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The anti-inflammatory effect of Dexamethasone against xylene-induced inflammation in male albino Balb/C mice

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Abstract

Nitric oxide (NO) is a small molecule that has a multifaceted role in inflammation, influencing both pro-inflammatory and anti-inflammatory processes. generated by the enzyme nitric oxide synthase (NOS). Excessive production of NO in inflammation leads to a chronic phase. In contrast, dexamethasone (DEXA) is a common anti-inflammatory steroid medication. In this study, the anti-inflammatory effects of dexamethasone treatment was investigated in male albino Balb/C mice. Thirty male albino Balb/C mice weighing between 18 g to 25 g were categorized into: a negative control group received normal saline IV (in tail vein) at a dose 0.1 mL for 6 days, a positive control group (Xylene-induced model) ; mice were injected with normal saline IV at a dose 0.1 mL for 6 days, One hour after the sixth day of administration, animals received a 50-µLsmearing of xylene on both the anterior and posterior surfaces of the right ear lobe and a DEXA group animals were injected with DEXA (1.25 mg/kg) IV for 6 days. Two hours later, samples from ear pinna and sera from all groups were separated for histopathological & biochemical analysis respectively. treatment with 50-µL smearing of xylene on both the anterior and posterior surfaces of the right ear lobe resulted in a significant rise in the levels of serum NO when compared with control group data, while the treatment with DEXA for 6 consecutive days revealed a high-significant decline in the elevated serum level of nitric oxide. The dermis shows dermatitis represented in extensive mast cell infiltration as well as broadening tissue space, edema, discrete and fractured striated muscle fiber in the ear tissue of the xylene-induced model group while DEXA treatment showed thickened-epithelium, cytoplasmic vacuolation in the basal and spinous layer.

Keywords: Nitric oxide, inflammation, dexamethasone

Introduction: Inflammation is a complex and intricate biological process [1] that accompanies numerous diseases [2]. One of the key mediators of inflammation is Nitric Oxide (NO) [3-4], which often exacerbates

the inflammatory response and contributes to the progression of inflammation toward a chronic state [5], NO is generated through a chemical process involving the activation of the enzyme inducible nitric oxide synthase (iNOS). This process, known as the nitric oxide synthesis pathway, involves the conversion of the amino acid L-arginine into nitric oxide and citrulline. iNOS is stimulated by inflammatory signals, such as cytokines, and produces NO as a signaling molecule in response to inflammation [6]. The exact details of the chemical steps and interactions involved in this process are complex and are still the subject of ongoing scientific research. NO molecule plays a crucial role in regulating various physiological processes and immune responses [7], but excessive or prolonged production of light NO can lead to detrimental effects, including tissue damage, disrupts cellular processes, causes cell death, inhibits the immune response against pathogens, contributes to the development of autoimmune diseases, and affects negatively of the cardiovascular system[8, 9, 10].

To address inflammation and its associated symptoms, commonly used medications include Dexamethasone (Dexa). Dexa is widely utilized for the treatment of inflammatory conditions due to its anti-inflammatory properties. By inhibiting certain enzymes involved in the production of inflammatory mediators, (Dexa) helps alleviate inflammation and reduce pain [11].

It is important to note that while Dexa can provide relief from inflammation, its use should be guided by medical professionals, as it may have potential side effects and interactions with other medications. Proper dosage and monitoring are crucial to ensure its safe and effective use in managing inflammatory conditions. understanding the complex mechanisms of inflammation and the role of mediators such as NO is essential for developing effective treatments. Medications like dexa play a significant role in managing inflammation and improving the quality of life for individuals with inflammatory diseases [12]. The present study aimed to investigate the effect of dexa on NO level at inflammation condition.

Materials and methods

A. Materials

***I.Experimental animals*:**

Thirty adults male albino Balb /C mice weighing between 18 g - 25 g were obtained from pharmacy college at Qassim university. All mice were housed in cages of 10 mice per cage and the environment of the laboratory was 19-25 C ° and 25-40% for humidity with 12 hour-controlled light-dark cycle. Mice were provided with a standard chow diet (General Organization for Grain Silos & Flour Mills, Riyadh) and purified water(13).

II.Chemical

The nitric assay kit was purchased from sigma Aldrich co., USA

A 1 mL syringe, normal saline and dexamethasone were obtained from a local pharmacy in Qassim region. Xylene was obtained from the laboratories of biology department, college of science at Qassim University.

III.Experimental design

Thirty adult male albino Balb/C mice were partitioned into 3 groups; **G I**: negative group was injected with normal saline at a dosage of 1.25 mg/kg for 6 days.

G II: Xylene-induced model mice were injected with normal saline by IV at a dose 0.10 mL for 6 days by using restrainer **[13]** One hour after the sixth day of administration, animals received a 50-µLsmearing of xylene on both the anterior and posterior surfaces of the right ear lobe. **GIII**: Mice were injected with dexamethasone (1.25 mg/kg) IV for 6 days **[13]**. Two hours later, samples from ear pinna and sera from all groups were separated for histopathological & biochemical analysis respectively.

B. Methods

I.Sampling

The mice were euthanized in humanly manner, adhering to the ethical principles of scientific research by using formaldehyde, the blood was collected in collecting tube. 9-mm sections were taken from both ears with a cork borer and tissue subsequently weighed. The study was approved by the Committee of Research of Ethics, Deanship of scientific Research Qassim University.

II.Analysis

The serum was separated by centrifugation at 12,000 rpm for 5 min. NO analysis was performed using an ELISA kit, with the Griess reagent as the principal method. The ear tissues were obtained using a biopsy tool, followed by preservation in formalin solution the histological examination was conducted using the hematoxylin and eosin staining technique.

II.Statistical analyses

Data were performed using the one-way analysis of variance (ANOVA) followed by the post hoc Tukey test. The General Linear Model Procedure of the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC, USA; copyright©1998) was employed for these analyses. Multiple comparisons were conducted at a significance level of $P \leq 0.05$, as determined by Waller and Duncan [14].

Results:

The treatment of control mice with 50-µL smearing of xylene on both the anterior and posterior surfaces of the right ear lobe for an hour resulted in a significant (($p \leq 0.01$) rise (**55.6**%) in the levels of serum **nitric**

oxide when this group was compared to the corresponding value of the control animals' group (26.6 vs 17.1 respectively). In contrast, the IV treatment of mice with both **DEXA** (1.25mg/Kg b.wt.) for six consecutive days resulted in a low-significant decline (**-15.8**% and **-7.5**% respectively) in the serum **nitric oxide** level when was compared to the model animals' group (**figure 2 &Table 1**). and histopathological examination of ear pinna of the control group revealed normal histological appearance of the keratinized epidermal layer, dermal structures with conjunctive tissue and vessels, as well as adnexal structures with normal architecture and thickness without any sign of inflammatory changes **(figure A)**. Meanwhile, the DEXA-induced model group showed the maximum thickness compared to the negative control group and other groups. In comparison with other groups, the epithelium was thickened, exhibited cytoplasmic vacuolation in the basal and spinous layer **(Figure B)**. The dermis shows dermatitis represented in extensive mast cell infiltration **(Figure C)**, as well as broadening tissue space, edema, discrete and fractured striated muscle fiber in the ear tissue of xylene-induced model group **(Figure D)**.

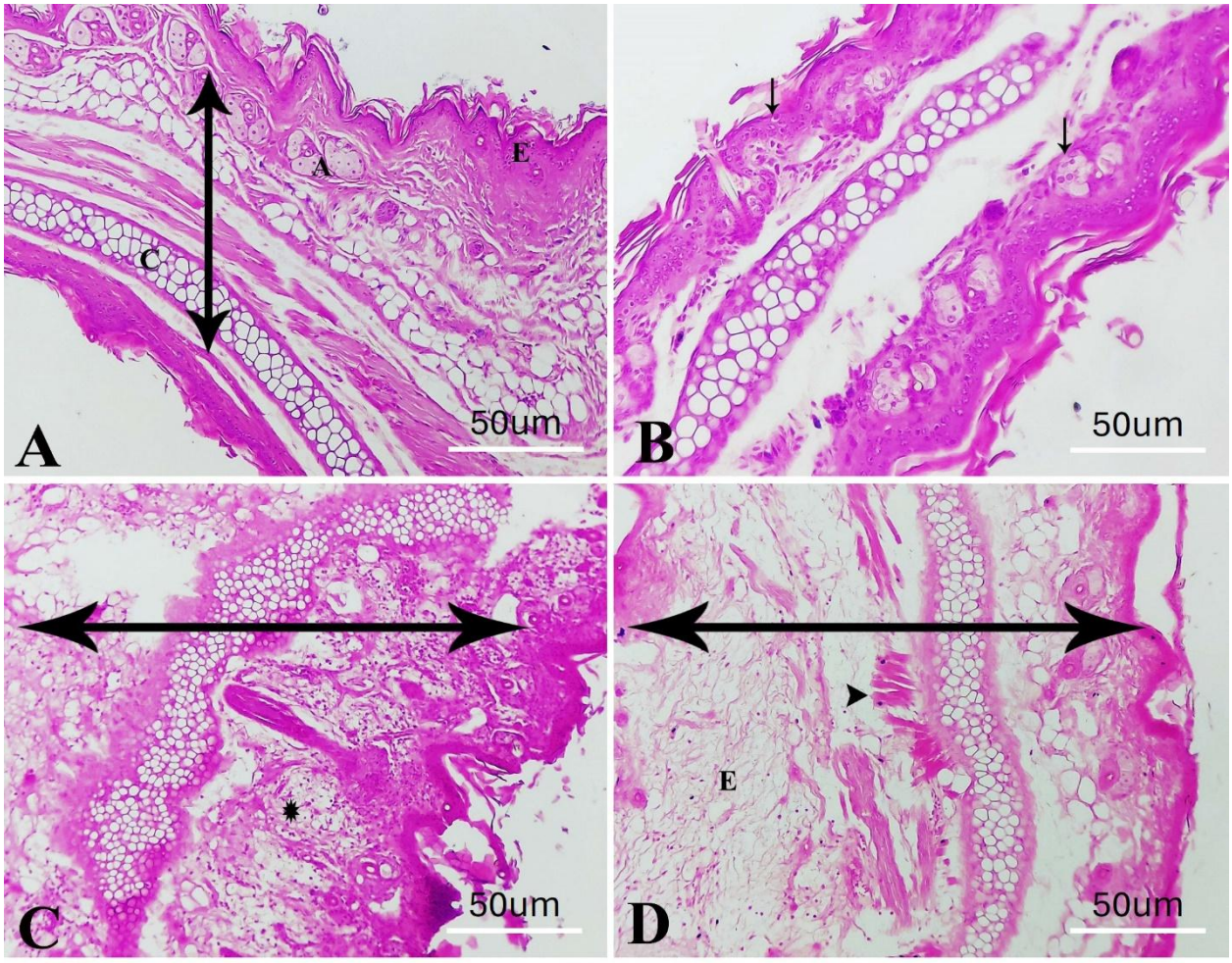

Fig 1: Histopathological samples

A. Photomicrograph of a section of Ear pinna of control group (I) showing normal histological appearance of ear pinna; keratinized epidermal layer (E), dermal structures with conjunctive tissue and normal adnexal structures (A) and elastic cartilage (C).

B. Photomicrograph of a section of Ear pinna of DEXA group, (II) showing thickened epithelium with cytoplasmic vacuolation in the basal and spinous layer (arrow).

C. Photomicrograph of a section of Ear pinna of group treated with xylene (III) showed diffuse mast cell infiltration (asterisk) in the dermis. (D) increased dermal thickness with ear tissue edema (e) and discrete breaks in the striated muscle fiber (arrowhead).

Table 1 . Mean values of serum nitric oxide of model and anti-inflammatiory treated -animals' groups as compared to normal control animals' group.

Groups \ Parameters		NO (nmol/µL)
Control	M ±SE	17.1±0.707^{A}
Model	M ±SE	26.6±0.412^{B}
	% Change A	**55.6%**
DEXA	M ±SE	22.4±0.832^{C}
	% Change B	**-15.8**
RfIP1 → Model (2.4mg/kg bwt.)	M ±SE	24.6±0.101^{C}
	% Change B	**-7.5%**
RfIP1 → Model (4.8mg/kg bwt.)	M ±SE	19.2±0.345^{A}
	% Change B	**-27.8%**

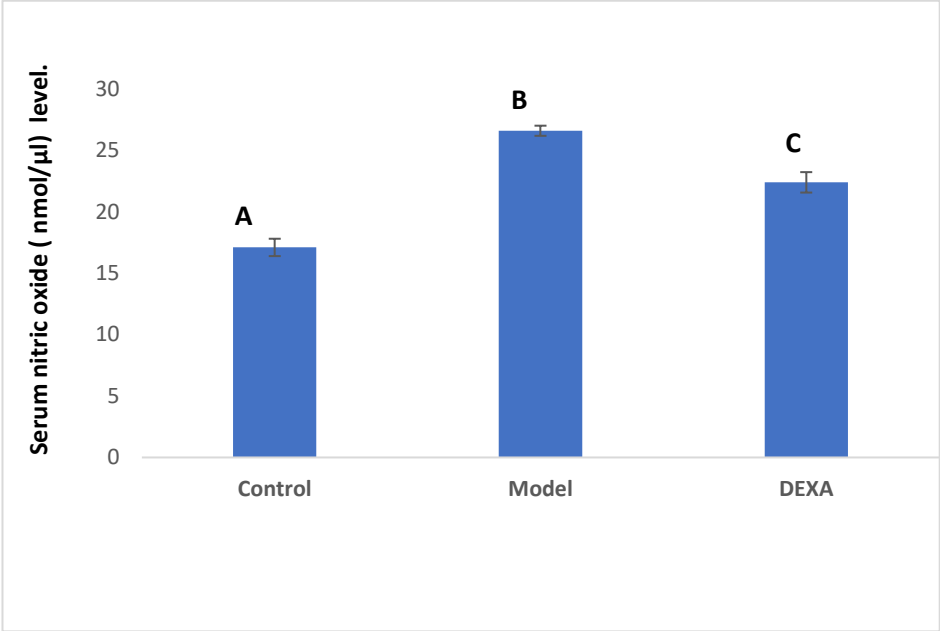

Fig.2 : Means of serum nitric oxide level (nmol/ul) of adult male albino Balb/C mice.

Discussion:

Dexamethasone (DEXA) is a medication that belongs to the class of corticosteroids, which are cortisone-like medicines or steroids. It is used to treat various conditions characterized by inflammation [15].

Nitric oxide (NO is a gaseous molecule with important properties, in human NO acts in vasodilator, neurotransmitters and immune regulation [15-18].

The current study investigated the inflammatory response induced by xylene exposure and its impact NO levels in mice. It was observed that xylene exposure led to a significant increase in serum nitric oxide levels. However, subsequent treatment with DEXA resulted in a notable reduction in NO levels.

Our study aligns with previous findings that demonstrate the inhibitory effect of DEXA on NO synthesis, which has been shown to enhance the survival rate in Plasmodium berghei-infected mice [19]. In the context of critically ill COVID-19 patients, DEXA was associated with a decrease in NO levels and a reduction in the risk of severe acute kidney injury (AKI) [20]. Furthermore, our study revealed a significant reduction in NO levels during xylene-induced inflammation with DEXA treatment, suggesting the potential therapeutic implications of DEXA in modulating NO levels and improving survival outcomes in various pathological conditions. Our current study has shown an increase in the volume of ear tissue due to inflammation caused by Xylene, with no change in size observed when using Dexa. This finding is consistent with a previous study which demonstrated that there are no tissue alterations as a result of Dexa usage [21].

Further investigation is warranted to elucidate the underlying mechanisms and explore potential therapeutic interventions.

Conclusion*:* Our study demonstrates that DEXA treatment reduces NO levels in a xylene-induced inflammatory model in mice. These findings support previous research and suggest the therapeutic potential of DEXA in modulating NO levels and improving outcomes in various pathological conditions. However, further research is needed to fully understand the mechanisms involved and explore additional therapeutic interventions.

Acknowledgment

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Conflicts of Interest:

The authors declare no conflicts of interest related to this research study

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مستخلص البحث

أكسيد النيتريك ((NOجزيء صغير يلعب أدوارًا متعددة في الاستجابة الالتهابية ، حيث يؤثر على الاستجابة المتعلقة بتأييد الالتهاب والاستجابة التي تعمل بشكل مضاد للإلتهاب. يتم إنتاج اكسيد النتريك بواسطة إنزيم مزيج أكسيد النيتريك (NOS)تؤدي زيادة إنتاج أكسيد النتريك في الاستجابة المؤيدة للإلتهاب إلى دخول الالتهاب الى المرحلة المزمنة. بالمقابل، يُعد ديكساميثازون (DEXA)دواء مضاد للالتهاب يُستخدم على نطاق واسع. في هذه الدراسة، تمت دراسة تأثيرات العلاج بالديكساميثازون المضاد للالتهاب المستحث بالزايلين في ذكور الفئران البيضاء البالغة من نوع Balb/C.