# PROTECTIVE EFFECT OF N-ACETYLCYSTEINE (NAC) AGAINST DI- ETHYLHEXYL PHTHALATE (DEHP) INDUCED PULMONARY TOXICITY IN MALE ALBINO RATS (HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY)

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### ABSTRACT

Di-Ethylhexyl phthalate (DEHP) is a global environmental pollutant. DEHP is ingested by people through environmental sources. Both community exposure (food, air, and water) and exposure in hospital settings have a significant impact on human health. DEHP had been reported to have cytotoxic, immunotoxic, genotoxic, and reproductive toxic properties. This study uses bodyweight and relative lung weight measurements to investigate the potential harmful effects of DEHP on the adult albino rats' lungs and the potential protective effects of N-acetylcysteine (NAC). Utilizing biochemical, histological, and immunohistochemical techniques, the toxicity of DHEP is evaluated. Fifty male adult albino rats were divided into five equal groups as follows: Group Ι (Negative control group), Group ΙΙ (Positive control group), Group IΙΙ (NAC-treated group): was given NAC orally (200 mg/kg/day), Group IV (DEHP-treated group): was given DEHP orally (3gm/kg once daily for 4 weeks) and Group V: (DEHP + NAC-treated group): was treated with DEHP concomitantly with NAC at the same previous doses. The present study's findings showed that DEHP has significantly increased the lipid peroxidation level and significantly reduced glutathione content (GSH), superoxide dismutase (SOD) activity, and catalase activity. The inter-alveolar septal thickness, bleeding, and inflammatory cellular infiltration of the lungs seen in group IV's histological results were significantly diminished in group V. Also, group V, compared to group IV, exhibited a significant reduction in the accumulation of collagen fibres and caspase-3 expression. Conclusion: By reducing oxidative stress, inflammation, and apoptosis, NAC therapy can defend against DEHP-induced lung damage in rats.

**Keywords:** Di-Ethylhexyl Phthalate, N-acetylcysteine, lung, lipid peroxidation; glutathione.

### INTRODUCTION

Di-Ethylhexyl phthalate (DEHP) is a diester of phthalic acid (PAE) that is used globally, as a plasticizer to improve the flexibility and elasticity of plastics. Numerous polyvinyl chlorides (PVC) goods, including plastic sheets, wire covers, faux leather, agricultural vinyl films, adhesives, and medical supplies, employ DEHP **(JCIA, 2018).**

Di-Ethylhexyl phthalate is a viscous, colorless substance that is soluble in lipophilic liquids. Under high temperatures, it can easily disperse and collect in the environment. or through contacting hydrophobic substances. However, little DEHP can be detected in the air because it does not evaporate easily **(JCIA, 2018 and Rowdhwal & Chen 2018).** Over 470 million pounds of PAE are manufactured annually throughout the world, and they can be found in many consumer goods such are goods used in the packaging of food, building supplies, toys for children, and other things **(Muczynski et al., 2012; Bernard et al., 2014).**

Di-Ethylhexyl phthalates are bound to the polymer they are associated with by non- covalent bonds. This allows for leaching of these compounds to occur leading to increased risk of environmental pollution and eventually adverse effects on human health **(Junaid et al., 2018).**

In living organisms, DEHP is metabolized to mono-Ethylhexyl phthalate (MEHP), by glucuronidation as well as by the lipases’ action. MEHP further broken down to phthalic acid and other metabolites and it represents around 12% of DEHP metabolism that is recognized as the key metabolite **(Ticker et al., 2001).**

Different ways of human exposure to DEHP through inhalation, oral intake, and skin contact. Orally ingested DEHP is mainly absorbed through the intestinal mucosa into the blood and consequently, it can reach the lungs **(Engel and Wolff, 2013).**

Di-Ethylhexyl phthalate or its metabolites have been demonstrated to induce various harmful health impacts on the heart, lungs, kidneys, liver, and genitalia. In humans, DEHP seems to have a comparatively low risk of liver cancer. Therefore, it is not possible to rule out the development of DEHP-related cancers in people **(Ticker et al., 2001).**

An estimation of the exposure dose of DEHP that is taken by inhalation is about

2.5 mg/kg/day for newborns and children. As regards to the adults, the estimated dose is about 0.14- 9.5 mg/kg/day

### (Sathyanarayana, 2008).

Respiratory issues have frequently been related to phthalate exposure in the environment and at workplace, according to epidemiological research **(Hoppin et al., 2013).** Researches have discussed the possible relationship and mechanisms of respiratory diseases such as asthma and allergy among plastic industry workers and the children exposed to phthalates and PVC **(Jaakkola and Knight, 2008).**

The parent compound (DEHP) and its byproducts cause hazardous effects in the airways of neonatal rats, particularly in the alveoli. Multiple effects have been noted such as alveolar simplification where the alveoli become larger but fewer in numbers with decreased septation, impaired alveolarization process, and a decrease in the levels of the surfactant protein. Such effects suggest that the lung and the alveolar

epithelium could be target sites for phthalate toxicity **(Rosicarelli and Stefanini, 2009).**

Superoxide dismutase (SOD), catalase (CAT), and glutathione-dependent enzymes (GSH) represent an antioxidant defense system that can control the reactive oxygen species (ROS) levels in the tissues **(Eren et al., 2007; Arikan et al., 2010).** One of the lung principal enzymes responsible for catalyzing the dismutation of O2 to H2O2 is SOD. CAT enzymes or the GSH redox cycle are used to further degrade H2O2 **(Beers, 2008)**.

the tissues' synthesis and ROS degradation are out of equilibrium leads to oxidative stress phenomena. These ROS- induced tissue injuries are characterized by oxidative damage of the DNA, lipids, and proteins. Malondialdehyde (MDA) is recognised as a symptom of oxidative stress since it is created during lipid peroxidation in tissues **(Laskin et al., 2010).**

Studies done on aquatic organisms have shown that exposure to DEHP results in lipid peroxidation as well as to changes in the activities of the enzymatic antioxidant (**Yuan et al., 2017).**

N-Acetylcysteine(NAC) is characterized by being an inexpensive and commonly used drug. It is an amino acid that comes before L-cysteine **(Mokhtari et al., 2016).** NAC metabolites are good sources of sulfhydryl groups which stimulate GSH production to enhance detoxification and to directly act as a scavenger of the free radicals **(Saha et al., 2013).** It is one of the suggested solutions for treating disorders linked to the production of ROS because of its potent antioxidant action (**Shahin et al., 2009).**

In light of the above-mentioned background, This study evaluates the pulmonary toxicity of DEHP in adult albino rats and potential protective benefits of NAC against its toxicity (biochemical, histological, and immunohistochemical study).

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# التأثيرات الوقائية لإضافة ان اسيتيل سيستايين ضد الآثار السامة للداى إيثيل هكسيل فثالات على رئتي الفئران البيضاء البالغة) دراسة هستولوجية - مناعية هستوكيميائية(

# أمينة فرج 1، ايمان فاروق2، نشوى حسن أبورية3، تغريد خربوش 4، هايدى فخر1

# 1 قسم الطب الشرعى والسموم االكلينيكية، كلية الطب، جامعة بنها

# 2 قسم األنسجة و الخاليا، كلية الطب، جامعة بنها

# 3 قسم الفارماكولوجيا االكلينيكية، كلية الطب، جامعة بنها

# 4 قسم الميكروبيولوجى، كلية الطب، جامعة بنها

# يعتبر الداى إيثيل هكسيل فثالات ملوث بيئي عالمي يتعرض له الإنسان من خلال المصادر البيئية. إن التعرض لهذا الملوث يسبب تأثيرات واضحة على صحة الإنسان سواء كان التعرض له في المجتمع (كالطعام والهواء والماء) أو في المجال الطبي. وقد وجد أن الداى إيثيل هكسيل فثالات له سمية خلوية، ومناعية، وجينية، وتناسلية. لذلك نهدف بهذا العمل لتقييم الآثار السامة المحتملة للداى إيثيل هكسيل فثالات على رئتي الفئران البيضاء البالغة وتقييم الآثار الوقائية المحتملة لدواء أسيتيل سيستايين باستخدام وزن الجسم ومعايير وزن الرئة النسبي. تم تقييم سمية الداى إيثيل هكسيل فثالات باستخدام الطرق البيوكيميائية والهستوباثولوجية والهستوكيميائية. قُسم خمسين فأر أبيض من الذكور البالغين إلى خمس مجموعات متساوية على النحو التالي: المجموعة الاولى (مجموعة ضابطة سلبية): تم اعطائها الوجبة العادية والماء بدون أي علاج لقياس المعايير الأساسية لمدة 4 أسابيع. المجموعة الثانية (مجموعة ضابطة إيجابية): تم اعطاء كل فأر زيت ذرة عن طريق الفم 1مل /يوميا" لمدة 4 اسابيع. المجموعة الثالثة (المجموعة المعالجة ب ان أسيتيل سيستايين: (أعطيت هذه المجموعة ان أسيتيل سيستايين عن طريق الفم (200 مجم / كجم / يوم). المجموعة الرابعة (المجموعة المعالجة ب الداى إيثيل هكسيل فثالات): تم اعطاء كل فأر الداي ايثيل هيكسيل فثالات بعد اذابته بزيت الذرة بالفم 3 جم/ كيلوجرام/ مرة واحدة يوميا لمدة 4 أسابيع. المجموعة الخامسة: (المجموعة المعالجة بكل من الداى إيثيل هكسيل فثالات وان أسيتيل سيستايين): تم عالجها بـ كل الدوائين بنفس الجرعات السابقة. كشفت نتائج الدراسة أن ب الداى إيثيل هكسيل فثالات قد زاد بشكل ملحوظ من مستوى بيروكسيد الدهون وقلل بشكل ملحوظ في محتوى الجلوتاثيون ونشاط سوبر أكسيد ديسموتاز ونشاط انزيم الكاتالاز. أظهرت النتائج النسيجية للمجموعة الرابعة ارتشاحا خلويًا التهابيًا للرئتين، وذمة خلالية، نزيف وسماكة الحاجز السنخي على عكس المجموعة الخامسة، التي أظهرت كذلك انخفاضا كبيرا في تراكم ألياف الكولاجين وانتاج انزيم كاسبيز ٣ بالمقارنة مع المجموعة الرابعة. الخلاصة: العلاج ب ان أسيتيل سيستايين يمكن أن يحمي ضد السمية الرئوية التي يسببها ب الداى إيثيل هكسيل فثالات في الفئران عن طريق تقليل الاجهاد التأكسدي، الالتهاب، و الموت المبرمج للخلايا

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