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# Alpha-lipoic acid inhibits oxidative DNA damage of some organs induced by 4-tert-octylphenol exposure in rats

**Abd El-Kader M. Abd El-Kader**
Department of Zoology, Faculty of Science, Aswan University, Aswan, Egypt

**Eatemad A. Awadalla**
Department of Zoology, Faculty of Science, Aswan University, Aswan, Egypt

**Mohammed H. Hassan**
Department of Medical Biochemistry, Faculty of Medicine, South Valley University, Qena83523, Egypt
*Corresponding author email: mohammedhosnyhassaan@yahoo.com, mohammedhosnyhassaan@med.svu.edu.eg

**Samia A. Gbr**
Department of Zoology, Faculty of Science, Aswan University, Aswan, Egypt

**Amna H. Nour**
Department of Zoology, Faculty of Science, Aswan University, Aswan, Egypt

**Ahmed Alamir Mahmoud Abdallah**
Medical Biochemistry Department, Faculty of Medicine, Sohag University, Sohag, Egypt

***Abstract***---Endocrine disputer chemicals (EDCs) such as 4-tert-octylphenol (OP), are a class of exogenous pollutants that can interfere with normal physiological processes, resulting in a number of health issues. Therefore, the goal of the current study is to determine how well α -lipoic acid (LA) protects against DNA damage brought on by OP. Forty male albino rats were randomly assigned into four groups. Group I (control group) not received any material, group II (αLA-group) received α-lipoic acid (40 mg/kg/ day), group III (OP-group) received 4-tert-octylphenol (50 mg/kg/ day), and group IV(protective group) received 4-tert-octylphenol (50 mg/kg/ day) plus α-lipoic acid (40 mg\ kg/ day) for a period of 30 days. The DNA fragmentations were detected in tissues of hypothalamus, pituitary gland and testis using agarose gel electrophoresis and bands were visualized under UV light and the total DNA concentration in samples were also measured

spectrophotometrically. Rats administered OP showed significant ($p < 0.05$) rising of DNA damage levels in pituitary gland and testis tissues and non-significantly ($p > 0.05$) increase in hypothalamus tissue. On other hand, αLA treatment showed significant ($p < 0.05$) improvement in DNA damage levels. Our findings concluded that αLA could ameliorate the oxidative DNA damage occurs in various organs that induced by OP exposure.

***Keywords***---4-tert-octylphenol, α -lipoic acid, DNA fragmentations, agarose gel electrophoresis.

## Introduction

The environmental pollution is deliberated as a growing problem in the whole world, but the levels of pollution are varied in different parts of the world (Guerreiro et al.,2014). The advanced countries have been able to notice and governor pollution by execution the environmental laws and performing district regulations, while developing countries are not paying enough attention to the current environmental problems (Koukal et al.,2004). Many of pollutants that relief to the ecosystem causing health problems belong to a group of pollutants that known as endocrine-disrupting compounds which affect the endocrine system and disrupt many physiological functions (Xu et al.,2018).The endocrine-disrupting chemicals (EDCs) defined as an exogenous substance disturbing the hormonal balance (Darbre, 2017). One of the most interesting compounds is alkyl phenols including octylphenols (OP) which have numerous consumptions in our present life. OP including 4-tert-octylphenol are a group of compound that are very much use in our life, it use in an enormous number of products that are used in everywhere and every time. OP can damage oxidative homeostasis through direct or indirect mechanisms, including the increase of reactive oxygen species (ROS), inhibiting antioxidant enzymes, decisive mitochondrial dysfunction, alteration in cell signaling pathways and induction of apoptosis (Themmen et al., 2000).

Much of the latest research on OP toxicity focuses on the capability of OP to induce oxidative stress. Some authors hypothesize that OP can directly produce ROS. Other studies indicate the essential role of the indirect mechanisms, such as OP induced alteration of the antioxidant defense system function. The oxidative damage to DNA is mainly caused by the hydroxyl radical. This radical forms adducts to double bonds of DNA bases and extracts a hydrogen atom from the methyl group of thymine and each of the carbon–hydrogen bonds of 20-deoxyribose. These reactions cause DNA bases modification and sugar moieties, alkali-labile sites and breakdown of double strand. Many studies revealed the vital role of antioxidant supplements such as α--lipoic acid (αLA) as a fire wall against oxidative stress induced DNA damage. αLA is a powerful micro-nutrient vitamin-like substance (Kim et al.,2013). It is commonly exist in dietary components such as vegetables, meats, mainly viscera and also in many dietary supplements (Szeląg et al.,2012). So we aimed in the current experimental study to explore the extent of oxidative DNA damage in the hypothalamus, pituitary and

testis following exposure to OP and the possible alleviating effect of αLA in protecting the DNA from damage in rat model.

## Material and Methods

### Chemicals

Octylphenol (Cat. No.140-66-3) and Alpha lipoic acid (Cat.No.1077-28-7) were purchased from Sigma Aldrich Co. USA. Proteinase K (Cat. No.PK0100) was purchased from Viogene co. USA. Agarose powder (Cat. NO. 9012-36-6) was purchased from Starchemie co. India. Ethidium bromide(Cat. No. 190202) was purchased from Biomedicals France. 1Kb DNA ladder (Cat. No. DM010-R500) was purchased from GeneDirex co. USA. DNA loading dye (Cat. No. R0611)was purchased from thermo scientific co. USA.

### Experimental Design

A total of forty male albino rats, weighing 180 ± 20 g, were purchased from South Valley University's animal house. Animals were kept in clean, well-ventilated cages that were kept at a constant temperature of 25± 2 °C with a relative humidity of 50 ± 5%. Before the study started, rats were kept for around two weeks to allow for acclimatization. The animals were given a pellet meal and unlimited access to water. The Ethical Committee of South Valley University, Qena, Egypt gave its approval to each experimental protocol before it was carried out. Four groups of 10 rats each were formed by randomly allocating the animals into groups. For 30 days, the rats received the following daily treatments:

- Group I (control group): the rats in this group did not inject with any material.
- Group II (αLA group): interperitoneally injected with α-lipoic acid (40 mg / Kg) (Wang et al.,2018), dissolved in alkaline saline.
- Group III (OP group): subcutaneously injected with 4-tert-octylphenol(50 mg / Kg) (Laws et al.,2000; Mikkilä et al.,2006), dissolved in corn oil.
- Group IV (protective group): interperitoneally injected with same dose of α-lipoic acid one hour before 4-tert-octylphenol injection at the same dose.

### Estimation of DNA damage by electrophoresis

DNA was extracted according to phenol\chloroform protocol (Geng et al.,1999). The tissues (hypothalamus, pituitary and testes) were crushed with 1 mL of DNA extraction solution containing 20 mmol/L Tris-HCl, pH 7.4, 0.1 mol/L NaCl, 5 mmol/L EDTA, and 100µl of 0.5% SDS. The cell lysates were incubated with 100mg/mL proteinase K at 37°C for 16 hours. After incubation, 1 mL of phenol/chloroform (1:1) was added then centrifuged at 20000 rpm for 20 minutes; DNA in the upper (aqueous) phase was extracted with phenol/chloroform again. DNA was collected by precipitation with 1 mL of isopropanol and 0.1 mL of 5 mol/L NaClat –20°C.overnight. After centrifugation, the resulting DNA pellets were washed with 75% ethanol and air dried. DNA was dissolved in 10 mmol/L Tris-HCl buffer with 1 mmol/L EDTA, and its concentration was determined at 260 nm by T70 single split beam UV\Vis

spectrophotometer (Cat. NO. MO0033004) manufactured by PG instruments limited co., England (Hassan et al.,2022).

DNA electrophoresis was carried out according to Geng et al. (1999), 1.5 % agarose gel melting in TAE buffer, containing1 mg/mL ethidium bromide staining. The DNA ladder (1kb) was mounted in the first left well; the control, αLA, OP and protective groups samples were mixed with DNA loading dye then mounted in the same order. The DNA samples were electrophoresed at 95-100 V for 1 h and DNA bands were visualized under UV light by UVP-BioDov-IT220 imaging gel documentation system (Cat. No. 97-0182-01) manufactured by Capitol scientific co., USA. The total DNA concentration is directly proportionate with the DNA fragmentations (Shokere et al., 2009). The mild DNA fragmentation well demonstrated as little numbered band near the loading well, the bands of moderate fragmentation located between the loading wells and the end of the gel. While the sever DNA fragmentation well observed as multiple bands (characteristic step ladder appearance) away from the loading well (El-Bendary et al.,2014).

**Statistical analysis**

Results of all quantitative data were expressed as means ± SD. Differences between means were tested by one-way analysis of variance ANOVA followed by the Student-Newman-Keuls T-test using Minitab 12 software so that the data obtained can be compared and statistically evaluated. Statistical significance was considered when $p < 0.05$.

**Results and Discussions**

**Results**

Table (1) revealed that, the mean ± SD values of the pituitary gland and testis DNA concentrations (ng/μL) in OP group (2.679 ± 0.191 & 2.519 ± 0.24 respectively) were significantly higher with $p < 0.05$ for all. While, the mean ± SD values of the hypothalamus DNA levels (1.258 ± 0.090) were non-significantly change with $p > 0.05$ when compared with the control group (1.335 ± 0.43, 1.120 ± 0.05 & 1.1436 ± 0.021 respectively). However, non-significant differences in the mean values of the hypothalamus, pituitary gland and testis DNA levels in LA group (1.123 ± 0.542, 1.121 ± 0.034 & 1.116 ± 0.186 respectively) with $p >0.05$ for all when compared with the control group. The mean ± SD values of the pituitary gland and testis DNA levels in protective group (1.650 ± 0.34 &1.464 ± 0.012 respectively) were significantly lower ($p < 0.05$) than those of OP group (2.679 ± 0.191 & 2.519 ± 0.24 respectively) with non-significant differences in the mean of hypothalamus DNA damage levels in the protective group (1.258 ± 0.090) with $p > 0.05$.

Table 1
Mean total DNA concentrations in hypothalamus, pituitary and testis tissues of various study groups

| Parameters (ng/µL) | Control group | αLA group | OP group | protective group |
|---|---|---|---|---|
| Hypothalamus DNA damage | 1.1436 ± 0.021 | 1.123 ± 0.542 # | 1.258 ± 0.090 # | 1.165 ± 0.030 * |
| Pituitary DNA damage | 1.335 ± 0.43 | 1.121 ± 0.034 # | 2.679 ± 0.191 ## | 1.650 ± 0.34** |
| Testis DNA damage | 1.120 ± 0.05 | 1.116 ± 0.186 # | 2.519 ± 0.24 ## | 1.464 ± 0.012 ** |

Values are means ± S.D, of animals in each group. #non-significant compared with the control group ($p>0.05$). ## significant compared with the control group ($p<0.05$). *non-significant compared with the OP group ($p>0.05$).**significant compared with the OP group ($p<0.05$).

Regarding the patterns of DNA fragmentations among the study groups, all were presented in (Fig. 1-3).

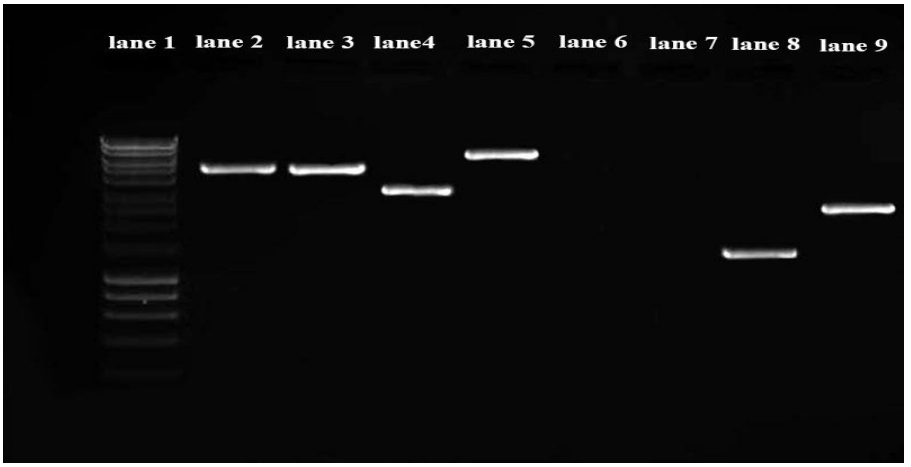


Fig .1. DNA fragmentation detection by agarose gel electrophoresis in the hypothalamus tissues of the control and treated groups.

Lane 1: 1Kb DNA ladder, lanes 2&3: control group, lanes 4&5: αLA group, lanes 6&7: NTC (no template control), lane 8: OP group and lane 9: protective group. Control, αLA and protective groups showed mild DNA fragmentations. However, OP group displayed moderate DNA fragmentation.

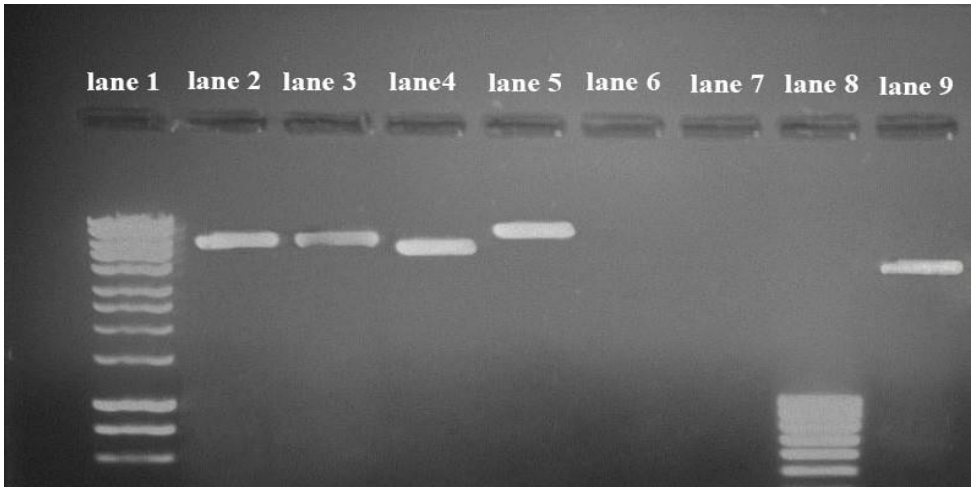


Fig .2. DNA fragmentation assay by agarose gel electrophoresis in the pituitary gland tissues of the control and treated groups

Lane 1: 1Kb DNA ladder, lanes 2&3: control group, lanes 4&5: αLA group, lanes 6&7: NTC (no template control), lane 8: OP group and lane 9: protective group. Control and αLA groups showed mild DNA fragmentations. While, OP group displayed sever DNA fragmentation bands away from the loading well with characteristic step ladder appearance. However, the protective group displayed moderate DNA fragmentation.


Fig .3. Agarose gel electrophoresis image of DNA fragmentation of the testes of the control and treated groups

Lane 1: 1Kb DNA ladder, lanes 2&3: control group, lanes4&5: αLA group, lanes6&7: NTC (no template control), lane 8: OP group and lane 9: protective group. Control and αLA groups showed mild DNA fragmentations. While, OP group displayed sever DNA fragmentation bands away from the loading well with characteristic step ladder appearance. However, the protective group displayed moderate DNA fragmentation.

## Discussion

Accordingly, in our model rat DNA damage increased after OP induction in the pituitary gland and testis tissues, although not significantly increased in hypothalamus tissue; this effect may attributed to the oxidative stress induced by OP as had proofed in previous studied (Othman et al.,2012). The oxidative stress due to excessive generation of reactive oxygen species (ROS) has been associated with affected cellular lipids, proteins and DNA. This explanation is in agreement with Li et al. (2011) and Bahreinian et al.(2015), who revealed that OP induced oxidative stress and also interfered with DNA integrity. Another theory of DNA damage induced by OP was stated by Al-Azawi and Asker (2017), who reported that, high cytochrome c levels released from mitochondrial inner space that accelerate the process of apoptosis, possibly leading to DNA damage. Alternative hypothesis stated that, the lipid peroxidation product 4HNE can bind to mitochondrial proteins triggering electron leakage and formation of further ROS. These events initiate apoptotic cascade, beginning with a loss of mitochondrial membrane potential and ending in DNA damage; this theory is also mentioned by Hamada et al. (2013). Likewise, Othman et al.(2012), showed that, the onset of mitochondrial permeability transition is paralleled by increased release of ROS and fragmentation of DNA. In addition, DNA structure alteration could be attributed to the induction of oxidative stress and depletion of antioxidant defense mechanisms.

Regarding αLA treatment, enormous improvements were noted in the protective group. αLA which been used as a nutritional supplementation, has several potential benefits including therapeutic potential and capable of scavenging free radicals. In the present study, α LA supplementation managed to inhibit DNA damage in OP rats. This theory is in agreement with Budin et al. (2009) , who found α LA has the capability in preventing DNA-damage. Supplementation of α LA had caused a substantial increase in nucleic acid content and protein. However, according to Jana et al.(2014), αLA induction can also inactivate caspase 3; this enzyme cleaves cytoplasmic structural proteins, such as actin and cytokeratins, or nuclear proteins, such as poly-ADP-ribose polymerase and lamins leading to induce activation of caspase-activated deoxyribonuclease (also called DNA fragmentation factor-40), which is integrally involved in degrading DNA and apoptosis in different cells.

## Conclusion

Our findings suggest that that supplementing with α-LA could be a useful treatment strategy for preventing DNA damage caused by 4-tert-octylphenol in the hypothalamus, pituitary gland and testis.

## Author Declarations section

**Funding:** NA.

## Conflicts of interest/Competing interests

The authors report no conflicts of interest in this work.

**Availability of data and materials**

The data will be available from the corresponding author upon reasonable request.

**Authors' contributions**

Conception and study design: Abd El-Kader M. Abd El-Kader, Eatemad A. Awadalla, Samia A. Gbr, Amna H. Nour, Ahmed Alamir Mahmoud Abdallah, and Mohammed H. Hassan. Tissue sampling and molecular assays: Mohammed H. Hassan and Ahmed Alamir Mahmoud Abdallah. Literature research: Abd El-Kader M. Abd El-Kader, Eatemad A. Awadalla, Samia A. Gbr, Amna H. Nour, Ahmed Alamir Mahmoud Abdallah, and Mohammed H. Hassan. Statistical analysis and interpretation of results: Abd El-Kader M. Abd El-Kader, Eatemad A. Awadalla, Samia A. Gbr, Amna H. Nour, Ahmed Alamir Mahmoud Abdallah, and Mohammed H. Hassan. First drafting of the manuscript: Mohammed H. Hassan and Amna H. Nour. All authors gave final approval of the version to be published; agreed on the journal to which the article was submitted; and agreed to be accountable for all aspects of the work.

**Ethics approval**

The Ethical Committee of South Valley University, Qena, Egypt gave its approval to each experimental protocol before it was carried out.

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