

**Original Article****Role of Clove Essential Oil on Benzo(a)pyrene Induced Lung Toxicity in Adult Male Albino Rats****Manar Ali Elsayed<sup>1</sup>, Maha Zayed Mohammed Hassan<sup>2</sup>, Marwa Tawfeek Abaza<sup>1</sup>**

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**ABSTRACT**

**Background:** Environmental exposure to benzo(a)pyrene (BP) elicit pulmonary inflammation and impair lung functions. Clove oil (CLV) possesses antioxidant, anti-inflammatory besides anticancer effects. **The aim of this study:** To evaluate the possible protecting role of clove oil (CLV) against lung injuries induced by benzo(a)pyrene (BP) in albino rats. **Material and methods:** Thirty rats were spilt randomly into 4 groups, control group (12 rats were divided equally into 2 smaller groups; negative and corn oil) as well as 3 experimental groups (each with 6 rats) (BP group: received a daily dose of (50 mg/kg) orally for 4 weeks, CLV group: received a daily dose of (100 µl) orally for 4 weeks, and CLV/BP group: received both CLV and BP in the same doses orally for 4 weeks). After 4 weeks, blood specimens then lung tissue were collected. Lactate dehydrogenase (LDH), reduced glutathione (GSH), Malondialdehyde (MDA), plus cancer marker carcinoembryonic antigen (CEA) were determined. Inflammatory mediators interleukin-1 $\beta$  (IL-1 $\beta$ ) besides tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) were estimated in lung tissues. Histopathological examination by hematoxyline and eosin stain plus immunohistochemical examination of proliferating cell nuclear antigen (PCNA) expression were evaluated. **Results:** GSH was decreased but LDH, MDA, IL-1 $\beta$ , TNF- $\alpha$  and CEA were elevated in BP treated group. Clove oil co-administration ameliorated the biochemical parameters and the histopathological alterations in lung tissues. Also, it reduced the PCNA expression caused by BP in the immunohistochemical examination. **Conclusion:** Clove oil might ameliorate BP elicited pulmonary dysfunction. **Recommendations:** Additional studies are required to explain the molecular mechanisms fundamental to clove oil antitumor activity.

**Key words:** Clove essential oil, Benzo(a)pyrene, Oxidative stress, Carcinoembryonic antigen, Proliferating cell nuclear antigen, Lung toxicity.

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## I. Introduction:

Benzo(a)pyrene (BP) is one of the main agents of polycyclic aromatic hydrocarbons (PAH) which are selected as environmentally chemical pollutants that cause genetic damage through high bioaccumulation potentials (Bukowska et al., 2022). Human are commonly exposed to BP via inhalation, ingestion, and dermal contact as it has been frequently found in the surface water, air, dusts, sediments, cigarette smoke in addition to dietary products, particularly smoked and grilled types (Sicińska and Bukowska, 2021). The European Commission has declared the allowed levels of BP in some food compounds; 6µg/kg in smoked meats and fish, 3µg/kg in fats, and 2µg/kg in cereals (European Commission, 2005).

Researches have shown increasing evidence about toxic effects induced by BP as it is metabolized by cytochrome P450 enzyme system to the more toxic metabolite; BP-7,8-diol-9,10-epoxide (BPDE); triggering mutations and carcinogenesis. Furthermore, BPDE is a neurotoxic and teratogenic compound besides affecting mammals' fertility (Saravanakumar et al., 2022). Other studies on the lungs showed that environmental exposure to BP initiates structural and functional changes in the lungs due to oxidative stress and apoptosis. Emphysema, inflammation, edema, and surfactant damage are common toxic effects that result in the occurrence of several chronic pulmonary disorders as lung tumors and pulmonary fibrosis. It is also observed that BP exposure accelerates the multiplication of pathogens as viruses, which may be a critical problem with the worldwide spread of COVID-19 pandemic (Bukowska et al., 2022).

Recently, a great awareness towards the use of essential oils was recognized, to combat several types of pathological activities particularly clove essential oil. The aromatic clove plant (*Syzygium aromaticum* L. Myrtaceae) is commonly present in tropical countries and it is rich with volatile products plus antioxidants such as eugenol and β-caryophyllene (Haro-González et al., 2021). The FDA recognized clove oil as a generally safe compound so, it is used in many applications such as perfumes, cosmetics, hygienic products, medications, and in food industries (Golmakani et al., 2017). Previous studies demonstrated that eugenol, the main component of clove oil, has antioxidant and anti-inflammatory properties, and prevents lipid peroxidation. The anticancer properties of eugenol were examined via several pathways as cell cycle arrest and inhibition of cell proliferation, migration, apoptosis, angiogenesis, and metastasis on multiple cell line models. Moreover, eugenol is used as an adjuvant treatment in patients who are receiving chemotherapy as this combination has an enhanced efficacy without major side effects or toxicity (Zari et al., 2021). So, the aim of the work was to evaluate the possible protective role of administering clove essential oil on pulmonary injuries provoked by BP exposure in adult male albino rats.

## II. Material and Methods:

### II.1 Chemicals:

II.1.1 Benzo(a)pyrene (96% purity), molecular weight 252.32. CAS Number: 50-32-8, in the form of white fine powder. It is produced by Sigma -Aldrich Chemical Company, USA and obtained from Sigma, Cairo, Egypt.

II.1.2 Clove oil was extracted from clove bud, without any biochemical treatment, and analyzed in the Egyptian Scientific Center, National Research Center (NRC), Cairo, Egypt.

II.1.3 Corn oil was obtained as a solvent agent for both BP and clove oil, and purchased from Cairo Pharmaceutical Company, Zagazig, Egypt.

## II.2 Experimental animals:

The experiment was done on animals, and ethically permitted through Institutional Animal Care and Use Committee of Zagazig University (Number: ZU-IACUC: 3/F/409/2022). Experiment fulfilled rules of the National Institute of Health. Thirty adult male albino rats, each 160–180 g weight and about 7–8 weeks age, were adapted for 14 days before the experiment in the standard laboratory conditions (temperature  $[22 \pm 3^\circ\text{C}]$ , humidity  $[52 \pm 3\%]$ ). Rats were nourished on a regular diet and tap water ad libitum.

## II.3 Experimental Design and Animal Grouping:

The experimental design included thirty albino rats, randomly branched into four groups, the experiment extended for 4 weeks and sample size was calculated using OpenEpi biostatistics program. Groups were divided as follow: Group I, contained 12 rats, was divided equally into 2 smaller groups; group Ia (negative control group) received basic diet and water and group Ib (corn oil group): received a daily dose of 10 mg/kg of corn oil (a solvent for both BP and CLV). Group II (CLV group): received a daily dose of (100  $\mu\text{l}$ ) clove oil dissolved in corn oil orally through nasogastric tube for 4 weeks (Banerjee et al. 2006). Group III (BP

group): received a daily dose of (50 mg/kg) benzo(a)pyrene dissolved in corn oil orally through nasogastric tube for 4 weeks, this utilized dose of BP equals (1/20  $\text{LD}_{50}$ ), oral  $\text{LD}_{50}$  of BP in rats =1g/kg BW (U.S. Environmental Protection Agency (EPA), 2013). Group IV (CLV/BP): received both clove oil and benzo(a)pyrene in the same previous doses, manner and duration.

## II.4 Specimens collection:

After 4 weeks, rats were anesthetized via intra-peritoneal pentobarbital injection (60 mg/kg), blood specimens were collected along with Joslin (2009) method. After centrifugation of blood, the sera were separated for the biochemical analysis of lactate dehydrogenase (LDH), interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and carcinoembryonic antigen (CEA). Subsequently, lungs were obtained through thoracic incision and dissected out. A part of tissue was ice-covered at  $-18^\circ\text{C}$ , transported, then stored at  $-85^\circ\text{C}$  to get homogenates for the chemical analysis of oxidative stress markers (MDA in addition to GSH) while other parts of lung were well-preserved in (10% saline formalin) for histopathological studies.

## II.5 Methods:

### II.5.1 Determination of clove oil chemical composition

It was done in the Egyptian Scientific Center located in the National Research Center, Egypt through the following steps:

a) Extraction of clove essential oil from clove bud through steam distillation

It was conducted through using steam distillation technique according to Amelia et al., (2017). A dried cut clove sample, with a

weight of 100 g, was placed on distillation flask containing distilled water as a solvent for 6 hours. Using magnesium sulfate, the attained distillate was dried then stored in a closed vial, as a pure clove oil, in the refrigerator (5 °C) before its analysis.

#### (b) Gas Chromatography/Mass Spectrometry (GC/MS) analysis

Clove essential oil analysis has been obtained through Trace 1300 gas chromatography interfaced with a detector ISQ LT single quadrupole mass spectrometer activated in electron impact method in 75 eV, column used was TG-5MS capillary column (film thickness 0.25mm). Oven temperature was 210 °C while injector temperature was 260 °C, helium is the carrier gas with a speed of 1.5 mL/min. Analysis was performed via injecting 2 µL of specimen (0.10 % in methyl alcohol) and using scan mode then splitless mode. Biochemical components of clove oil were recognized via correlation between the consequences of chromatogram with reference retention period via Wiley (W9N12) library. The chromatogram of clove oil analysis showed three peaks indicating the presence of three compounds; Eugenol (C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>, 85.4%), Caryophyllene (C<sub>15</sub>H<sub>24</sub>, 11.93%), and 3-Carene (C<sub>10</sub>H<sub>16</sub>, 2.67%) (Table 1, Figure 1)

#### II.5.2 Lactate dehydrogenase enzyme (LDH) estimation (U/L)

LDH is an enzyme found in all body cells; plays a vital role in cellular respiration, the process by which glucose is converted into energy needed for our cells. LDH activity depends on its capability to convert lactate into pyruvate in the presence of co-enzyme nicotinamide adenine dinucleotide. LDH level was estimated

spectrophotometrically in the sera in accordance to the method of Kamaraj et al. (2009).

#### II.5.3 Reduced glutathione (GSH) estimation (µm/L)

It was estimated in accordance to Rahman et al. (2006) method, using kits acquired from Myobiosource Co. (Cat. Number, MB265966). Lung tissue was homogenized then supernatant was mixed with distilled water, glacial metaphosphoric acid (50.2 mg), EDTA (6.3 mg), and sodium chloride (0.8 mL), this reaction mixture was incubated then filtered. Finally, the collected filtrate (0.6 mL) was mixed 3mL of disodium phosphate. Absorbance was read at 413 nm spectrophotometrically.

#### II.5.4 Malondialdehyde (MDA) estimation (nmol/L)

The level of MDA was estimated in accordance to the method of Ohkawa et al. (1997) by using kits acquired from Biodiagnostic Co. (Cat. Number, MDA2529). Lung tissue was homogenized then the supernatant was mixed with 2 mL trichloroacetic acid, heated for 2 h at 80°C then well-ventilated, supernatant was centrifuged at 2000 rpm for 15 min, organic layer absorbance was estimated at 535 nm spectrophotometrically.

#### II.5.5 Interleukin IL-1β and Tumor necrosis factor-α (TNF-α) estimation (pg/ml)

Both were estimated in plasma, through using IL-1β and TNF-α ELISA kits (CAT. Number, P01584) and (CAT. Number, P01375), (RayBiotech Co., USA)

respectively following the manufacturer's instructions.

### **II.5.6 Carcinoembryonic antigen (CEA) estimation (ng/ml)**

CEA levels were determined in the sera via ELISA method according to the manufacturer instructions. Kits were obtained from Sigma-Aldrich chemical company, (CAT. Number, SE120025). Depending on a solid phase direct sandwich ELISA technique; following the manufacturer's instructions.

### **II.5.7 Histological examination**

After fixation of lung tissues by using 10% saline formalin for 50 h, lung tissues were dehydrated, cleared, impregnated, and lastly embedded forming paraffin blocks, then divided at consecutive sections by a biocutting rotary microtome according to Bancroft and Gamble (2002) technique. Sections were fixed on slides to be stained by hematoxyline and eosin (H&E) stain then examined by light microscope.

### **II.5.8 Immunohistochemical examination**

Recognition of proliferating cell nuclear antigen (PCNA) antibodies were carried out for the assessment of proliferation of lung cells. It is done through the streptavidin–biotin complex immunoperoxidase system according to the method of Ramos-Vara et al. (2008), using kits obtained from Thermo Scientific company (CAT. Number, MS-106-R7). Paraffin-embedded lung sections were deparaffinized on charged slides then preserved in 0.2% H<sub>2</sub>O<sub>2</sub> for 30 min in order to stop endogenous peroxidase activity then washed by phosphate buffered saline for three times. Antigen retrieval is determined

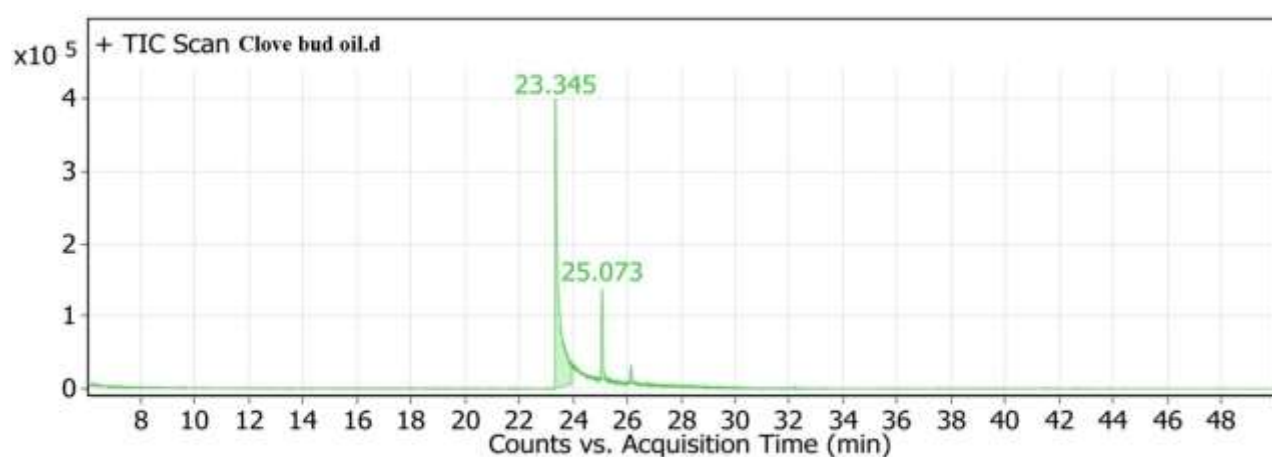
via microwave handling (25 min, 0.02 Mol/L citrate buffer, at pH 6). Then, slides were incubated by the main antibody for 30 min at room temperature.

### **II.6 Statistical analysis**

For all groups, statistics were expressed as a mean  $\pm$  standard deviation ( $X \pm SD$ ). Data interpretation was done by software SPSS (Statistical Package for the Social Sciences, form 26). Tests of (Kolmogorov Smirnov (A distribution type) plus Levene (for homogeneity of variances)) were utilized to validate hypothesis in parametric tests. Whereas, for comparison between more than 2 groups, one way ANOVA test was utilized. If the difference is significant, Tukey HSD comparison was used to determine the difference between each two individual groups. Statistically significant level is present if  $P < 0.05$ , while high significant difference is determined at  $P \leq 0.001$  (Petrie and Sabin, 2005).

**Table (1):** Percentage of clove oil components after GC/MS analysis:

Peak	Retention time	Name	Formula	Area	Area sum%
1	23.345	Eugenol	C10H12O2	3420044.76	85.4%
2	25.073	Caryophyllene	C15H24	477761.21	11.93%
3	26.137	3-Carene	C10H16	106744.76	2.67%

**Figure (1):** GC/MS chromatogram showing clove oil components

### III. Results:

#### III.1 Biochemical results:

Statistically, no significant differences were present among mean values of LDH, GSH, MDA, IL-1 $\beta$ , TNF- $\alpha$ , and CEA in CLV group compared with control group. Whereas, a high significant ( $P < 0.001$ ) difference of LDH, GSH, MDA, IL-1 $\beta$ , TNF- $\alpha$ , and CEA levels was observed in BP and CLV/BP groups when compared with control group. Also, a high significant ( $P < 0.001$ ) increase in LDH mean value was detected in BP group when compared with control group, while a significant decrease in LDH level was detected in the CLV/BP

group when compared with BP group. Also, a high significant decrease in GSH and an increase ( $P < 0.001$ ) in MDA were showed in BP group when compared with control group, however a significant elevation in GSH besides a decrease in MDA were detected in CLV/BP compared with BP group. Also, high significant elevation in IL-1 $\beta$ , TNF- $\alpha$ , and CEA mean values were detected in BP group compared with the control group, whereas a significant decline in IL-1 $\beta$ , TNF- $\alpha$ , and CEA ( $P < 0.001$ ) were observed in CLV/BP group compared with BP group (Tables 2,3).

**Table (2):** Comparison between mean values of LDH, GSH, MDA, IL-1 $\beta$ , TNF- $\alpha$  and CEA among the studied groups by using ANOVA test

Group (N=6)	Group Ia	Group Ib	Group II	Group III	Group IV	F	p
Parameter	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
LDH (U/L)	127.33 $\pm$ 4.13	124 $\pm$ 3.58	113.17 $\pm$ 1.94	455.67 $\pm$ 4.03	126.17 $\pm$ 2.48	1188.3	<0.001**
GSH ( $\mu$ m/L)	9.33 $\pm$ 0.48	8.83 $\pm$ 0.34	9.22 $\pm$ 0.48	0.25 $\pm$ 0.1	9.35 $\pm$ 0.33	695.41	<0.001**
MDA (nmol/L)	4.48 $\pm$ 0.21	4.47 $\pm$ 0.27	3.2 $\pm$ 0.18	18.63 $\pm$ 1	4.65 $\pm$ 0.31	1017.78	<0.001**
IL-1 $\beta$ (pg/ml)	1.18 $\pm$ 0.15	1.2 $\pm$ 0.14	0.58 $\pm$ 0.08	16.77 $\pm$ 0.8	3.12 $\pm$ 0.44	1609.52	<0.001**
TNF- $\alpha$ (pg/ml)	4.4 $\pm$ 0.13	4.78 $\pm$ 0.23	3.02 $\pm$ 0.08	19.82 $\pm$ 0.63	12.87 $\pm$ 0.26	2872.46	<0.001**
CEA (ng/ml)	0.15 $\pm$ 0.08	0.15 $\pm$ 0.08	0 $\pm$ 0	7.4 $\pm$ 0.53	2.5 $\pm$ 0.09	999.51	<0.001**

F: One way ANOVA test, \*\*p $\leq$ 0.001 is statistically highly significant, SD: standard deviation, N: number of rats, LDH: lactate dehydrogenase, GSH: reduced glutathione, MDA: Malondialdehyde, IL-1 $\beta$ : interleukin-1  $\beta$ , TNF-  $\alpha$ : tumour necrosis factor-  $\alpha$ , CEA: carcinoembryonic antigen, Group Ia (negative control, group Ib (corn oil group), group II (clove oil group), group III (benzo(a)pyrene group) and group IV (clove oil/benzo(a)pyrene), N: number of rats per each group

**Table (3):** Post hoc Tukey high significant difference analysis among the studied groups

Groups	LDH (U/L)	GSH ( $\mu$ m/L)	MDA (nmol/L)	IL-1 $\beta$ (pg/ml)	TNF- $\alpha$ (pg/ml)	CEA (ng/ml)
P1	0.438	0.253	0.994	>0.999	0.285	>0.999
P2	<0.001**	0.54	0.004*	0.112	<0.001**	0.826
P3	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
P4	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
P5	<0.001**	0.982	<0.001**	0.128	<0.001**	0.826
P6	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
P7	0.973	>0.999	0.977	<0.001**	<0.001**	<0.001**
P8	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
P9	0.794	0.223	0.86	<0.001**	<0.001**	<0.001**
P10	<0.001**	0.97	<0.001**	<0.001*	<0.001**	<0.001**

P1 difference between group Ia and Ib, P2 difference between group Ib and II, P3 difference between group II and III, P4 difference between group III and IV, P5 difference between group Ia and II, P6 difference between group Ia and III, P7 difference between group Ia and IV, P8 difference between group Ib and III, P9 difference between group Ib and IV, and P10 difference between group II and IV, LDH: lactate dehydrogenase, GSH: reduced glutathione, MDA: Malondialdehyde, IL-1 $\beta$ : interleukin-1  $\beta$ , TNF- $\alpha$ : tumour necrosis factor-  $\alpha$ , CEA: carcinoembryonic antigen, group Ia (negative control), group Ib (corn oil group), group II (clove oil group), group III (benzo(a)pyrene group) and group IV (clove oil/benzo(a)pyrene)

### III.2 Histological and immunohistochemical results:

The examined lung sections of groups Ia, Ib & II, by light microscopy using H&E stain, showed normal tissue architecture with regular sized alveolar spaces, normal interalveolar septa and bronchioles with intact bronchial epithelium (Figures: 2a, 3a). Weak positive nuclear immunoreaction for PCNA in the nuclei of few alveolar cells (Figure: 4a). While examination of H & E-stained sections of Group III (BP-treated group) revealed disturbed lung architecture with thick interalveolar septa, cellular infiltrations, congested blood vessels and bronchioles with detached bronchial epithelium. Area of hyaline degeneration was also seen (Figures: 2b, 3b&c). A strong positive immunoreaction for PCNA in the nuclei of lung epithelial cells and bronchiolar epithelium (Figure: 4b). Examined sections of Group IV (CLV/BP treated group) showed areas of normal lung tissue architecture with regular sized alveolar spaces and normal inter alveolar septa and bronchiole with intact bronchial epithelium and other areas appeared with disturbed lung architecture with thick interalveolar septa (Figures: 2c, 3d). Positive immunoreaction for PCNA in the nuclei of some alveolar epithelial lining (arrow) was noticed (Figure: 4c).

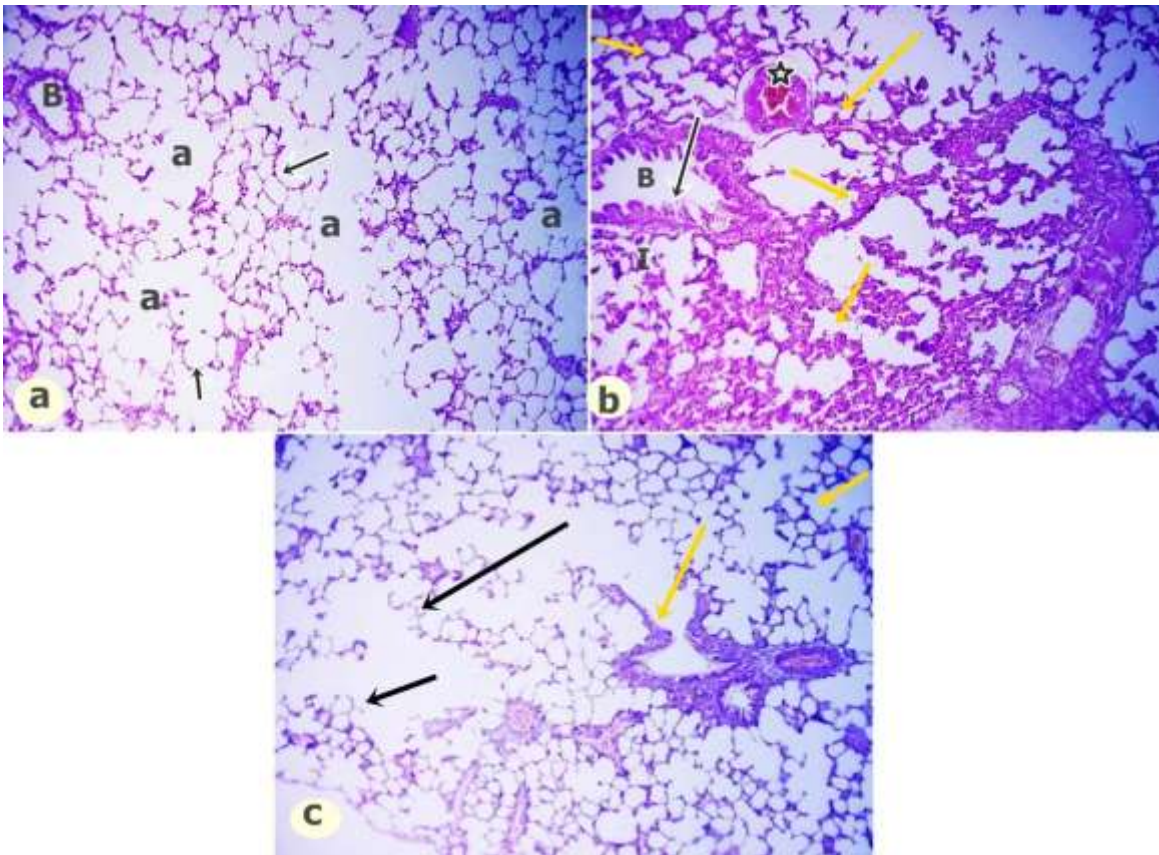
### IV. Discussion:

Human exposure to benzo(a)pyrene (BP) is increasing currently than before

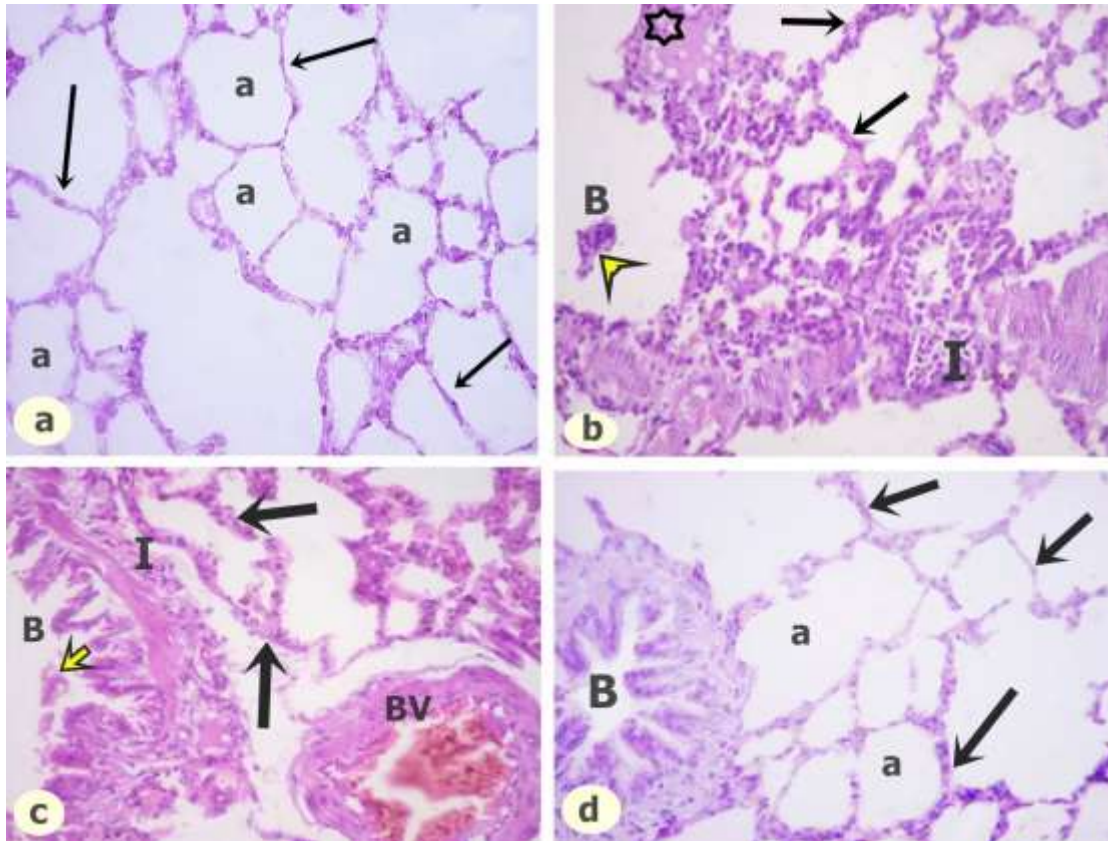
because it is produced as a consequence of several thermal procedures as tobacco smoking, organic materials burning, diesel exhausts and industrial wastes. Monitoring of BP level during occupational exposure is a high priority as there is increasing risk of lung malignancy (Bukowska et al. 2022). Clove oil (CLV) is a food flavor owing antibacterial, analgesic and anti-inflammatory properties, studies showed that clove oil has antioxidant effects that encouraged its application in food industries (Haro-González et al. 2021). So, the aim of this experiment was to evaluate the possible protective role of administering CLV against lung injuries elicited by BP in albino rats.

In the present study, oral exposure to BP caused a significant decline in GSH and an increase in MDA levels which are oxidative stress markers and reflect the extent of toxic cell injury. These results are in agreement with Johirul et al. (2020) in which BP exposure exhibited a significant decrease in the total antioxidant level plus increasing MDA level. Disruption in the redox homeostasis is the main mechanism of BP induced- pulmonary dysfunction, because BP exposure produces excess reactive oxygen species that require to be antagonized by GSH plus other antioxidants (Deng et al. 2018). However, in this study, clove oil administration leads to a significant improvement of GSH level in the CLV/BP group together with a high significant decline of MDA level compared with the BP group.

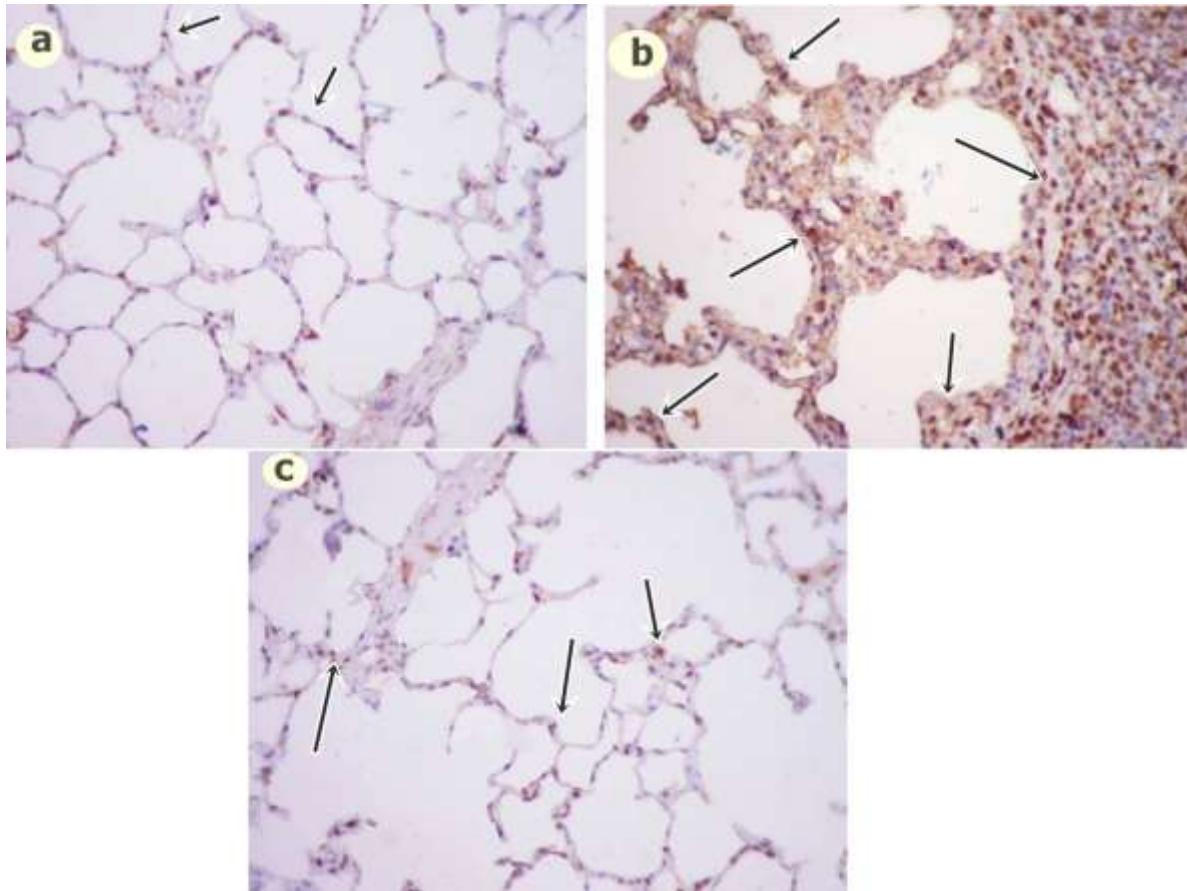




**Figure (2):** H&E-stained sections of rat lung of the experimental groups: **a)** Control groups showing normal lung architecture. Alveolar spaces (a) are regular in size with normal inter alveolar septa (arrows). Bronchiole (B) with intact bronchial epithelium is seen. **b)** Group III Benzo(a)pyrene (BP)-treated group showing alveoli with thick interalveolar septa (yellow arrows), cellular infiltrations (I), congested blood vessel (\*) are noticed. Bronchiole (B) with detached epithelium is also noticed (black arrow). **c)** Group IV (CLV/BP) showing areas of normal tissue architecture with regular sized alveolar spaces, normal inter alveolar septa (black arrows) beside other areas of disturbed lung architecture with thick interalveolar septa (yellow arrows) (100× H&E).



**Figure (3):** H&E-stained sections in rat lung of the experimental groups: a) Control groups showing normal tissue architecture. Alveolar spaces (a) are regular in size with thin interalveolar septa (arrows). b) and c) Group III Benzo(a)pyrene (BP)-treated group showing disturbed lung architecture. Sections show thick interalveolar septa (arrows), cellular infiltrations (I), congested blood vessel (BV) and bronchiole (B) having detached epithelium (yellow arrow head). Area of hyaline degeneration is also seen (\*). d) Group IV (CLV/BP) showing normal tissue architecture with alveoli regular in size having normal interalveolar septa (black arrows). Bronchiole with intact epithelium (B) is also noticed (400× H&E)



**Figure (4):** Immunoperoxidase technique for PCNA immunoreaction of the control and experimental groups: a) Control group showing weak positive immunoreaction in the nuclei of few alveolar cells (arrows). b) Group III Benzo(a)pyrene (BP)-treated group showing a strong positive immunoreaction in the nuclei of both alveolar epithelial cells and bronchiolar epithelium (arrow). c) In Group IV (CLV/BP), positive immunoreaction in the nuclei of some alveolar epithelial cells (arrow) was noticed (Immunoperoxidase technique  $\times 400$ )

In the current work, BP exposure caused a significant increase in LDH level compared to that of control groups, this finding is in the same line of Ali et al. (2017). But, in this study, CLV administration leads to a significant decline of LDH level in CLV/BP group compared to that of BP group. The current study revealed that BP has carcinogenic potentials evidenced by a significant increase in CEA in BP group

when compared with control group. Similarly, Islam et al. (2022) proved this evidence of cancer risk in Swiss albino mice after 4 weeks of exposure. Whereas, co-treatment with CLV showed a significant decline in CEA in CLV/BP group compared with BP group. Also, in another study by Du et al. (2021), BP significantly increased LDH and CEA levels, these effects are in line with this experiment. This occur due to

higher level of LDH activity is present in the proliferating tumor cells compared with normal ones and this may be caused by increased glycolysis during hyperplasia and/or overgeneration of malignant cells (Stabile et al. 2012). CEA is considered a significant tumor marker, also it is a good prognostic sign of lung malignancy. Excess CEA commonly provoked outside the malignant tumor cell and rises risk of metastasis (Abdelaziz et al., 2018). The current study results showed a significant elevation of the inflammatory mediators IL-1 $\beta$  and TNF- $\alpha$  concentrations in BP group, these findings are in accordance with Arjumand et al. (2011). Inflammation, due to the expression of inflammatory mediators showed a major role in lung injury onset, these mediators are secreted from lung parenchyma and vascular endothelial cells (Rahimi et al. 2017). Also, chronic inflammation has a major role in tumors development; TNF- $\alpha$  is responsible for inflammation related- cancer and IL-1 $\beta$  is a critical player in growth and deviation of cells. Additionally, CEA may be linked to inflammation as persistent increase of pro-inflammatory cytokines is dependable with CEA level that is indicative of carcinogenesis (Chauhan and Trivedi, 2020). However, in the existing study, clove oil co-administration leads to a significant decline in IL-1 $\beta$  plus TNF- $\alpha$  values in CLV/BP group when compared to BP group.

The histological examination of the present study proved that BP-induced lung injury. Lung tissues revealed congested dilated vessels, abnormal thickening of alveolar septa, detached bronchial epithelium, and mononuclear cellular infiltrates are present in alveolar wall besides interstitial tissues. Empty deflated

alveoli and area of hyaline degeneration were also seen. In the same context, Hu et al. (2021) observed that rat's exposure to BP provoked histopathological changes in the lung tissues of rats such as interstitial inflammatory cell infiltrates, hemorrhage and/or odema with collagen precipitation. This can be clarified by the overproduction of large quantities of free radicals induced by BP exposure, which, in turn, interact with membrane lipids and subsequently induced lipid peroxidation in the lung tissues of rats. Moreover, progressive cellular architectural alterations due to oxidative stress and lipid peroxidation produced during the cytochrome p450 dependent metabolism of BP is concerned in the pathogenesis of lung carcinogenesis (Brihoum et al. 2018). On the other side, co-administration of CLV in the present study showed slight improvement of lung tissue architecture with regular sized alveolar spaces, normal inter alveolar septa, and bronchiole with intact bronchial epithelium in the CLV/BP group compared to BP group. This outcome is compatible with Banerjee, et al. (2006) study which stated that clove oil co-administration offered chemoprotection against BP induced- lung toxicity, as clove can be efficient in inhibiting cell proliferation and inflammation in BP-induced lung carcinogenesis.

Furthermore, immunohistochemical studies for PCNA revealed an increase and proliferation of the positive immunoreactive cells in the BP group when compared with control group, whereas immunoreactivity for PCNA was enhanced in CLV/BP group compared to that of BP group. In the current study, these findings proved the expression level of preneoplastic changes, hyperplasia and/or proliferating cells in lung tissues.

Clove oil is a natural compound, recognized to employ protecting role through antagonizing free radicals; enhancing the antioxidant defense mechanisms and pollutant decontamination. All these favorable properties of clove oil are related to its biochemical structure and its role in cell membrane stabilization via diminishing membrane fluidity employing antioxidant, anti-inflammatory plus anti-proliferative properties (Vicidomini et al. 2021). So, pulmonary toxic effects provoked by BP were proved by; oxidative stress evidenced by increasing MDA and decreasing GSH level, inflammatory reaction evidenced by elevated inflammatory mediators IL-1 $\beta$  as well as TNF- $\alpha$  levels plus carcinogenic potential evidenced by elevated LDH and CEA levels and positive reaction to PCNA protein which is a marker for cellular proliferation, besides disrupted lung architecture in histological examination, however the administration of CLV offered protection with better outcome.

## V. Conclusions

In the present experimental study, clove essential oil showed a well-measured *in-vivo* investigations which indicate valuable actions towards BP-provoked lung injury and inflammatory-oxidative damage, clove oil might be a promising chemoprevention candidate against lung cancer.

## VI. Recommendations

Additional investigations are required to explain the molecular mechanisms fundamental to clove oil antitumor activity. Also, using various doses of clove oil co-treatment to determine the dose/response correlation against benzo(a)pyrene toxic insults.

## VII. Disclosure statement:

No conflict of interest was declared by the authors.

## VIII. References:

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## المخلص العربى

## تقييم دور زيت القرنفل على سمية الرنتين المحدثة بالبنزو(أ)بيرين فى ذكور الجرذان البيضاء البالغة

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**مقدمة:** يؤدي التعرض البيئي لمادة البنزو (أ) بيرين إلى العديد من امراض الجهاز التنفسى فهو يضعف من كفاءة ووظائف الرنتين. **الهدف من البحث:** تهدف الدراسة الى تقييم الدور الوقائى المحتمل لزيت القرنفل ضد التسمم الرئوى المحدث بمادة البنزو(أ)بيرين فى الجرذان البيضاء البالغة. **طريقة البحث:** تشمل الدراسة عدد ثلاثون جرد من الجرذان البيضاء البالغة وتم تقسيمها الى 4 مجموعات رئيسية. المجموعة الاولى(أ) لم تتلقى سوى الطعام والشراب فقط والمجموعة الاولى (ب) تم اعطاؤها المادة المذيبة وهو زيت الذرة بينما تلقت المجموعة الثانية زيت القرنفل يوميا بواسطة التغذية الانبوبية. اما المجموعة الثالثة فتم اعطاؤها مادة البنزو(أ)بيرين بواسطة التغذية الانبوبية بينما تم اعطاء المجموعة الرابعة مادتي زيت القرنفل والبنزو(أ)بيرين بواسطة التغذية الانبوبية يوميا ولمدة 4 اسابيع وهى مدة الدراسة. **النتائج:** اوضحت الدراسة ارتفاعا ملحوظا فى انزيم اللاكتات ديهيروجينيز والمستضد السرطانى المضغى والسيتوكينات المحفزة للالتهاب وكذلك ارتفاع المألونداهديد وهو احد مؤشرات الاجهاد التاكسدى فى المجموعة الثالثة (مجموعة البنزو(أ)بيرين) بالاضافة الى وجود اضطراب وتدهور بنسيج الرئة واحتقان وتجلط بالاوعية الدموية مع وجود ارتفاع بمستضد الخلية النووية الذى ظهر فى الفحص المناعى لخلايا الرئة. ولكن اعطاء زيت القرنفل اظهر قدرا من التحسن على مستوى الدلالات البيوكيميائية و فحص الهستوباثولوجيا وكذلك الفحص المناعى لمستضد الخلية النووية. **ملخص البحث:** ان التعرض لمادة البنزو(أ)بيرين يؤدي الى تدهور بوظائف الرنتين وحدوث سرطان بالرئة، ان اعطاء زيت القرنفل العطرى وفر قدرا من الحماية ضد الاثار السمية على الرنتين فهو من مضادات الاكسدة ومضادات الالتهاب وقد يحمى الخلايا ضد الاورام السرطانية. **التوصيات:** ينصح باجراء أبحاث تستخدم جرعات مختلفة من زيت القرنفل لتحديد مدى ارتباط جرعة زيت القرنفل بالوقاية ضد التأثير السام لمادة البنزو(أ)بيرين.