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## Assessment anti-granuloma activity of Alpha-lipoic acid in chicks' models

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**ABSTRACT:** Background: Alpha-lipoic acid is an anti-inflammatory and antioxidant agent that has free radical scavenging characteristics as well as better effects on the recycling of other cellular antioxidants.

Aim: The present study was made to assess the anti-granuloma of Alpha-lipoic acid by the cotton pellet granuloma method.

Method: The chicks weighing around 100-150 g were selected, the Alpha-lipoic acid was administered at the following dose of 20, 40 & 80 mg/kg body weight respectively after insertion of cotton pellets into the groin region of chicks.

Results: By using the up-and-down technique, it was found that the Median Lethal Dose ( $LD_{50}$ ) of the Alpha-lipoic acid was 451.8 mg/kg, IP. Alpha-lipoic acid at 20, 40 and 80 mg/kg per body weight daily for consecutive seven days revealed that the ALA shows dose-dependent inhibition of weight of both wet and dry cotton pellets. The inhibition percentage for wet cotton pellets was 5, 11 and 14 respectively whereas the inhibition percentage for dry cotton pellets was 9, 15 and 25 respectively.

Conclusion: We conclude that Alpha-lipoic acid has notable anti-granuloma activity as compared to control in animal models of chronic inflammation. Additional studies must be done to find the molecular role of Alpha-lipoic acid as anti-granuloma remedies

**Keywords:** Alpha-lipoic acid, Cotton pellets granuloma, Chronic inflammation, Chicks, anti-granuloma.

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## INTRODUCTION

**A**lpha-lipoic acid (ALA) is a natural particle made up of a five-membered cyclic disulfide and a hydrocarbon tail that ends in a carboxylic acid group. Alpha-lipoic acid, as a result, is a predominantly hydrophobic particle with an amphipathic charisma because of the carboxylic acid group involved in the ring structure(Gomes & Negrato, 2014). Lipoic acid is found in our food primarily in animal organs like muscles and liver tissues, and at low or untraceable levels in plant foods like potatoes. Alpha-lipoic acid, on the other hand, is considered desirable when used as a food additive due to its antioxidative activity, which has initially been described, and many articles have indicated its preventive properties in cases such as aging, type 2 diabetes, and neuropathy(Perera, Tan, Jeevathayaparan, Chakravarthi, & Haleagrahara, 2011). Many studies have confirmed that ALA has analgesic effects for pain. In a study conducted on rats, it was found that alpha-lipoic acid had analgesic effects in the postoperative pain model in two ways. Cotton pellet-induced granuloma formation is the most suitable method for studying the efficacy of drugs against the proliferative phase of inflammation(Abraham & Agshikar, 1972). The subcutaneous implantation of a cotton pellet into a rodent results in the formation of a granuloma at the site of the implant(Abraham & Agshikar, 1972). The initial events include the accumulation of fluid and proteinaceous material together with infiltration of macrophages, neutrophils, and fibroblasts, and multiplication of small blood vessels, which are the basic sources of the highly vascularized reddish mass termed granulation tissue(Oljade, Makinde, & Awe, 1999). This method has been widely used to assess the transudative, exudative, and proliferative phases of subacute inflammation. The fluid adsorbed by the pellet greatly influences the wet weight of the granuloma, whereas the dry weight correlates well with the amount of granulomatous tissue formed(Subash, Veeraraghavan, Sali, Bhardwaj, & Vasanthi, 2016). In the cotton pellet-induced chronic inflammation model, the cotton pellet, when applied in the interscapular area, induces a chronic inflammation process. In this process, monocyte migration, liquid accumulation, apoptosis, damage, and so on will occur in the surrounding tissue of the pellets, and these accumulations will produce a granulation tissue that covers the pellets(Uzkeser et al., 2012). Oxidative stress is closely related to inflammation in the body, which initially appears in changes that occur in

blood vessels, such as atherosclerosis, as a result of increased synthesis of vascular adhesion molecules. In inflammatory processes and increases endothelial cell migration(Acaroz et al., 2019; Arslan, Keles, Siuda, Rostami, & Bollwein, 2021; Liguori et al., 2018; Tibullo et al., 2017) Reactive Oxygen Species stimulate the production of nuclear factor-kappa B, activator protein-1, and mitogen-activated protein kinase(Lj et al., 2015; Puvača et al., 2015). Nuclear factor-kappa B is one of the most important inducers for the production of inflammatory cytokines such as TNF-alpha. And interleukins and cyclooxygenase II (COX2)(Román-Pintos, Villegas-Rivera, Rodríguez-Carrizalez, Miranda-Díaz, & Cardona-Muñoz, 2016). The anti-inflammatory mechanism of ALA can be summarized through its inhibition of nuclear factor kappa B(Çakir et al., 2015), and the anti-inflammatory action of ALA is different from that of it. The anti-inflammatory effect of ALA is attributed to its inhibition of nuclear factor-kappa B, tumor necrosis factor-alpha and interleukin-6 and its activation of anti-inflammatory proteins, the most important of which is a nuclear erythroid2-related factor (Nrf2) (Andrea Moura, Queiroz de Andrade, Celia Farias dos Santos, & Oliveira Fonseca Goulart, 2015).

To our knowledge, there was no study on evaluating the effect of alpha-lipoic acid on chronic inflammation in the broiler chick model, and the chicks were not used in the test for cotton pellets induce granuloma. Therefore, our study aimed to evaluate the anti-granuloma activity of ALA in cotton pellets induced granuloma models in broiler chicks.

## MATERIALS AND METHODS

### Ethical approval

We obtained the official approval for the study protocol from the Committee of Postgraduate Studies at the College of Medicine, University of Mosul, Iraq according to institutional regulations on animal handling and use in research.

### Drugs and vehicle

The Alpha lipoic acid 600mg (Lipoic-Forte by AMS®) dissolved in propylene glycol (99% Thomas baker, India) and Meloxicam 15mg/1.5ml (Boehringer Ingelheim,Germany) injected intraperitoneally.

### Animals:

37 Ross broiler chicks of unsexes were obtained at age of one day from a local hatchery in Nineveh,

Iraq. They were housed in batches of 20-25 chicks. Ambiental conditions were following broiler chickens hybrid recommendations, while wood shavings were used as floor litter. The supply of water and feed were *ad libitum*. Experiments were conducted when the ages of the chicks were between 7-9 days. This is a suitable experimental animal (chick) model used previously in pharmacological and toxicological studies(Alatrushi & Naser, 2021; Naser & Albadrany, 2021)

### Doses selection

The doses were chosen according to the acute toxicity trials. The LD<sub>50</sub> of the Alpha lipoic acids was calculated by the up and down method described by Dixon(Dixon, 1980). Hence the authors selected the three doses of Alpha-lipoic acid as 20, 40 and 80 mg/kg.

### Calculation of the median lethal dose LD<sub>50</sub> of alpha-lipoic acid in chicks

We determine the LD50 of ALA in the method described by Dixon(Dixon, 1980). Rudimentary dosage of Alpha-lipoic acid at 1000 mg/kg, IP which depends on an initiative test. The chicks were observed for 24 hours marked at the appearance of Alpha-lipoic acid toxic signs afterward, the doses of Alpha-lipoic acid should be reduced or amplified 200 mg according to the death presented by Alpha-lipoic acid in the chicks. The toxicity signs observed in the chicks were recumbency, defecation, tachypnea, Tonic and clonic convulsion, and the outcome was death during 10-15 min.

The LD50 value was calculated as follows:

LD50 value = xf + K d were:

xf = The last dose used

K = Table's value which extracted from Dixon, 1980

(Depend on X and O symbols obtained)

d = ± in the dosage

### Screening of anti-granuloma action (Cotton pellet granuloma model)

#### Experimental procedure.

This study was carried out as described by Ismail et al (1997)(Ismail, Gopalakrishnan, Begum, & Elango, 1997). With the modified animals model we used chicks instead of rats. A sterilized cotton pellet Weighing 10 ± 1 mg was inserted subcutaneously into

the thigh region of anesthetized chicks with propofol at 80mg/kg intraperitoneally after which five groups were injected (once daily) with 20,40 and 80mg/kg for seven sequential days. Chicks in negative control received only propylene glycol whereas chicks in positive control groups received meloxicam at 5mg/kg respectively. The chicks were sacrificed on the 8th day. Afterward, the pellets were dissected out cautiously and the weight of wet cotton pellets was noted thereafter the cotton pellets were dried in the oven at 40°C for 12 h to get a dry cotton pellet weight, the mean weight of granuloma tissue formed around each pellet was obtained and the percentage inhibition was determined.

Percentage inhibition % = Control - Treated/ Control X100

#### Statistical analysis

Data are shown as mean ± SEM. Statistical analysis was carried out using a one-way analysis of variance and then subjected to an LSD test. P<0.05 was deemed significant

## RESULTS

### Calculation of the median lethal dose LD<sub>50</sub> of alpha-lipoic acid in chicks

Table 1 show Alpha-lipoic acid LD<sub>50</sub> value in chicks determined to become 451.8 mg/kg, IP.

### Screening of anti-granuloma action (Cotton pellet granuloma model)

The chicks were injected intraperitoneally with different doses of ALA at 20, 40 and 80 mg/kg per body weight daily for consecutive seven days. The results revealed that the ALA shows dose-dependent inhibition of weight of both wet and dry cotton pellets.

Table 2 reveals that ALA at 20mg/kg decreases in wet and dry weight of cotton seeds in comparison with the control groups At inhibition percentage 5 and 9% respectively .ALA at 40mg/kg showed a significantly lower wet and dry weight in comparison with the control group and meloxicam group at inhibition percentage 11 and 15 respectively. ALA at 80mg/kg show significantly lower wet and dry weight in comparison with the control group and groups of ALA At 20mg/kg at 14 and 25 inhibition percentage respectively The ALA was found to be most effective at a dose of 80 mg/kg body weight (Table 2 and in Figs. 1 and 2, Figs).

**Table 1:** Median lethal dose ( $LD_{50}$ ) of ALA injected IP in chicks

Variables	Results
$LD_{50}$ mg/kg	451.8
Average of the doses utilized mg/kg	1000-600=40
first dose mg/kg	1000
final dose mg/kg	600
Increase or decrease in the dose mg/kg	200
Number of chicks involved	(XXXXXX) 7
Sequence of doses	1000,800,600,400,600,400,600

X: death, O: no death, The  $LD_{50}$  was calculated by the up-and-down method

**Table 2:** Effect of Alpha-lipoic acid on cotton pellet granuloma.

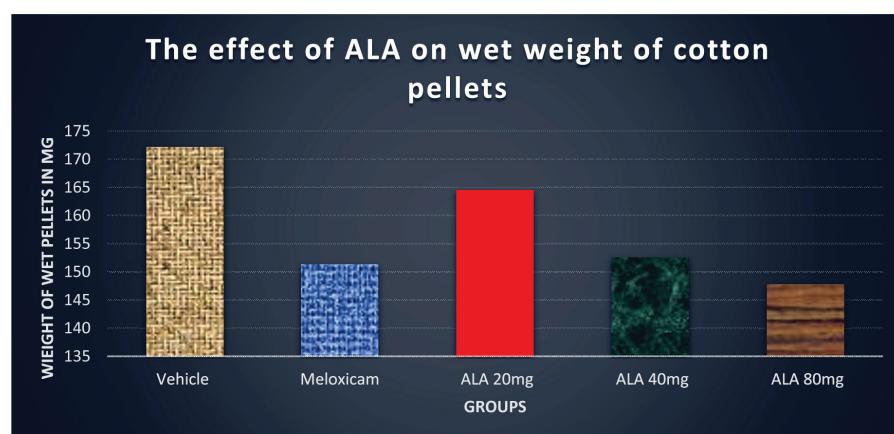
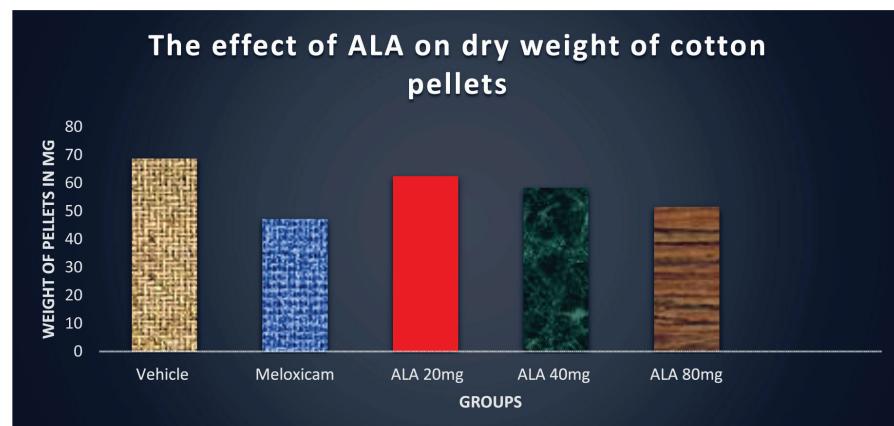
Treatment group	Mean of wet cotton pellets (mg)	Percentage inhibition	Mean of dry cotton pellets (mg)	Percentage inhibition
Vehicle	172.2 $\pm$ 2.75	-	3.38 $\pm$ 68.8	-
Meloxicam 5mg/kg	5.81 * $\pm$ 151.4	12	0.80* $\pm$ 47.8	31
ALA 20mg/kg	4.23*a $\pm$ 164.6	5	2.40*a $\pm$ 62.6	9
ALA 40mg/kg	4.94*a $\pm$ 152.6	11	3.23*a $\pm$ 58.4	15
ALA 80mg/kg	3.44*b $\pm$ 147.8	14	5.05*b $\pm$ 51.6	25

Values are mean  $\pm$  SE of 6 chicks/group.

\*Significantly different from the respective vehicle,  $p < 0.05$ .

a Significantly different from the respective value of Meloxicam 5mg/kg  $p < 0.05$ .

b Significantly different from the respective value of the group treated with Alpha-lipoic at 20 mg/kg,  $p < 0.05$ .

**Figure 1:** The effect of ALA on the wet weight of cotton pellets**Figure 2:** The effect of ALA on the dry weight of cotton pellets

## DISCUSSION

The inflammatory process is the essential portion of the body's protection mechanism (Román-Pintos et al., 2016). It is recognized that acute inflammation involves 3 chief vascular properties such as notable vasodilation, vascular permeability increment, and migration of leukocytes and other inflammatory cells to the damaged part of the body (Ching, Omogbai, Okpo, & Ozolua, 2009). The chronic inflammatory process consists of fibroblasts proliferation, connective tissue augmentation, and mononuclear cells infiltration (Kapsokefalou et al., 2001). These chemical mediators of the inflammatory process, if go unrestrained, can lead to the destruction of the tissue also, finally subsequent to a variety of sicknesses e.g. rheumatoid arthritis. Drugs that have anti-inflammatory properties have long been involved to treat this variety of inflammatory diseases. Anti-inflammatory drugs, in use today, though have efficacy, causes a variety of adverse effects producing major health problem during their therapeutic use (Kapsokefalou et al., 2001).

The assessment of ALA was done using cotton pellets granuloma, which has several advantages for drug anti-granuloma validation. Initially, the reaction is local and involves only the skin, so topical application excludes drug metabolism and excretion. Second, this model employs a very small amount of remedies; analogously, granulomatous tissue creation is associated with a chronic inflammatory reaction that is distinguished by several processes. Cotton pellet-induced granuloma model comprised at least 3 stages, i.e., transudative stage, exudative stage and proliferative stage. The proliferative stage is distinguished as the increment in the dry weight of the granuloma (Ching et al., 2009; Kapsokefalou et al., 2001). In the cotton pellet-induced granuloma model, we measured mean wet and dry granuloma weight. A decrease in the mean wet and dry granuloma weight is considered an anti-inflammatory property. In this model, the anti-inflammatory activity of Alpha-lipoic acid as

shown by a decrease in mean wet and dry granuloma weight was significant ( $p > 0.05$ ) as compared to control (Table 1). Percentage inhibition of mean wet and dry granuloma weight shown by Alpha-lipoic acid was very greater as compared to meloxicam (Table 1). Several mechanisms have been suggested to clarify this anti-inflammatory reaction of Alpha-lipoic acid. Recent articles refer to that Alpha-lipoic acid has the ability to suppress the creation of numerous cytokines, nitric oxide, tumor necrosis factor- $\alpha$ , leukotrienes, and prostaglandins (Çakir et al., 2015; Román-Pintos et al., 2016; Tibullo et al., 2017). The greatest apparent mechanisms of chronic anti-inflammatory action of Alpha-lipoic acid could be due to that, they suppress the proliferation of fibroblasts and collagen synthesis in chronic inflammation. Previous studies, such (Bulut et al., 2011; Gomaa, Abd El-Mottaleb, & Aamer, 2018; Shen et al., 2015; Tastekin et al., 2007) have also shown that Alpha-lipoic acid has anti-inflammatory Action using rodent models. Our findings are associated with the outcomes in the above research. So from our study, we suggest that Alpha lipoic acid has chronic anti-inflammatory action. These results need to be confirmed in other animal models studies for further founding of the role of Alpha-lipoic acid in the management of chronic inflammation.

## CONCLUSION

We conclude that Alpha-lipoic acid has notable anti-granuloma activity as compared to control in animal models of chronic inflammation. Additional studies must be done to determine the molecular role of Alpha-lipoic acid as anti-granuloma remedies.

## ACKNOWLEDGMENT

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## CONFLICT OF INTEREST

None declared.

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