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Effects of solanum macrocarpon (African eggplant) on haematological parameters of wistar rats exposed to urban air pollution

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Abstract. Our study investigated the effects of *Solanum macrocarpon* (African eggplant) on haematological and biochemical parameters of male albino rats exposed to urban air pollution (O_3, PM_{10}, CO, SO_2, NO_2 and VOC). Male albino Wistar rats were exposed for 63 days either to urban air pollution without treatment (A); oral supplementation with *Solanum macrocarpon* given at 36 and 75 mg/kg body weight (BW), representing C and D respectively and exposed to air pollution; oral supplementation with *Solanum macrocarpon* given at 75 mg/kg BW after exposure to air pollution (B); or kept in animal house without exposure to air pollution (E). Animals exposed to air pollution showed significant alterations in haematological and biochemical parameters signaling that the blood and organs were badly injured. There were significant elevations in white blood cells (WBC) and its indices, reduction in red blood cells (RBC) and significant depletions of non-enzymic antioxidants, total protein and increase in lipid peroxidation values. *Solanum macrocarpon* supplementation in the feed of animals halted significantly the deleterious effects of air pollution, with co-administration during exposure given better results.

Keywords: *Solanum macrocarpon*; urban air pollution; haematological indices; biochemical indices; wistar rat

1. Introduction

The ambient air of urban centers is polluted with potentially toxic chemicals mostly arising from the combustion of fuels used for transport and industrial power plants. The composition of ambient air is complex and depends on the quality of fuel, the type of engine and engine maintenance (Leong *et al.* 2002, Hrelia *et al.* 2004). Vehicle exhaust generally contains polycyclic aromatic hydrocarbons (PAHs), particulate matters (PM) and volatile organic compounds (VOC), carbon monoxide (CO), nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and ozone (O_3). Diesel-powered engines in industries and trucks are the major source of particles whereas two-stroke motorbikes and petrol powered cars emit more gaseous pollutants (Enemeri 2001, Ajayi and Dosunmu 2002, Abam and Unachukwu 2009, Syd

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Bom *et al.* 2001, Avogbe *et al.* 2011).

The urban atmosphere contains a wide variety of harmful substances, many of which are carcinogenic, genotoxic and haematotoxic compounds. Air pollution is the source of many hazardous substances that may enter the human bloodstream through the nose, mouth, skin, and the digestive tract. Most air pollutants reach the blood quickly without previous bio-transformation and have been shown to produce harmful effects on the blood, bone marrow, spleen, and lymph nodes (Nikolic *et al.* 2008, Shrey *et al.* 2011, Macchionea and Garcia 2011). Air pollutants can interfere with the body functions. Most commonly, they act at cellular level, influencing chemical reactions within cells, changing cell functions or killing cells. The pollutants affect different organs in mammalians and cause several health problems, including respiratory, cardiovascular, neurological, reproductive and developmental ailments and even cancer. In addition, exposure to air pollutants has been reported to alter immune response and enhance respiratory infection (Madamanchi *et al.* 2005). Many air pollutants exert their major effects by causing oxidative stress in cells and tissues. Gaseous pollutants and PM are known to form reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals that may damage proteins, lipids and DNA directly, and form distinct products (Lirong *et al.* 2004). Oxidative stress can trigger redox-sensitive pathways that lead to different biological processes such as inflammation and cell death (Madamanchi *et al.* 2005, Shrey *et al*. 2011, Macchionea and Garciab 2011).

Epidemiological studies have consistently shown that regular consumption of vegetables is strongly associated with reduced risk of developing chronic diseases (Gunawardena and Silva 2006). A major benefit from diet rich in vegetables may be increased consumption of antioxidants including carotenoids, ascorbate, tocoherol and phenolics (Lui 2004, Huang *et al.* 2005). *Solanum macrocarpon*, one of the commonly consumed vegetables in Nigeria has been analyzed and reported to contain high phenolic, flavonoid, vitamin C and antioxidant ability which enhance its ability to be used as anti-inflammatory, anti-glucoma, anti-asmathic, anti-allegic, anti-cancer and anti-viral agents (Cushnie and Lamb 2005, de Sossa *et al.* 2007, Chinedu *et al.* 2011, Olajire and Azeez 2011). However, very little is known about the influence of dietary supplementation of vegetables, especially *Solanum macrocarpon* during oxidative stress induced by urban air pollutants. The present study was undertaken to assess the effect of *Solanum macrocarpon* on haematological such as WBC, RBC, platelet (PLT), lymphocytes (LYM), neutrophils (NEU) and biochemical parameters such as lipid peroxidation, total protein, non-enzymic antioxidants (vitamin C, glutathione, β-carotene) and antioxidant enzymes (superoxide dismutase, glutathione reductase, catalase) of male albino rats exposed to urban air pollution.

2. Materials and methods

2.1 Materials

2.1.1 Study area and air pollution data

Sampling location (SP_2) along Oba Akran, Lagos-Nigeria as described by Olajire *et al*. (2011) was used (Fig. 1). SP_2 (Lat. 06°36'39.13''N and Long. 003°20'08.41''E) is located at heart of Oba Akran and is the most populated with the highest level of commercial and industrial activities. The location houses companies such as Dangote Agro Sack, May and Baker, Dunlop Tyres and Shampoo Company. Air pollution data for CO, NO_2, SO_2, PM_{10} and O_3, benzene, toluene, ethylbenzene, xylenes (mixed isomers), trichloroethene, carbontetrachloride and tetrachloroethene were determined (Olajire *et al.* 2011).

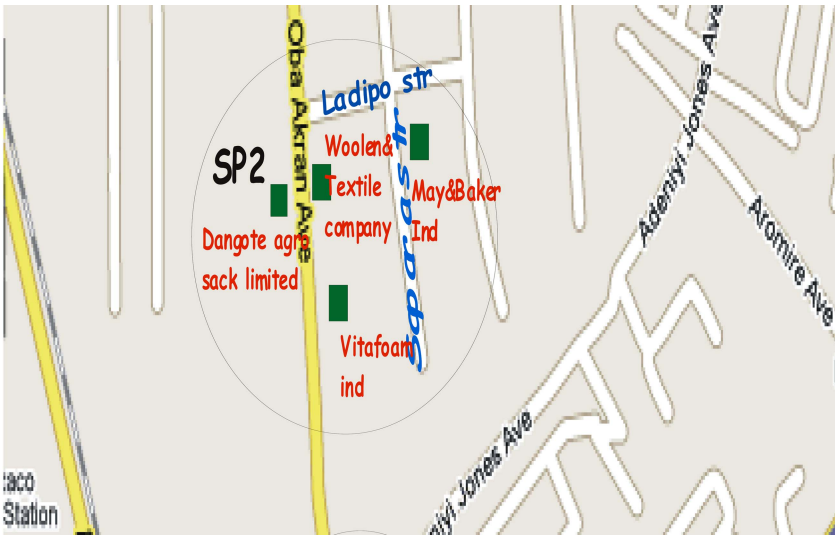

Fig. 1 Map of Oba Akran road showing the sampling location

2.1.2 Chemicals

Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Hydrochloric acid (HCl), Glutathione(GSH), ascorbic acid, β-carotene, 2,4-dinitro-phenylhydrazine (DNPH), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), H_2SO_4, sodium citrate, K_2HPO_4, NaH_2PO_4, bovine serum albumin were used. All reagents were purchased from Sigma-Aldrich (USA).

2.1.3 Vegetable

Solanum macrocarpon was bought in Osogbo market and identified by Dr. Awodoyin of Botany Department, Fountain University Osogbo. It was washed with tap water, cut into pieces, lyophilized, ground and stored in a dessicator to prevent absorption of moisture, until needed.

2.1.4 Animals

Experiments were carried out on male albino rats (*Rattus norvegicus*) of Wistar strain weighing 160-210 g body weight (BW). The animals were purchased from Biochemistry Department of University of Ibadan and were maintained at 24 ± 2ºC with day and night cycles of 12 h each and given food and water adlibitum. The animals were housed in cages made of wire except the basement which was plank so as to allow air in and out. Food and tap water were made available and good hygiene was maintained by cleaning the cages of feces and spilled-off food every day.

The effect of *Solanum macrocarpon* was studied in 25 rats, divided into five groups of five rats each. Group A was exposed to air pollution without treatment (negative control). Group E served as positive control (kept in the animal house without exposure to air pollution and without supplementation). Groups C and D were supplemented orally with *Solanum macrocarpon* given at 36 and 75 mg/kg body weight (BW) respectively and exposed to air pollution. Group B supplemented orally with *Solanum macrocarpon* given at 75 mg/kg body weightt (BW) after exposure to air pollution.

These animals were exposed to air pollution for nine (9) weeks at the location for 8 hours

(8.00am-4.00pm) everyday of the study area. They were placed 75 m away from the road and were sacrificed after nine weeks of exposure. The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals (NIH 1985) in accordance with the principles of Good Laboratory Procedure (GLP) (WHO 1998).

2.2 Preparation of serum, plasma and tissue homogenates

The procedure described by Yakubu *et al*. (2005) was used in the preparation of serum and tissue homogenates. Briefly, the animals were sacrificed under diethylether anesthesia to prevent total death and enough blood collection. Blood was collected through jugular vein into two tubes; first with EDTA anticoagulant for hematological indices of the blood and the second without anticoagulant for separation of serum from whole blood. Blood in the first tube was used for hematological indices. Blood in the second tube was allowed to clot and then centrifuged at 1000 rpm for 15 min. to obtain clear supernatant for colorimetric determinations of biochemical parameters and was kept frozen until needed.

Kidney, lung, liver and heart were dissected out, freed of surrounding tissues and fats with sharp blade and dipped into ice-cold 0.25 M sucrose solution. 0.5 g of each organ was weighed and homogenized in ice-cold 0.25 M sucrose solution. The homogenates were centrifuged at 1000 rpm for 15 min. to obtain clear supernatants, which were frozen until needed.

2.3 Haematological indices analysis

White Blood Cells (WBC), Lymphocytes (LYM), Neutrophils (NEUT), Red Blood Cells (RBC), Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Red Blood cell Distribution Width (RDW), Platelet (PLT), Platelet Distribution Width (PDW) and Mean Platelet Volume (MPV) were all assayed as described by the manufacturers manual using hematological analyzer system X model KX-2IW.

2.4 Biochemical assay

The total protein was measured in serum and homogenates using Biuret test (Gornall 1949). 1 mL of each sample was added to 4 mL of Biuret reagent and allowed to stand for 30 min at room temperature. The absorbance of brick red solution was measured at 540 nm. Bovine serum was used as standard and the values are expressed as mg/L. Lipid peroxidation in serum and homogenates was estimated colorimetrically by measuring acid reactive substances (TBARS) using the method described by Pari and Amali (2005). 0.1 mL of each sample was added to 2 mL of TBA-TCA-HCl reagent (TBA, 0.37%; 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged. The clear supernatant was measured at 535 nm against a reference blank and the values are expressed as nMolMDA/mg protein. Reduced glutathione (GSH) and vitamin C were determined by the method of Pari and Amali (2005). For reduced glutathione, 1 mL of supernatant was taken and 0.5 mL of Ellman's reagent and 3 mL of phosphate buffer (pH 8.0) were added. The yellow color developed was read at 412 nm using glutathione as standard. The values are expressed as mMol/dL. For vitamin C, 1.5 mL of 6% TCA was added to 0.5 mL of serum homogenates and centrifuged. To 0.5 mL of supernatant, 0.5 mL of DNPH reagent was added and incubated for 3 h at room temperature. After incubation, 2.5 mL of 85% tetraoxosulphate (VI) acid was added and red

color developed was read at 530 nm after 30 min. The values are expressed as mg/dL. Ascorbic acid was used as the standard. β-carotene was measured using the method of Suzuki and Katoh (1990). To 1 mL of serum homogenates, 1mL of 10% ascorbic acid solution was added followed by 3.5 mL of hexane and the tube was centrifuged for 10 min. 3 mL of resultant solution was removed from the tube and absorbance was read at 453 nm. The values are expressed as mg/mL. β-carotene was used as standard.

2.5 Statistical analysis

Data were expressed as mean ± standard deviation of five replicates. They were subjected to one-way ANOVA followed by Duncan Multiple Range Test (DMRT). Two-tailed bivariate Pearson correlation was used to evaluate the contribution of each pollutant to haematological and biochemical alterations of the exposed animals. SPSS 15 version was used for the statistical analysis. Significant differences were tested at $p < 0.05$.

3. Results

Air pollution data in both the studied location and the animal house are given in Table 1. Concentrations of VOCs and ozone in the control group that were kept in the animal house were below the detection limit. Except for PM_{10} and CO, other gaseous pollutants were also below the detection limit (0.002 ppm) of the instruments used.

WBC, Neutrophils, RDW and PDW values of animals exposed to air pollution without treatment (A) were significantly increased ($p < 0.05$) compared with the control (Table 2). In all except for PDW, the values of these indices in Group A were significantly higher ($p < 0.05$) compared with the animals in Group D. Supplementation with *Solanum macrocarpon* did not affect significantly ($p < 0.05$) the values of WBC indices when compared with the control, however values of RBC, lymphocytes, HGB, HCT and PLT in animals in Group A were significantly ($p < 0.05$) lower compared with the control (Table 2).

The levels of lipid peroxidation in animals exposed to air pollution without treatment were significantly ($p < 0.05$) higher compared with the control in serum and homogenates (Table 3). Air pollutants had its strongest effects on serum followed by liver, lung, kidney and heart. The difference between the control and animals supplemented with *Solanum macrocarpon* given at 75 mg/kg BW and exposed to air pollution was not significant ($p < 0.05$). In heart and kidney, *Solanum macrocarpon* supplementation given at 36 mg/kg BW was not significantly different ($p < 0.05$) from control.

The levels of protein measured in all the organs of animals exposed to air pollution without treatment decreased significantly ($p < 0.05$) compared with the control. There was a significant difference ($p < 0.05$) in protein levels of animals in Group B and the control (Table 3). There is no significance difference ($p < 0.05$) in protein levels of animals in Groups C and D.

Levels of non-enzymic antioxidants (glutathione, vitamin C and β-carotene) were significantly ($p < 0.05$) depleted in animal in Group A. There was a significant ($p < 0.05$) reduction in levels of non-enzymic antioxidants in animals exposed to air pollution without treatment compared with the control and Group D (Table 3). Lung has the highest depletion of GSH while liver and serum were mostly depleted of vitamin C and β-carotene respectively.

Table 1 Concentration of air pollutants in the study location and animal house

Pollutants	Concentration	
	Study location	Animal house
PM_{10} ($\mu g/m^3$)	307.29 ± 35.34^e	5.01 ± 0.01
NO_2 (ppb)	85.7 ± 18.7	BDL
SO_2 (ppb)	198.6 ± 23.7	BDL
CO (ppm)	21.57 ± 3.46^a	0.2 ± 0.01
O_3 (ppb)	0.47 ± 0.11	N.D
Benzene ($\mu g/m^3$)	3.22 ± 0.88	BDL
Toluene ($\mu g/m^3$)	2.43 ± 0.49	BDL
Ethylbenzene ($\mu g/m^3$)	11.62 ± 3.17	BDL
Trichloroethene ($\mu g/m^3$)	242.08 ± 32.22	BDL
Carbon tetrachloride ($\mu g/m^3$)	478.81 ± 157.50	BDL
Tetrachloroethene ($\mu g/m^3$)	529.7 ± 142.61	BDL
Xylene* ($\mu g/m^3$)	503.03 ± 117.08	BDL

AL, Acceptable level; CO, carbon monoxide (AL, 9 ppm); NO_2, nitrogen dioxide (AL, 0.05 ppb); O_3, ozone (AL, 0.08 ppb); SO_2, sulphur dioxide (AL, 0.03 ppb); PM_{10}, particulate matter < 10 μm (AL, 50 $\mu g/m^3$); [a]significantly different from animal house ($p < 0.05$), [*]Xylene (mixed isomer), BDL-below detection limit, ND-not detected

4. Discussion

4.1 Air pollution data

Mean levels of O_3, NO_2 and SO_2 at the studied location were a much higher than acceptable values, and the average PM_{10} level was remarkably high, (307.29 vs. 50 $\mu g/m^3$) (Table 1). PM_{10} and CO at the studied location were highly traffic-related with possibly severe health consequences (Olajire *et al.* 2011). The VOC concentrations, especially chlorinated VOCs, xylene and to a lesser extent ethyl benzene were high. The chlorinated VOCs are dominated by trichloroethylene, tetrachloroethylene and carbon tetrachloride. The concentration of PM_{10} and CO obtained in the animal house showed good air quality; and VOC concentrations were at below detection limit. These pollutants are well known to produce oxidative stress in organisms. If not neutralized, various oxyradicals can trigger redox-sensitive pathways that lead to different biological processes such as inflammation and cell death (Shrey *et al.* 2011, Macchionea and Garciab 2011).

Supplementation with diet rich in vegetables may increase consumption of antioxidants including carotenoids, ascorbate, tocoherol and phenolics (Liu 2004, Huang *et al.* 2005), which were found to inhibit the cellular damage induced by oxidative stress (Burton and Traber 1990, Simon-Schnass 1992, Palozza *et al.* 1997). *Solanum macrocarpon* has been reported to have potent antioxidant properties among the commonly consumed vegetables in Nigeria (Olajire and Azeez 2011).

4.2 Haematological studies

This study investigated haematological changes in male albino rats exposed to urban air pollution.

Table 2 Haematological parameters* of animals' exposure to air pollution without treatment, with concurrent supplementation with *Solanum macrocarpon* during and after exposure, and the control

Parameter	A	B	C	D	E
WBC ($10^3/\mu L$)	18.9 ± 2.9^{b}	$15.0 \pm 1.0^{a,b}$	$14.9 \pm 1.1^{a,b}$	13.2 ± 0.6^{a}	11.0 ± 0.6^{a}
LYM (%)	79.0 ± 6.6^{b}	83.5 ± 3.2^{b}	84.3 ± 2.4^{c}	85.5 ± 1.0^{a}	90.8 ± 2.0^{a}
NEUT (%)	21.0 ± 6.6^{b}	16.5 ± 3.2^{b}	15.7 ± 2.4^{c}	14.5 ± 1.0^{a}	9.2 ± 2.0^{a}
RBC ($10^6/\mu L$)	7.6 ± 0.5^{b}	8.0 ± 0.3^{b}	8.2 ± 0.5	8.2 ± 0.1^{a}	8.7 ± 0.1^{a}
Hb (g/dL)	13.7 ± 0.4^{b}	13.9 ± 0.4^{c}	14.0 ± 0.0^{c}	14.6 ± 0.6^{c}	15.0 ± 0.7^{a}
HCT (%)	43.3 ± 2.0^{b}	45.2 ± 1.6^{b}	46.9 ± 0.7^{c}	48.5 ± 2.4^{a}	49.6 ± 1.3^{a}
MCV (fl)	59.1 ± 1.1^{c}	57.0 ± 1.8^{c}	55.7 ± 1.3^{c}	56.7 ± 0.4^{c}	57.9 ± 0.4^{c}
MCH (pg)	17.0 ± 0.2^{c}	17.7 ± 0.3^{c}	17.5 ± 0.4^{c}	17.5 ± 0.1^{c}	17.6 ± 0.4^{c}
MCHC (g/dL)	29.8 ± 0.7^{c}	30.0 ± 0.4^{c}	31.5 ± 0.6^{c}	30.9 ± 0.2^{c}	30.3 ± 0.5^{c}
RDW (%)	16.2 ± 1.6^{b}	15.7 ± 0.5^{b}	15.0 ± 0.4^{b}	12.1 ± 0.8^{a}	10.6 ± 0.1^{a}
PLT (μL)	689.0 ± 50.3^{b}	653.7 ± 65.4^{b}	690.3 ± 40.5^{c}	718.0 ± 80.0^{c}	728.7 ± 9.2^{a}
PDW (%)	13.8 ± 1.1^{b}	12.6 ± 1.0^{c}	12.1 ± 0.4^{c}	10.9 ± 0.5^{c}	11.0 ± 0.7^{a}
MPV (fl)	9.3 ± 0.4^{c}	9.0 ± 0.2^{c}	8.9 ± 0.2^{c}	8.2 ± 0.2^{c}	8.1 ± 0.9^{c}

A, exposure alone; B, exposure + treatment; C, 36 mg/kg BDW supplementation; D, 75 mg/kg BDW supplementation; E, control; *All values are mean ± SD, ($n = 5$); $p < 0.05$; [a]significantly different at $p < 0.05$ from Group A of each group across the row; [b]significantly different at $p < 0.05$ from control (Group E) of each group across the row; [c]not significantly different from Groups A and E at $p < 0.05$; Number of animals 5 male rats / group; WBC, white blood cell; LYM, lymphocyte count; NEUT, neutrophils count; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, Red cell distribution width; PLT, platelet count;PDW, platelet distribution width; MPV, mean platelet volume

In the present study the animals exposed to urban air pollution without treatment showed a significant increase in WBC levels (Table 2). This increase may be attributed to the inability of the animal's defense mechanism and the immune system to counteract the toxic effects that could result from the deposition of these toxic substances (NO_2, SO_2, CO) into alveolar surface and to fight any infection resulting from the exposure (Tan *et al.* 2000). The significant increase in WBC could also be due to SO_2 as reported by Gorriz *et al.* (1996). In the present study, *Solanum macrocarpon* supplementation did not change the rise in WBC count. This shows that *Solanum macrocarpon* acts as an antioxidant only in reducing the oxidative stress by exposure to urban air pollution and did not alter the body's adaptive phenomenon to stress.

Lymphocytes and neutrophils are white cell indices, which help in fighting diseases. Low levels of lymphocytes may indicate presence of diseases like hepatitis and lymphoma and elevated levels neutrophils may suggest presence of bacterial and viral infections (Blum *et al.* 1998, Jassim and Hassan 2011). The animals exposed to urban air pollution without treatment showed significant increase in neutrophils level and decrease in lymphocytes level (Table 2). The decrease in lymphocytes and increase in neutrophils could be due to irritant effects produced under the influence of these toxic gases (Agarwal and Guleria 2008). The decrease in lymphocytes levels may also be attributed to direct toxicity of benzene, a constituents of the polluted air to which the animals were exposed. As reported by some authors (Leea *et al.* 2002, Avogbe *et al.* 2011), benzene reduces lymphocytes because benzene is known to be immunotoxic and as blood cell carcinogen inducing anaemia, leukemia and blood formula modification. When animals were supplemented with *Solanum*

Table 3 The changes in TBARS*, total protein, GSH, vitamin C and β-carotene in serum and homogenates of animals' exposed to air pollution alone, with concurrent supplementation with *Solanum macrocarpon* during and after exposure and the control

Group/BP	Kidney	Heart	Lung	Liver	Serum
TBARS (nMolMDA/mgprotein).					
A	1.7 ± 0.3^{b}	1.6 ± 0.2^{b}	2.6 ± 0.7^{b}	3.4 ± 1.0^{b}	4.9 ± 0.6^{b}
B	$1.0 \pm 0.1^{a,b}$	0.9 ± 0.1	$1.3 \pm 0.20^{a,b}$	$1.2 \pm 0.1^{a,b}$	$2.7 \pm 0.2^{a,b}$
C	0.8 ± 0.1^{a}	0.8 ± 0.1	$1.2 \pm 0.0^{a,b}$	$1.6 \pm 0.2^{a,b}$	$1.3 \pm 0.1^{a,b}$
D	0.4 ± 0.1^{a}	0.5 ± 0.1	0.6 ± 0.1^{a}	0.9 ± 0.1^{a}	$1.9 \pm 0.1^{a,b}$
E	0.1 ± 0.0^{a}	0.2 ± 0.1	0.4 ± 0.0^{a}	0.4 ± 0.2^{a}	0.2 ± 0.0^{a}
TP (mg/ml)					
A	7.7 ± 0.5^{b}	4.5 ± 0.2^{b}	11.8 ± 1.0^{b}	10.4 ± 0.7^{b}	12.2 ± 1.2^{b}
B	8.1 ± 0.3^{b}	5.5 ± 0.5^{b}	13.0 ± 1.1^{b}	12.3 ± 0.4^{b}	12.6 ± 0.7^{b}
C	10.3 ± 0.3^{a}	6.2 ± 0.6^{a}	$15.6 \pm 2.2^{a,b}$	12.4 ± 1.5^{b}	$14.8 \pm 1.1^{a,b}$
D	11.9 ± 0.2^{a}	6.3 ± 0.6^{a}	21.6 ± 1.6^{a}	15.2 ± 1.1^{a}	18.3 ± 1.5^{a}
E	13.2 ± 0.8^{a}	11.5 ± 1.2^{a}	25.7 ± 0.9^{a}	22.9 ± 1.8^{a}	23.2 ± 1.0^{a}
GSH (mM/dl)					
A	17.5 ± 1.0^{b}	14.6 ± 0.2^{b}	3.1 ± 1.0^{b}	5.8 ± 0.8^{b}	6.3 ± 0.6^{b}
B	20.7 ± 0.8^{b}	20.2 ± 0.4^{b}	3.8 ± 1.1^{b}	7.2 ± 0.2^{a}	7.1 ± 0.1^{a}
C	$22.3 \pm 0.2^{a,b}$	24.4 ± 1.8^{a}	4.8 ± 0.5^{b}	5.9 ± 0.8^{b}	7.6 ± 0.1^{a}
D	22.2 ± 0.6^{b}	26.7 ± 0.8^{a}	$7.4 \pm 0.6^{a,b}$	9.3 ± 0.1^{a}	9.3 ± 0.1^{a}
E	30.6 ± 0.2^{a}	29.3 ± 0.8^{a}	20.7 ± 2.4	13.6 ± 0.1^{a}	11.7 ± 0.1^{a}
VIT. C (mg/dl)					
A	15.4 ± 0.9^{b}	19.7 ± 0.1^{b}	14.0 ± 1.1^{b}	8.1 ± 0.2^{b}	20.7 ± 0.2^{b}
B	21.0 ± 0.2^{a}	20.3 ± 0.2^{b}	13.7 ± 1.0^{b}	10.8 ± 0.5^{b}	22.3 ± 0.3^{b}
C	20.6 ± 0.2^{a}	20.5 ± 0.2^{b}	16.9 ± 0.4^{a}	12.6 ± 0.6^{b}	24.8 ± 0.7^{a}
D	24.4 ± 0.2^{a}	24.5 ± 0.7^{a}	18.0 ± 0.4^{a}	15.7 ± 0.5^{a}	27.4 ± 1.1^{a}
E	25.0 ± 0.2^{a}	25.5 ± 0.9^{a}	19.6 ± 0.3^{a}	16.6 ± 0.5^{a}	28.4 ± 0.8^{a}
β-carotene (mg/l)					
A	5.8 ± 0.5^{b}	5.3 ± 0.6^{b}	1.6 ± 0.2^{b}	3.2 ± 0.4^{b}	0.8 ± 0.1^{b}
B	10.4 ± 0.8^{b}	5.2 ± 0.2^{b}	1.6 ± 0.4^{b}	$4.2 \pm 0.2^{a,b}$	1.2 ± 0.3^{b}
C	11.0 ± 0.6^{b}	7.4 ± 1.2	2.6 ± 0.7^{a}	$4.9 \pm 0.6^{a,b}$	1.6 ± 0.2^{b}
D	24.2 ± 3.2^{a}	8.4 ± 0.6	3.2 ± 0.7^{a}	5.4 ± 0.6^{a}	3.5 ± 0.9^{a}
E	26.9 ± 2.3^{a}	8.8 ± 1.0^{a}	3.4 ± 0.6^{a}	5.9 ± 1.8^{a}	7.8 ± 1.0^{a}

*All values are mean ± SD, ($n = 5$); $p < 0.05$; [a]significantly different at $p < 0.05$ from group A of each group across the row; [b]significantly different at $p < 0.05$ from control (Group E) of each group across the row. TBARS, thiobarbituric acid reactive substances

macrocarpon and exposed to air pollution (Groups C and D), the potent antioxidant contained in *Solanum macrocarpon* quenches the reactive free radicals and fight against the infection caused to the neutrophils and lymphocytes by the air pollutants (Table 2). Vitamins, polyphenols and flavonoid content of this vegetable might have been responsible for inhibition of the cellular damage induced by oxidative stress since vitamin C has been shown to improve immune system by its transfer to the nuetrophils and lymphocytes during infection (Romieu *et al.* 2008). Supplementation with *Solanum macrocarpon* after exposure (Group B) could not adequately fight the infection because the values obtained were significantly ($p < 0.05$) different from the control. *Solanum macrocarpon* shows its anti-inflammatory and antioxidant power containing polyphenols and vitamins to boost the immunity of animals that were fed with *Solanum macrocarpon* and exposed to air pollution (Yamamoto and

Table 4. Correlation data* between biochemical parameters and the pollutants

Biochem./Poll.	PM_{10}	CO	NO_2	SO_3	O_3	B	T	EB	TCE	CCl_4	TeCE	XY
TBARS												
Kidney	-0.00	-0.30	0.25	-0.35	-0.91	-0.93	-0.03	0.60	-0.32	-0.06	-0.81	0.52
Heart	0.97	-0.75	0.86	0.96	-0.30	0.45	-0.68	-0.08	0.28	0.55	-0.50	0.76
Lung	-0.73	0.72	-0.80	-0.53	0.87	0.27	0.65	-0.45	0.11	-0.29	0.96	-0.98
Liver	0.85	-0.80	0.88	0.67	-0.76	-0.10	-0.66	0.29	0.04	0.42	-0.89	0.97
Serum	0.52	-0.42	0.54	0.42	-0.87	-0.30	-0.80	0.72	-0.45	-0.07	-0.91	0.97
TP												
Kidney	0.01	0.13	-0.15	0.22	0.93	0.80	0.36	-0.86	0.63	0.33	0.83	-0.66
Heart	-0.08	0.35	-0.18	0.13	-0.43	-0.11	-0.81	0.90	-0.91	-0.73	-0.36	0.51
Lung	0.50	-0.31	0.46	0.50	-0.76	-0.14	-0.90	0.74	-0.53	-0.17	-0.80	0.93
Liver	-0.81	0.72	-0.72	-0.76	-0.24	-0.64	0.05	0.74	-0.84	-0.88	-0.22	-0.16
Serum	-0.66	0.58	-0.68	-0.53	0.86	0.23	0.78	-0.58	0.28	-0.11	0.92	-1.00
GSH												
Kidney	-0.75	0.74	-0.81	-0.54	0.86	0.26	0.64	-0.43	0.08	-0.32	0.95	-0.98
Heart	0.81	-0.99	0.95	0.46	-0.58	-0.18	-0.10	-0.19	0.54	0.82	-0.72	0.65
Lung	-0.12	0.13	-0.21	0.00	0.91	0.61	0.60	-0.91	0.69	0.36	0.84	-0.78
Liver	-0.17	-0.30	0.15	-0.60	-0.50	-0.86	0.59	0.03	0.18	0.25	-0.41	0.01
Serum	-0.50	0.56	-0.62	-0.26	0.98	0.52	0.56	-0.64	0.31	-0.09	0.99	-0.93
VIT. C												
Kidney	0.12	-0.43	0.38	-0.26	-0.93	-0.88	-0.03	0.52	-0.21	0.08	-0.86	0.58
Heart	0.81	-0.99	0.95	0.46	-0.58	-0.18	-0.10	-0.19	0.54	0.82	-0.72	0.65
Lung	-0.12	0.13	-0.21	0.00	0.91	0.61	0.60	-0.91	0.69	0.36	0.84	-0.78
Liver	-0.17	-0.31	0.15	-0.60	-0.50	-0.86	0.59	0.03	0.18	0.25	-0.41	0.01
Serum	-0.50	0.56	-0.62	-0.26	0.98	0.52	0.56	-0.64	0.31	-0.09	0.99	-0.93
***β*–Car.**												
Kidney	0.52	-0.06	0.22	0.85	0.32	0.88	-0.70	-0.10	0.02	0.05	0.16	0.26
Heart	0.30	-0.05	0.21	0.40	-0.63	-0.09	-0.93	0.83	-0.71	-0.41	-0.64	0.80
Lung	-0.24	0.35	-0.39	0.00	0.99	0.70	0.46	-0.77	0.48	0.12	0.94	-0.81
Liver	-0.61	0.39	-0.55	-0.62	0.70	0.03	0.93	-0.64	0.42	0.06	0.78	-0.95
Serum	-0.94	0.92	-0.93	-0.77	0.15	-0.36	0.18	0.50	-0.74	-0.92	0.36	-0.48

*$p < 0.05$

Gaynor 2001, Olajire and Azeez 2011).

RBC contains hemoglobin which carries oxygen through the body system and reduction may indicate the presence of anemia, hemorrhage and cancer (Mansour *et al.* 2007). There was a significant ($p < 0.05$) reduction in RBC, Hb and HCT of animals exposed to air pollution without treatment (Table 2). The reduction may be linked to the exposure to air pollution containing gases like SO_2, NO_2 and CO (Agarwal and Guleria 2008). These gases make contact with the internal environment of the body through respiratory tract and interfere with the metabolism of red blood cells and its haemoglobin contents which results in haemolytic anaemia in albino rat (Saadat and

Bahaoddini 2004, Golalipour *et al.* 2007, Poursafa *et al.* 2011). Decrease in RBC may also be indicative of excessive damage to erythrocytes or inhibition of erythrocyte formation (Aljumaah and Hussein 2011). Values of MCHC, MCV, and MCH are not significantly ($p < 0.05$) different from control, however the significant ($p < 0.05$) increase in RDW may be a reflection of disruption in erythropoiesis that may occur during some haematological disorders such as anaemia (Docci *et al.* 1989, Laso *et al.* 1990). Studies have also shown that animals exposed to air pollution containing these gases lead to reduction in RBC, Hb and HCT (Leea *et al.* 2002, Hrelia *et al.* 2004, Agarwar and Guleria 2008). The decrease in hematocrite and hemoglobin in association with NO_2 exposure has been observed and previously reported (Gorriz *et al.* 1996, Nikolic *et al.* 2008). PM_{10} has been shown to cause significant damage of red blood cells such as reduced hemoglobin concentrations, the number of erythrocytes and hematocrite, thus leading to anemia (Syd Bom *et al.* 2001). *Solanum macrocarpon* supplementation clearly prevented erythropoiesis of RBC and its indices by mopping up the toxic effects of air pollutants with its inherent antioxidative power. Vitamins and polyphenol constituents in *Solanum macrocarpon* provided essential growth to haemopoietic organs and erythropoiesis which could have been responsible for the increase RBC, Hb, HCT and PLT in animals fed with *Solanum macrocarpon.*

PLT are for blood clotting and low values may indicate antibody formation, liver disease and blood clotting problems (Yilmaz *et al.* 2004). Platelet indices (i.e., PDW and MPV) are potentially useful markers for the early diagnosis of thromboembolic diseases (Vagdatli *et al.* 2010). PDW levels were significantly ($p < 0.05$) higher in animals exposed to air pollution without treatment compared to control (Table 2). MPV levels were not significantly ($p < 0.05$) different but they were higher in exposed animals than control (Table 2). Increase in PDW occurs in sickle cell anaemia (Amin *et al.* 2004) which may contribute to erythrocyte abnormalities in platelet dysfunction. High levels of MPV are a potential predictor of vascular disorders like atherosclerosis, coronary artery disease and ischemic stroke. (Yilmaz *et al.* 2004, Karagöz *et al.* 2009). All animals that were fed with *Solanum macrocarpon* and exposed to air pollution had boosted PLT. This could be as a result of vitamins and polyphenols that are present in this vegetable (Olajire and Azeez 2011).

The correlation coefficient between the pollutants and the heamatological parameters are calculated in order to deduce the pollutant responsible for the observed damages in cell and blood tissues (Table 4). Considerable correlations were obtained between Hb and HCT ($r = 0.99$, $p < 0.05$; 8 d.f), Hb and PLT ($r = 0.901$, $p < 0.05$; 8 d.f) and HCT and PLT ($r = 0.947$, $p < 0.05$; 8 d.f). This shows that reduction in one might lead to reduction in the other.

Elevation in WBC of animals exposed to air pollution without treatment could have possibly been due to ethylbenzene because of significant correlation ($p < 0.05$) between WBC and ethylbenzene (Table 5). Ethylbenzene has been shown to be toxic and its toxicity could have caused the increase in the WBC (Henderson *et al.* 2007). Synergistic combination of PM_{10}, SO_2 and benzene may be the major contributors to reduction in RBC, as they correlated significantly ($p < 0.05$) with the reduction in RBC of animals exposed to air pollution without treatment. PM_{10}, trichloroethene and carbontetrachloride could have been responsible for the decrease in Hb and HCT levels because of the significant correlations ($p < 0.05$) between these pollutants and the blood indices (Table 5). PM_{10} and carbon tetrachloride could possibly be responsible for the decrease in PLT levels as they significantly correlated ($p < 0.05$) with the reduction in PLT of animals exposed to air pollution without treatment.

Table 5 Correlation data* between the hematological parameters and the pollutants

Heam./Poll.	PM_{10}	CO	NO_2	SO_2	O_3	B	T	EB	TCE	CCl_4	TeCE	XY
WBC	-0.18	0.44	-0.29	0.06	-0.24	-0.01	-0.74	0.78	-0.93	-0.75	-0.17	0.33
RBC	0.66	-0.37	0.47	0.88	0.18	0.61	-0.61	-0.26	0.31	0.37	-0.02	0.37
Hb	0.80	-0.28	0.19	0.59	0.13	-0.27	0.21	-0.78	0.74	0.98	-0.13	0.29
HCT	0.77	-0.35	0.24	0.51	0.02	-0.34	0.26	-0.73	0.74	0.98	-0.22	0.31
PLT	0.76	-0.39	0.29	0.42	-0.28	-0.54	0.17	-0.48	0.56	0.89	-0.49	0.50

*$p < 0.05$

4.3 Biochemical studies

Polyunsaturated fatty acids are the prime target of radical oxygen scavengers (ROS) for oxidation due to the presence of their methylene group. They yield lipid peroxide molecules known to further perpetuate ROS production. Malondialdehyde (MDA) is a marker of oxidative lipid damage and it is a major oxidative product of peroxidized polyunsaturated fatty acids. Its high value may signify mutagenic and carcinogenic effects of ROS (Pari and Amali 2005, Ziecha *et al.* 2010, El-Gendy *et al.* 2010). There was a significant increase in TBARS levels in all the organs of the animals exposed to urban air pollution without treatment (Yanga and Omayeb 2009). When the animals were supplemented with *Solanum macrocarpon*, the TBARS level in all the organs of the animal decreased significantly, which may be due to decreased oxidative stress. This could be due to the polyphenols and vitamins contents of *Solanum macrocarpon* as previously reported (Olajire and Azeez 2011). The decrease in oxidative stress when *Solanum macrocarpon* was supplemented after exposure was not as effective as observed for Groups C and D, this is because the free radical induced by air pollutants could not be effectively reversed as evidenced in Group B. Effectively, *Solanum macrocarpon* acted by inhibiting lipid peroxidation thereby stabilizing biomembranes and biostructures thus protecting the body against oxidative stress.

Exposure to urban air pollutants resulted in reduction of total protein values. It was reported that in stressful environment, protein catabolism takes place resulting in reduced protein levels, which signifies poor liver functions and some kidney diseases (Sarada *et al*. 2002). *Solanum macrocarpon* supplementation and exposure to air pollutants showed significant increase in total protein value. *Solanum macrocarpon* constituents especially vitamin C could have been responsible for the increase in total protein (Table 3). Vitamin C has been reported to halt protein oxidation (Romieu *et al.* 2008).

Non-enzymic antioxidants like glutathione (GSH), vitamin C and β-carotene were evaluated in the serum and homogenates. They play important role in preventing the oxidation of cellular macromolecules (Olajire and Azeez 2011). It is well established that they donate proton to free radical to inactivate them. GSH in blood keeps up the cellular levels of active forms of vitamins C and E. Vitamin C fights off these pollutants by stimulating enzymes in the liver that detoxify the body (Ziecha *et al.* 2010, El-Gendy *et al*. 2010). β-carotene is the most effective naturally occurring quencher of singlet oxygen and it inhibits lipid peroxidation better than vitamin E (Sarada *et al.* 2002). In the present study, levels of glutathione (GSH), vitamin C and β-carotene in all the organs were decreased in animals exposed to air pollution without treatment, while *Solanum macrocarpon* supplementation clearly enhanced the GSH, vitamin C and β-carotene levels (Table 3). The

enhancement could be due to antioxidative properties of *Solanum macrocarpon* (Olajire and Azeez 2011), which protected the organs against the deleterious effects of air's toxic constituents as well as their metabolites. Significant depletion of these non-enzymic antioxidants in animals exposed to air pollution without treatment could be due to the reaction between these pollutants and non-enzymic antioxidants. Ozone has been reported to react with ascorbic acid in the system leading to its depletion (Romeiu *et al.* 2008). Exposure of animals to NO_2 has been shown to decrease glutathione and vitamin C levels in animals (Elsayed 2001). Results of researches have shown that supplementation with vitamin C boosts intracellular antioxidant capacity (El-Gendy *et al.* 2010). The decrease in the non-enzymic antioxidants which correlates significantly (Table 4) with the concentration of pollutants determined at the study location might have been the results of toxic effects of these pollutants on the animals exposed without treatment.

The correlation coefficient between the pollutants and the biochemical parameters are also calculated in order to deduce the pollutants responsible for the observed damages in cell and organs of the animals (Table 4). High correlations of these pollutants with increase in lipid peroxidized products levels showed that they could possibly be the cause of lipid peroxidation of animals exposed to air pollution. Gaseous (CO, SO_2, NO_2, O_3) and particulate air pollutants have been reported to induce the release of oxidants such as superoxide anion, hydrogen peroxide and hydroxyl radicals among other free radicals to cause lipid peroxidation and protein catabolism (Oyarzún *et al.* 2005, Rom 2011). Carbon tetrachloride has been shown to damage liver, kidney, lung and intestine (Edewor *et al.* 2007). Significant correlations obtained between the decrease in non-enzymic antioxidants and pollutants could have resulted in the reduction in non-enzymic antioxidants. These pollutants have been reported to generate free radicals which these antioxidant molecules would have scavenged thereby reducing their levels (Kampa and Castanas 2008). The significant correlation obtained between reduction in total protein and pollutants showed that combination of these pollutant gases might be possibly responsible for the reduction in total protein.

5. Conclusions

Living on top of oxidative stress is a necessity in our increasingly toxic world. Taking care to avoid those toxins as much as possible and to enrich our diets with vegetable- rich antioxidants is a wise step to take in our endless quest for wellness. The present findings reveal that *Solanum macrocarpon* has potent antioxidant effect in reducing the oxidative stress induced by exposure to urban air pollution. Further, these data provide information regarding the possible use of *Solanum macrocarpon* as a nutritional supplement in amelioration of pollutants-induced oxidative stress at urban centres. Thus, sufficient dietary intake of vegetable-rich antioxidants by individuals who regularly come in contact with urban air pollution is beneficial in combating the adverse effects of these air pollutants.

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