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# Evaluation of Aromatherapeutic Potential of *Allium cepa* in Carbon Monoxide-induced Respiratory Tissue Toxicity in Wistar Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors IO and ANC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IO managed the analysis of the study. Authors IO and VV managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

This study evaluated the aromatherapeutic tendency of *Allium cepa* in carbon monoxide-induced lung parenchyma toxicity. *Allium cepa* and carbon monoxide served as the main treatments in this study. The time-dependent effect and ratio of pro-oxidant to antioxidant caused by each treatment and combination of both treatments was also determined. Thirty (30) experimental rats were used. The study design included six (6) groups with equal number of animals selected randomly. The test groups included *i* the control treated normal saline and feed; *ii Allium cepa* 50 mg/kg; *iii* carbon monoxide 3300 ppm for 1 hour; *iv Allium cepa* 50 mg/kg and carbon monoxide 3300 ppm; *v Allium cepa* 100 mg/kg and carbon monoxide 3300 ppm; *vi Allium cepa* 200 mg/kg and carbon monoxide 3300ppm. The assays included lung tissue homogenates and lung tissue stress enzyme and non-

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enzyme markers. *Allium cepa* alone and at moderate and high doses, effectively prevented carbon monoxide-induced lung tissue toxicity. From the outcome of this study, *Allium cepa* has the potential to be effective when used as an antitoxic and aromatherapeutic agent.

*Keywords: Allium cepa; carbon monoxide; ratio; lungs; toxicity.*

## 1. INTRODUCTION

We are what we expose ourselves to, whether consciously or subconsciously. Through the air we breathe, the respiratory tissues are exposed to various agents [1,2]. Nitrogen, being the most abundant component in the air [3] exists in the dissolved form in the body's adipose tissues [1]. Its concentration may as well be proportional to the fatty components of the visceral and most peripheral part of the human body [3]. Oxygen, the second most abundant in air [1,3], is the useful component of inspired air in humans [1]. The essence of breathing encompasses all the physiologic processes ranging from nutrition [3,4], metabolism, excretion, defence and homeostasis [1]. The respiratory tissues are well designed and strategically positioned to 'brain house' gaseous exchange. It is therefore of utmost importance to maintain the normal functional state of the respiratory tissues. Carbon monoxide (CO) is a clear, odourless and highly diffusible gas [1,3]. Some sources of carbon monoxide include exhaust engines, deep wells, gas flaring refineries, underground drainage system and gases from modern nuclear weapons [4]. According to United States National Center for Environmental Health, Carbon monoxide causes more pathologic manifestations than any other gas globally, at an estimated rate of 20,000 hospital emergency visits per annum [5]. The advent of aromatherapy has provided useful insight on the therapeutic nature of various plants. Aromatherapy has indeed posed a huge 'question mark' on conventional medicine and the usual scientific formulations including enlightening the general public on the need for an alternative form of medicine [6,7]. *Allium cepa* commonly called onion [8], of same genus as *Allium aggregatum* (shallot), *Allium ampeloprasum* (leek), *Allium schoenoprasum* (chive) and *Allium sativum* (garlic). They are known for their characteristic pungency [9,10]. It is a worldwide seasoning and a native to central Asia and northeastern part of iran [6]. *Allium cepa*, when crushed, liberates sulfuric phytoconstituents including allin, vinyldithiins, S-allylcysteine, ajoene and diallyl polysulfides [7,8]. *Allium cepa* has been reported to be effective against pathologic changes including, diabetes [6,8], hypertension, hemolytic anemia [11] and atherosclerosis [12]. This study evaluated the potential aromatherapeutic effect of *Allium cepa* on carbon monoxide induced respiratory tissue changes.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection

A quantity weighing 1lb of fresh, large and pungent onion was purchased from Fruit Garden in Port Harcourt, Rivers State. Immediately before each exposure, the vegetable was properly washed with distilled water ($H_2O$) and sodium chloride salt (NaCl), before the rinds were removed and discarded properly.

### 2.2 Plant Identification

Plant identification and authentication was carried out by Rivers state ministry of Agriculture.

### 2.3 Plant Preparation

The fleshy edible part of the plant was used for this study. It was grinded and churned into a paste form before administration. This preparation was done daily.

### 2.4 Carbon Monoxide Exposure

This experiment was carried out using an air-tight rectangular glass chamber manufactured by OK GOLD®, Nigeria. 3300 pm of Carbon monoxide was introduced into the glass chamber through an adjustable valve system at the left wall of the glass chamber.

### 2.5 Experimental Animals and Protocols

Thirty (30) adult male Wistar rats weighing 150 to 180 grams were obtained from the experimental animals unit, Department of Human Physiology, Madonna University. All experimental animals were physically healthy as confirmed by a veterinarian in same institution. Using simple random technique of sampling, the animals were divided into six (6) groups containing five (5) rats per group. The animals were allowed to

acclimatise for 2 weeks before the start of the experiment which lasted for 30 days. All animals had access to food and water *ad libitium*. The cages were properly cleaned twice daily to avoid infection due to coprophagy.

## 2.6 Plant Treatment

From previous report [8], The $LD_{50}$ of *Allium cepa* was 2000 mg/kg. *Allium cepa* was administered in 3 doses;

Treatment was performed using a gastric cannula.

Using same methods from previous studies [2] Dose for Carbon monoxide: 3000 ppm for 1 hour in 30 days. Timing was done using a Tag Heueur® stop watch.

## 2.7 Sacrifice and Collection of Blood Samples

Blood samples were collected in three (3) separate days (0, 15 and 30). The animals were carefully restrained to avoid stress alteration of the monitored parameters and then they were placed in prone position after which 5ml of blood was collected from the lateral tail vein using 'butterfly syringes' following standard protocols. The blood samples were collected into well-labeled heparinated bottles. Sacrifice was carried out several hours after treatment on day 30. The parenchyma of the lungs was collected after the mid-thoracic cage was carefully dissected. This procedure was carried out after anesthetising the animals with diethyl-ether from sigma® reagents. All samples were taken to the laboratory for haematology and histopathology, Madonna University Teaching Hospital, for analysis of oxidative stress biomarkers.

## 2.8 Lung Stress Markers

Lung stress markers assayed for include superoxide dismutase (SOD), catalase (CAT) protein carbonyl (PC), isoprostanes ($F_2$IsoP), Malondialdehyde (MDA) and reduced glutathione (GSH).

### 2.8.1 Superoxide dismutase (SOD) assay

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical (02•), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

XOD
Xanthine ----------> Uric acid + 02•
02• + I.N.T. ----------> Red formazan dye
SOD
02• + 02•+ 2H----------> 02 + H202

### 2.8.2 Catalase (CAT) assay

Catalase assay kit provided by Cayman Chemical Company, USA. Catalase is a ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase (CAT) is involved in the detoxification of hydrogen peroxide ($H_2O_2$). This enzyme catalyses the conversion of two molecules of $H_2O_2$ to molecular oxygen and two molecules of water (catalytic activity) [12]. Catalase also demonstrates peroxidatic activity, in which low molecular weight alcohols can serve as electron donors, while the aliphatic alcohols serve as specific substrates. In humans, the highest levels of catalase are found in the liver, kidney, and erythrocytes, where it is believed to account for the majority of hydrogen peroxide decomposition. The Cayman Chemical Catalase Assay Kit utilises the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal con-centration of H2O2. The formaldehyde produced is measured spectrophotometrically with 4-amino-3-hydrazino- 5-mercapto-1, 2, 4-trizazole as the chromagen. The assay can be used to measure CAT activity in plasma, serum, erythrocyte lysates, tissue homogenates, and cell lysates.

### 2.8.3 Protein carbonyl (PC) assay

IBL® assay kit was used for protein carbonyl assay. 2, 4-Dinitrophenylhydrazine (DNPH)) reacts with protein carbonyl in plasma to form a Schiff base to produce a corresponding hydrazone which can be analysed spectrophotometrically at an absorbance between 360-385 mm.

**Table 1. Treatment protocols**

| Tests | Protocols | Treatment | Dose |
|---|---|---|---|
| *i* | Normal saline + feed | Control | - |
| *ii* | *Allium cepa* | Low | 50 mg/kg |
| *iii* | Carbon monoxide | 1 hour | 3000 ppm |
| *iv* | *Allium cepa* + Carbon monoxide | Low + | 50 mg/kg |
| *v* | *Allium cepa* + Carbon monoxide | Moderate+ | 100 mg/kg |
| *vi* | *Allium cepa*+ Carbon monoxide | High + | 200 mg/kg |

#### 2.8.4 Isoprostanes ($F_2$IsoP) assay

The Eagle® Biosciences 8-Isoprostane ELISA Assay kit was used for the quantitative determination of 8-isoprostane in biological samples by enzyme linked immunoassay (ELISA). 8-Isoprostane ELISA Assay kit is for research use only and not to be used in diagnostic procedures.

#### 2.8.5 Malondialdehyde (MDA) assay

Malondialdehyde level of the plasma was measured by the following procedure.0.5 plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. 1ml of 0.6 % TBA was added to the mixture, shaken, and warmed for 30 min in a boiling water bath followed by rapid cooling. Then it was shaken into a 4 ml of n-butyl-alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 535 and 520 nm by spectrophotometer against butanol. The standards of 5, 10, 20 nmol/ml TEP were used.

#### 2.8.6 Glutathione peroxidase assay

The Cayman Chemical Glutathione Peroxidase Assay Kit measures GR activity by measuring the rate of NADPH oxidation:

$$2GSH + H_2O_2 \xrightarrow{GPx} 2GSSG + 2H_2O$$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm and is directly proportional to the GPx activity in the sample. The Cayman GPx Assay Kit can be used to measure GPx activity in plasma, erythrocyte lysates, tissue homogenates, and cell lysates. Glutathione peroxidase catalyses the oxidation of reduced glutathione (GSH) in the presence of $H_2O_2$, which is oxidised to GSSG [12]. The decrease in absorbance at 340 nm is measured.

### 2.9 Lung Tissue Homogenate

The lung tissue (Parenchyma) was cut into slices of appropriate sizes for analysis (100 to 300 mg) and placed into a micro centrifuge tube. The typical sample size was 100 mg [11]. The tissue was washed properly with 1 ml PBS. Glass beads (0.5 mm) equal to tissue mass followed by 0.1 to 0.6ml of buffer was added. The micro centrifuge tubes were placed into a Kutterman 240 blender at speed 6 and time 3 to homogenise. Complete homogenisation was achieved at the end of the laboratory session.

### 2.10 Statistical Analysis

Experimental data are presented in Mean±SEM, and Harmonic mean, Percentage change (%c), as well as Pro-oxidant/Antioxidant ratio, was also calculated to make the data well translated. SPSS 21.0 was used for all calculations and statistical analysis such as One-way analysis of variance (ANOVA). Values are significant at $p \leq 0.05$ or at confidence interval of 95%.

## 3. RESULTS

The following results were obtained from this study.

### 3.1 From Table 2

The effect of each treatment on lung antioxidant enzyme biomarkers was clearly presented. *i* test was the control with SOD 214.3±7.32, CAT 45.3±1.31 and GPx 142.4±2.1. *ii* test had a significant increase in all antioxidant enzymes SOD,CAT and GPx compared to control at $475.0\pm19.2^a$, $93.2\pm14.2^a$ and $248.2\pm3.0^a$ respectively. *iii* test had a significant decrease in all antioxidant enzymes SOD,CAT and GPx compared to control at $120.4\pm.8.21^{ab}$, $14.6\pm12.4^{ab}$ and $41.3\pm8.11^{ab}$ respectively. *iv* test had a significant decrease in antioxidant enzymes SOD, CAT and GPx at $140.2\pm11.4^{ab}$, $19.8\pm3.0^{ab}$ and $42.7\pm6.13^{ab}$. Test *v* had a

significant increase in antioxidant enzymes SOD, CAT and GPx at 230.2±4.43[ab], 47.2±7.10[b] and 94.6±8.10[ab] respectivcompared to control. Test *vi* had a significant increase in SOD, CAT and GPx at 412.2±12.1[ab], 63.4±8.23[ab] and 143.4±2.43[b] compared to control.

**Table 2. Effect of treatments on lung stress enzyme markers**

| Tests | SOD(IU/L) | *%c-i* | CAT(IU/L) | *%c-i* | GPx(µg/ml) | *%c-i* |
|---|---|---|---|---|---|---|
| *i* | 214.3±7.32 | 0 | 45.3±1.31 | 0 | 142.4±2.1 | 0 |
| *ii* | 475.0±19.2[a] | 121.7 | 93.2±14.2[a] | 106.0 | 248.2±3.0[a] | 74.3 |
| *iii* | 120.4±.8.21[ab] | -44.0 | 14.6±12.4[ab] | -68.0 | 41.3±8.11[ab] | -71 |
| *iv* | 140.2±11.4[ab] | -35.0 | 19.8±3.0[ab] | -56.3 | 42.7±6.13[ab] | -70 |
| *v* | 230.2±4.43[ab] | 7.42 | 47.2±7.10[b] | 4.20 | 94.6±8.10[ab] | -34.0 |
| *vi* | 412.2±12.1[ab] | 92.3 | 63.4±8.23[ab] | 40.0 | 143.4±2.43[b] | 0.70 |

*Key: Numerals=Treatments, SOD=Superoxide dismutase, CAT= Catalase, GPx= Glutathione Peroxidase, %c=Percentage change, [ab] values are significantly different at P≤0.05 to i and ii respectively*

**Table 3. Effect of treatments on lung stress non-enzyme markers**

| Tests | PC(µg/ml) | *%-i* | MDA(µg/ml) | *%-i* | $F_2$IsoP(µg/ml) | *%-i* |
|---|---|---|---|---|---|---|
| *i* | 20.4±1.40 | 0 | 57.4±3.2 | 0 | 60.3±8.1 | 0 |
| *ii* | 12.3±1.31[a] | -40.0 | 28.3±1.0[a] | -51.0 | 34.1±2.3[a] | -43.4 |
| *iii* | 47.2±3.40[ab] | 131.3 | 90.1±1.4[ab] | 57.0 | 84.2±3.0[ab] | 40.0 |
| *iv* | 38.6±2.41[ab] | 89.2 | 74.4±0.2[ab] | 30.0 | 64.2±7.1[ab] | 6.5 |
| *v* | 19.2±2.14[ab] | -6.0 | 48.6±2.0[ab] | -15.33 | 34.2±0.4[a] | -43.3 |
| *vi* | 13.0±3.10[a] | -36.3 | 28.1±1.2[a] | -51.0 | 21.4±2.1[ab] | -65.0 |

*Key: Numerals=Treatments, PC=Protein carbonyl, MDA=Malondialdehyde, $F_2$IsoP=Isoprostane $F_2$, %c=Percentage change, [ab] values are significantly different at P≤0.05 to i and ii respectively*

**Table 4. Effect of treatments on Pro-oxidant/Antioxidant ratio**

| Tests | Pro-oxidant | Antioxidant | Ratio |
|---|---|---|---|
| *i* | 138 | 402 | 1:3 |
| *ii* | 75 | 816 | 0.1:1$^{\downarrow}$ |
| *iii* | 222 | 176 | 1.3:1$^{\uparrow}$ |
| *iv* | 177 | 202 | 0.9:1$^{\downarrow}$ |
| *v* | 102 | 372 | 0.3:1$^{\downarrow}$ |
| *vi* | 63 | 619 | 0.1:1$^{\downarrow}$ |

*Key: ↑=Ratio increased, ↓=Ratio decreased*

**Table 5. Time-dependent effect of treatments on antioxidants**

| Tests | Day 0 | *% 0→15* | Day 15 | *%15→30* | Day 30 | (H) |
|---|---|---|---|---|---|---|
| *i* | 132.0±5.4 | -0.30 | 131.6±4.1 | 1.71 | 133.9±3.6 | 133 |
| *ii* | 147.2±3.1 | 38.0 | 203±3.4[a] | 34.0 | 272.1±12.1[ab] | 195 |
| *iii* | 69.3±4.0 | -13.3 | 60.1±1.2[a] | -2.20 | 58.8±9.6[ab] | 62 |
| *iv* | 43.1±1.2 | 40.1 | 60.4±1.40[a] | 12.0 | 67.6±3.23[ab] | 55 |
| *v* | 98.6±2.13 | 12.0 | 110.3±6.2[a] | 12.4 | 124.0±7.0[ab] | 110 |
| *vi* | 170.2±2.4 | 2.40 | 174.2±2.1[ab] | 18.4 | 206.3±8.0[ab] | 182 |

*Key: % 0→15= day 0 to 15, %15→30= day 0 to 30, (H)=Harmonic mean*

**Table 6. Time-dependent effect of treatments on pro-oxidants**

| Tests | Day 0 | *% 0→15* | Day 15 | *%15→30* | Day 30 | (H) |
|---|---|---|---|---|---|---|
| *i* | 46.4±3.21 | -0.22 | 46.3±4.20 | -0.64 | 46.0±4.23 | 46 |
| *ii* | 43.2±3.42 | -28.2 | 31.0±1.33[a] | -30.3 | 21.6±1.53[ab] | 29 |
| *iii* | 42.4±1.43 | 45.0 | 61.4±2.4[a] | 21.0 | 74.3±2.6[ab] | 56 |
| *iv* | 50.8±2.23 | 0.80 | 51.2±3.31 | 15.4 | 59.1±3.23[ab] | 53 |
| *v* | 48.1±4.41 | -14.1 | 41.3±7.1[a] | -18.0 | 34.0±1.51[ab] | 40 |
| *vi* | 53.2±1.43 | -43.2 | 30.2±1.0[ab] | -31.0 | 21.0±2.13[ab] | 30 |

*Key: % 0→15= Percentage change day 0 to 15, %15→30= Percentage change day 0 to 30, (H)=Harmonic mean*

### 3.2 From Table 3

The effect of each treatment on oxidative stress metabolites was presented clearly. Test *i* is the control group administered normal saline and feed. Test *ii* has a PC, MDA and $F_2$IsoP level significantly lower at $12.3\pm1.31^{a}$, $28.3\pm1.0^{a}$ and $34.1\pm2.3^{a}$ than that of control. The *iii* test group had $47.2\pm3.40^{ab}$ for PC, $90.1\pm1.4^{ab}$ for MDA, $84.2\pm3.0^{ab}$ for $F_2$IsoP; all test values are significantly higher than that of control. Test *iv* has $38.6\pm2.41^{ab}$, $74.4\pm0.2^{ab}$ and $64.2\pm7.1^{ab}$ for PC, MDA and $F_2$IsoP respectively, which are significantly higher than that of control. Test *v* had $19.2\pm2.14^{ab}$ for PC, $48.6\pm2.0^{ab}$ for MDA and $34.2\pm0.4^{a}$ for $F_2$IsoP. Test *vi* has PC at $13.0\pm3.10^{a}$, MDA at $28.1\pm1.2^{a}$ and $F_2$IsoP at $21.4\pm2.1^{ab}$, all values for test *v* and *vi* significantly higher than that of control.

### 3.3 From Table 4

The pro-oxidant/antioxidant ratio (Pro-anti-index) was calculated. The ratio of pro-oxidants against antioxidants was reduced in test *ii*, *iv*, *v* and *vi*. The ratio was increased in test *iii*. From a biochemical perspective, increased pro-oxidant/antioxidant ratio in a sample reveals that the level of free radical-induced oxidative tissue damage was much greater than the intrinsic reactive oxygen species scavenging property of the samples. It simply means the pro-oxidative reactions are greater than antioxidant capacity. The reverse is the case in samples with a reduced ratio.

### 3.4 From Tables 5 and 6

These tables reveal the time-dependent effect of all treatments on oxidative stress enzyme and non-enzyme markers. The harmonic mean for each test was also presented. Test *iii* had the highest harmonic mean of 56. This means carbon monoxide caused the highest reciprocal increase in oxidative metabolites, but test *iv* had the lowest reciprocal decrease in antioxidant enzymes.

## 4. DISCUSSION

This study revealed that *Allium cepa* has aromatherapeutic potential, but this potential is dose dependent. The histotoxic potential of carbon monoxide exposure was also established, and is in contribution to several scientific findings [1,4,5]; that the toxicity of carbon monoxide may also be related to the oxidative stress-induced tissue damage it inflicts on the respiratory tissues. *Allium cepa* may be able to prevent such tissue changes only at moderate to high dose ingestion. This study did not reveal that low dose ingestion of *Allium cepa* can be effective. The phytoconstituents of *Allium cepa* may be effective in preventing adverse changes in respiratory tissues [10]. Quercetin, kaempherol and myricetin, all flavonols [9,8], may be suspected agents in the aromatherapeutic potency of *Allium cepa*. At low dose treatment, the concentration of these phytoconstituents may be less than the amount required for its effectiveness against carbon monoxide exposure. The concentration of these phytoconstituents may probably be the strength behind their effectiveness in moderate and high dose. Carbon monoxide at the dose of exposure in this study reduced the blood level of anti-oxidant, SOD, CAT and GPx but increased oxidative stress metabolites PC, MDA and F2IsoP. This infers carbon monoxide is pro-oxidative and causes oxidative stress. The mechanism behind its pro-oxidative effect may be at the level of the mitochondria [1,4], or possibly by metabolic alterations [5] or other mechanisms still yet to be determined. *Allium cepa*, at moderate and high doses, increased antioxidant SOD, CAT and GPx level in blood but reduced oxidative stress metabolites PC, MDA and F2IsoP. This infers that at these concentrations, *Allium cepa* has antioxidant effect, this is in agreement with earlier reports [6,8], a characteristic which is valuable in aromatherapy.

## 5. CONCLUSION

From this study, prolonged carbon monoxide exposure may cause adverse changes in lung tissue. *Allium cepa*, at moderate and high dose treatments, has the potency of preventing adverse changes in lung tissue due to prolonged carbon monoxide exposure.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

This study was approved by Madonna University Research Ethics Committee. All experimental procedures were done strictly following the guidelines provided by the research ethics committee. The animals were sacrificed after exposure to diethyl ether according to EC

directives 86/609/EEC. In addition, the laid down standards according to the 1964 declaration of Helsinki were strictly adhered to.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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