysfunction by causing an unexpected extra effort on the central nervous system.

Alpha-lipoic acid caused an increased in the duration of propofol-induced anesthesia in a dose-dependent manner and decreased the recovery period, indicating

the inhibitory effect of alpha-lipoic acid in the central nervous system according to our findings. No such effect of alpha-lipoic acid was recorded with other anesthetic drugs in the scientific literature, especially in bird species or mammalian species. Our findings agree with a previous study conducted on rats model indicated the inhibitory effect of alpha-lipoic acid in nervous behavior and motor activity (Gupta et al., 2018) .The expected reason for the decrease in the recovery period attributed to the antioxidant effects of alpha-lipoic acid may be due to its scavenger activity on free radicals that arise accidentally through the

anesthesia process (Reinke et al., 1998), the increase in the period of anesthesia induced by propofol for chicks previously injected with alpha-lipoic acid may be due to the type of solvent used in dissolving alpha-lipoic acid is propylene glycol, which is considered to have depressant effects on the central nervous system as reported by many studies (Farber et al., 2010; Iltis et al., 2008; Moon, 1994). Therefore, the effect of this must be studied alpha-lipoic acid at the level of the central nervous system using other solvents to confirm its depressant effect on the central nervous system, furthermore using the other tests to determine the neurobehavioral effects of alpha-lipoic acids.

Chlorpyrifos has been widely used globally as an insecticide to control agricultural and household pests such as termites, reduce insect damage, and control mosquitoes. It was first introduced to the market in 1965 and its toxicity was first evaluated in 1969. The problems associated with it are not limited to the dangers of pesticides to humans and the environment in developing countries furthermore extended to developed countries (Nuckols et al., 2007)

Alpha-lipoic acid had a protective effect against the poisoning of the insecticide chlorpyrifos in chicks by increasing the median lethal dose of chlorpyrifos by 31.3% and 62.0% when alpha-lipoic acid was injected at doses of 20 and 40 mg/kg. Our study agreed with two studies conducted in rats about the protective effect of alpha-lipoic acid in chronic chlorpyrifos and deltamethrin poisoning (Uchendu et al., 2017, 2018). One of the most important toxic effects associated with exposure to insecticides

such as chlorpyrifos is oxidative stress and the generation of free radicals (Aly et al., 2010; Baba et al., 2016). Alpha-lipoic acid scavenges free radicals and has an antioxidant mechanism through its ability to on the recycling of endogenous glutathione (Patrick, 2002; Suh et al., 2005).

Conclusion

According to our result, we demonstrate that the alpha-lipoic acid has a depressant effect on the central nervous system furthermore the preanesthetic administration of it has shortened effect to the recovery time which has been a clinical benefit in clinical application. Alpha-lipoic acid also has a protective effect against toxicity with chlorpyrifos.

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Conflict of interest statement

The authors declare that they are not involved in any potential conflicts of interest.

Author's Contributions

Both ASN and MR are equally contributed in the planning, analysis, evaluation, writing, and final approval of this article.

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