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The Effect of Exhaust Fumes on Glutathione S-Transferase Enzymes in the Lung of Rats Supplemented with Natural Products

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Abstract: This study examined the effect of exhaust fumes on the lungs and the impact of dietary supplementation with natural products containing cancer chemopreventive agents in attenuating their effect. Thirty-two rats were grouped into eight groups of four rats each. Groups 1-3 were on non-supplemented diet and exposed to exhaust fumes from generator for time intervals of 5 mins, 1 h and 2 h, respectively at a distance of 2.5 m away from the generator. Groups 4-6 were fed on supplemented diet and exposed to exhaust fumes for time intervals of 5 min, 1h and 2 h, respectively at a distance of 2.5 m from the generator. Group 7 was positive control not exposed to exhausted fumes and fed on diet supplemented with natural products. Group 8 was positive control not exposed to exhaust fumes and not on supplement diet. Normal cellular architecture was observed in supplement positive control groups compared with non supplement positive control groups indicated that the integrity of tissues were not compromised following food supplementation. However, large deposit of dark spots were seen in lungs of non supplemented groups on 1h and 2 h exposure groups, respectively. The lungs also showed significant decrease in the Glutathione-S-Transferase (GST) level on exposure for 5 min, 1hour and 2 h ($p<0.05$) compared with their respective control groups. It was also observed that the level of Malondialdehyde (MDA) increased significantly ($p<0.05$) in non supplement groups compared with their control groups. Combination of natural products significantly reversed the effect of exhaust fumes on the level of GST ($p<0.05$) and MDA level ($p>0.05$ compared with non supplement groups. Supplementation of diet with natural products had no adverse effect on the integrity of the tissues under examination as demonstrated by histochemical analysis. Hence, combination of natural diet may provide a useful preventive measure against tissue injury consequent to exposed to exhaust fumes experienced in our houses and roads.

Keywords: Chemo preventive agents, carcinogenic, neurotoxic, endogenous antioxidant enzyme

INTRODUCTION

It has become a great concern to every Nigerian of the epileptic condition of the electricity supply in the country. The condition is worsening everyday as most Nigerians go without power supply for weeks and perhaps even months. This has hampered the nation's economic development as industries, companies, cooperate organizations, private establishment have to rely on generators (Emeka, 2008). Regular power supply is the prime mover of technological and social development. There is hardly any enterprise or indeed any aspect of human development that does not require energy in one form or the other. Nigeria is richly endowed with various energy sources, crude oil, natural gas, coal, hydro-power, solar energy, fissionable materials for nuclear energy. Yet the country consistently suffers from energy shortage-a major impediment to industrial and technological growth. The National Electric Power Authority (NEPA), a government parastatal, has the sole responsibility for managing the generating plants as well as distribution of power nationally. The total generating capacity is about 3000MW, approximately one-third the current level of national demand (Ajanaku, 2007; Adegbamigbe, 2007). However, the actual power available at any given time is less that 40% of the total capacity due to poor maintenance; hence, there is a perennial shortage (Ajanaku, 2007; Adegbamigbe, 2007). This situation is exacerbated by a grossly inefficient and poorly maintained distribution system. Industry can only cope with power outages by resorting to internal generating plants (Ajanaku, 2007; Adegbamigbe, 2007). It is disheartening that about 60% of the country still has no access to electric power supply (UNDP, 2001; Ajanaku, 2007; Adegbamigbe, 2007). Libya with a population of only 5.5 million has generating capacity of 4,600 megawatts, approximately

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the same as Nigeria which has a population of about 140 million (Odjaka, 2006; Ubani, 2012). Furthermore, South Africa with a population of only 44.3 million has a generating capacity of 45,000 megawatts, almost eleven times the generation capacity in Nigeria which has three times the population of South Africa (Agbo, 2007). Studies and experiences have shown that power generation in the country has been dismal and unable to compare with what is obtainable in smaller African countries. The recent survey on power distribution to the industrial sector in Nigeria showed that average power outage in the industrial sector increased from 13.3 h in January 2006 to 14.5 h in March 2006. In a worsening experience, the outage increased to 16.48 h per day in June. In other words, power distribution in the month of June, 2006 to the industrial sector, on the average, was 7.52 h per day (Odjaka, 2006).

Hence, industrial sector, business oriented minds and domestic home sort alternative source of electricity through the use generators (Ajanaku, 2007). In densely populated areas cities in Nigeria, generators are placed on doorsteps, top of houses and people live in this condition for years. The exhaust fumes produced by these generators contain some hydrocarbons and other toxic substances like oxides of carbon, nitrogen, sulphur, lead and carcinogenic benzo(a)pyrene which pose serious health problems (Timbrell, 1991). Cases of death sequel to use of generators at home are daily reoccurrences because of exhaust fumes inhalation (IPCS *et al*., 1999). Similarly traffic congestions in our major cities have led to acute exposure to exhaust fumes at traffic stop points. Hence this study attempted to evaluate the damage of exposure to exhaust fumes at different time interval using rat as model.

MATERIALS AND METHODS

Thirty-two male Albino rats weighing 220 g on the average were obtained from National Institute of Veterinary Research Von; Jos. The rats were housed in eight groups of four rats each. They were housed in animal house, Biological Science, Bayero University, Kano with 12-h light-dark cycle. The rats were allowed free access to standard drinking water and feed *ad libitum* for 7 days to acclimatize. After this period, the animals were subjected to generator exhaust fumes and fed with combined antioxidants supplement feed for a total period of 2 months (8 weeks)

Grouping of experimental animals:

Group 1: The rats were fed on normal diet and exposed to exhaust fumes 2.5 m from electric generator for 5 min.

Group 2: The rats were fed on normal diet and exposed to exhaust fumes 2.5 m from electric generator for 1 h.

Group 3: The rats were fed on normal diet and exposed to exhaust fumes 2.5 m from electric generator for 2 h.

Group 4: Rats were fed on normal diet supplemented with natural products and exposed to exhaust fumes 2.5 m from electric generator for 5 min

Group 5: Rats were fed on normal diet supplemented with natural products and exposed to exhaust fumes 2.5 m from electric generator for 1h

Group 6: Rats were fed on normal diet supplemented with natural products and exposed to exhaust fumes 2.5 m from electric generator for 2 h

Group 7: The rats were fed on normal diet supplemented with natural products and non-exposed exhaust fumes.

Group 8: The rats were fed on normal diet without dietary supplementation and not exposed to exhaust fumes.

Composition of supplemented diet: Dietary supplementation composed a combination of 0.25 g (green tea crude extract), 1.0 g (onion crude extract) and 1.0 g/100 g of normal diet

Preservation of organs and blood serum for analysis
Lungs and blood serum of 3 rats from each group were collected and preserved at -80°C for biochemical analysis. Lungs from the others three animals in the group were preserved in 10% formalin for histological analysis.

Preparation of onion (Allium Cepa) crude extract: Fresh red onions were purchased at Sabon Gari market in Kano State, Nigeria. Bulbs were cut into pieces, dried under shade away from direct sunlight to avoid possible damage to their phytochemicals. The dried onion was made into powder using pestle and mortar. To 40 g of the powdered onion, 250 cm^3 of methanol was added, shaken for 10 min and allowed to stand for 2 weeks at 4°C. The mixture was filtered and the filtrate placed on the rotary evaporator using water bath at 35°C-40°C to evaporate the solvent in order to obtain the crude extract rich in quercetin (Won *et al*., 2005).

Preparation of crude cabbage (*Brassica Oleracea*) extract: Cabbage was purchased at Sabon Gari market, Kano. Rinse with fresh water and cut into pieces, dried under shade. The dried cabbage was made into powder using pestle and mortar. To 40 g of the powdered sample, 250 cm^3 of methanol was added, shaken for 10 min and allowed to stay for 2 weeks. The mixture was filtered with a filter paper and the filtrate placed on the rotary evaporator using water bath at 35°C-40°C. The crude extract obtained was rich in indole-3-carbinol (Won *et al*., 2005).

Preparation of crude of Green tea extract (*Camellia sinensis*): Pure green tea (Twinings brand) was purchased from Shad stores, Zoo Road, Kano., Nigeria. To 5g of green tea 250 cm^3 of distilled water was added and heated at 60°C in 500 cm^3 round bottom flask, placed in a water bath an exact amount of 5.00 g green tea powder. The mixture was filtered through a 0.45µ membrane filter to remove dispersed minute green tea powder.

The sample was washed with the same amount of chloroform in a separatory funnel to remove caffeine, pigments and other non-polar impurities. This step was repeated three times and negligible catechins compounds were found in the chloroform phase owing to their low solubility in chloroform. The crude extract containing mainly catechins in the water phase was extracted to 250 cm^3 ethyl acetate and this step was repeated three times. The ethyl acetate phase was evaporated to dryness using rotary evaporator to obtain crude cabbage extracts containing catechin compounds (Won *et al*., 2005).

Procedure for histological analysis: Histological analysis was carried out at the Histopathological Department of Aminu Kano Teaching Hospital, Kano, Kano State, Nigeria. Physical examination of lung tissues was made. Likely diseased area of the tissue sample was selected for histochemical analysis (Bancroft and Stevens, 1982).

GLUTATHIONE REDUCTASE ASSAY

Glutathione reductase activity was assayed by the methods of Castro *et al*. (1990) in which 5ul of cytosolic extract was added to 125 uL of 0.2M potassium phosphate, buffer (pH 7.0) containing 0.1 mM NADPH the reaction was initiated by the addition of 25 uL of 10mM GSSG after a 30s incubation period at 30°C. Oxidation of GSSG was measured as a decrease in absorbance at 340 nm. Calculation of enzymes activity was based on consumption f NADH (ϵNADPH = 6300 L/mol/Cm.

Protein estimation: Estimation of protein concentration was carried out by the method of Bradford (1976). Bradford reagent for protein determination was prepared by dissolving 100 mg of coomassie Brilliant Blue in 50 mL of 95% ethanol, 100 mL of 85% phosphoric acid and made up to 1dm^3 with distilled water. The reaction was initiated by adding 16 µL of the sample to 640 µL of Bradford reagent and 60 µL of distilled water. Protein concentrations were determined from a calibration curve using Bovine serum Albumin as standard at varying concentrations.

Statistical analysis of data: Standard Error of Mean (SEM) from the mean standard deviation were obtained for all data collected during analysis and experiment. The students' unpaired t-test. (Test of significance of difference between two means) was utilized to determine increase in enzyme activity using statistical programme SSPS 14.0.

RESULTS AND DISCUSSION

Results:

Mean Weekly body weights: There was an observed weekly increase in weight of the animals in every group. This suggests there was no significant effect of exhaust fume in the weight of the rats. In addition, the administered chemopreventive agents do not retard weight of the animals.

Histological profile of tissues: The histological profiles of the lungs are shown in Fig. 1. Both the supplement and non-supplement control groups were compared with the exposed supplement and non-supplement test groups exposed to exhaust fumes for 5 min, 1hr and 2 h time intervals, respectively. Normal lymphocyte content of all the tissues in supplement control groups compared to non-supplement control groups indicate that the integrity of tissues were not compromised following food supplementation. However, large deposits of dark spots (likely carbon) were observed in the lungs of non supplemented exposed group at 1 h and 2 h exposure. Furthermore, supplementation did not reduce the deposits of dark spots in supplemented groups exposed for two hours

Effect of exhaust fumes and food supplements on Glutathione-S-Transferase (GST) in tissues: The results of the effect of exhaust fumes on GST activity in the various the lungs are presented in Table 1.

Exposure to fume at 5 min had no significant effect on the activity of GST between exposed non supplement group and non supplemented control group. Prolonged exposure at 1 h and 2 h significantly reduced the activity of GST ($p>0.001$) in non supplemented group compared with non supplemented control group. Interestingly, it was observed that supplementation significantly increase GST activity ($p<0.05$) in 5 min and 1 h exposure groups compared to exposed non-supplemented groups. Supplementation had no significant effect at 2 h compared to non supplemented exposed group on the activity of GST.

Effect of exhaust fumes and chemopreventive effect of natural food Supplements on lipid peroxidation product (MDA): There was a significant increase in the level of Malondialdehyde in serum ($p<0.05$) on exposure to exhaust fumes in non dietary supplemented group compared to the control non-supplemented (Table 2). The supplemented groups exposed to fume showed a significant decrease in MDA level ($p<0.05$)

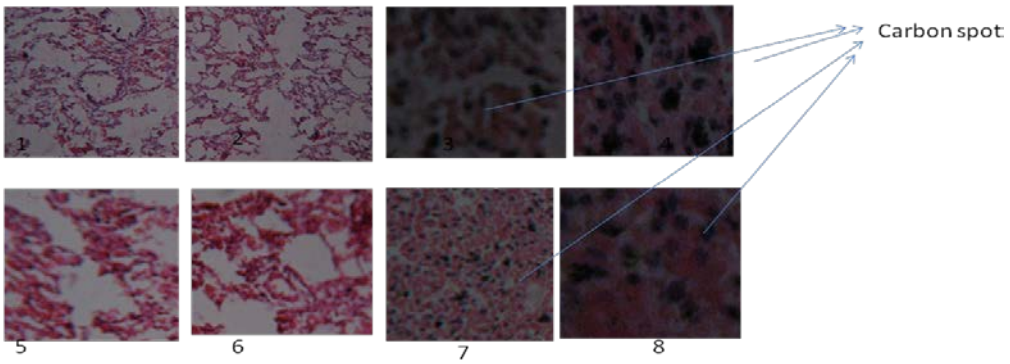

Fig. 1: Histochemical profiles of lung tissue stained with haemtoxyline; (1) Non supplemented control, (2) Non supplemented exposed for 5 min, (3) Non supplemented for 1 hr, (4) Non supplemented exposed for 2 hr, (5) Supplemented control, (6) Supplemented exposed for 5 min, (7) Supplemented exposed for 1hr, (8) Supplemented exposed for 2 hr

Table 1: Effect of exhaust fumes from generator on the activity of antioxidant enzyme in rat lungs

GST nmol/min/mg protein	Control	5 min	1 h	2 h
Non supplement	83.5±9.10	75.2±70	70±6.97[a]	60±12.05[a]
Supplement	95±6.44[b]	88±2.27[b,c]	82±6.26d	79±13.21be

Results are mean±Standard Error of Mean (n =3). 5 min, 1 h and 2 h groups represent groups 1-3, respectively under non-supplement , while they represent groups 4-6, respectively under dietary supplementation. Non supplement control represents group 8, while supplement control represents group 7; a = p>0.001 compared non-supplement control group; b = p<0.05 Compared non-supplement control group; c = p<0.05 5 min exposed non-supplemented and 5 min exposed supplemented; d = p<0.05 Compared between 1 h exposed non-supplement group and 1h exposed supplemented; e = p<0.05 Compared between 2 h exposed non-supplement group with 2 h exposed supplement group

Table 2: Concentration of serum malondialdehyde in rats exposed to exhaust fumes

MDA x 10^{-5} nmol/mL	Control	5 min	1 h	2 h
Non supplement	10.33±0.07	11.79±0.13[b]	12.41±0.10[b]	13.70±0.66[b]
Supplement	8.90±0.17x	9.26±0.13[b1b2]	9.10±0.70[b1b2]	9.07±0.75[b1b2]

Results are mean±Standard Error of Mean (n = 3). 5 min, 1 h and 2h groups represent groups 1-3, respectively under non-supplement , while they represent groups 4-6, respectively under dietary supplementation. Non supplement control represents group 8, while supplement control represents group 7; b = p<0.05 Compared non-supplement control group with non-supplement exposed group with respect to time; b1 = p<0.05 Compared non-supplement group with supplement group; b2 = p>0.05 Compared non-supplement control group with supplement exposed group with respect to time. X = p>0.05 Compared non-supplement control group with supplement control group

compared to the non- supplement groups exposed to fumes irrespective of time of exposure in lung tissues.

DISCUSSION

Toxicological studies in animal and epidemiological data suggest possible health risk to diesel exhaust fume exposure. Acute effects of exposure may lead to lung function decrements and altered brain function (Scheepers and Bos, 1992; Pope *et al*., 2002). Also Lung function decrements are reported as chronic effects among occupationally exposed persons (Pope *et al*., 2002). The components in the exhaust fumes associated with carcinogenic effect are soot particles, particle-associated organics and/or gas phase compounds. The particle load direct effect may include retardation of lung clearance, inflammation and increased cell proliferation. These effects were all demonstrated in rodents. The particles may also prolong the residence time of particulate organics or induce the generation of reactive oxygen species. These compounds are known to react with macromolecules, causing lipid peroxidation (Scheepers and Bos, 1992). There is also a reservoir of basic information on effect of high exposure to diesel exhaust as occupational hazard (Groves and Cain, 2000).

Our study used rats as animal model to evaluate possible impact of exhaust fumes that the generality of population are exposed but not associated with occupation; and the possible impact of supplementation of diet with natural products containing cancer chemopreventive agents in attenuating these adverse effects. The population is exposed to exhaust fumes especially in urban centres from a variety of sources. With the collapse of power sector, individuals have resorted to the use of generators either to provide electricity to their homes or use the energy to run businesses. Furthermore, increased vehicles on our roads would also increase the exposure of people at traffic junctions to exhaust fumes.

There was an observed weekly increase in weight of rats in all the groups. This indicates that the exhaust fumes have no effect on the weight of the rats for the period of exposure. In addition, the administered chemopreventive agent did not retard weight of the animal; this is in accordance with the research on the effects of green tea on weight maintenance after body-weight loss (Kovacs *et al*., 2004).

Studies in several laboratory animal species provide strong evidence that carbon monoxide (CO) exposure produce reductions of birth weight, cardiomegaly, delays in behavioral development and disruptions in cognitive function (IPCS *et al*., 1999). Nevertheless, a diet rich with natural food supplements (Quercetin, indole-3-carbinol, kampferol and catechins) can go a long way to avert the severity of the damage. It has been reported that Glucobracissin; a phytochemical in cabbage is metabolized *in vivo* to indole-3-carbinol, a powerful inducer of glutathione-s-transferase (specially the class Mu-enzyme, GST M2-2) which plays a vital role in xenobiotic metabolism (Fong *et al*., 1999). Hence increase in GST activity observed in this study may have contributed to reduced production of MDA, consequent to metabolism of agents in the fumes which could contribute to the generation of reactive oxygen species. Furthermore Certain GST isozymes (GST-A and GST-M) show catalytic activity of Conjugation of 4-Hydroxy- 2enals (HNE), a product of lipid peroxidation to GSH in rat and human liver cells as well as related endogenous electrophiles (Esterbauer *et al*., 1991; Schaur *et al*., 1991).

Another study showed that Quercetin has health promoting effects such as improvement of cardiovascular health and reducing risk of cancer. It also have anti-inflammatory and anti-allergic effect due to its strong anti-oxidant action; it help to combat free radical molecules which can damage cells and prevent the oxidation of LDL cholesterol (Luo *et al*., 1997). People with high intake of apple (rich in quercetin) have low risk of certain respiratory diseases (Suganuma *et al*., 1999a, b).Other laboratory studies show that catechins act, as a powerful inhibitor of cancer growth is several ways. They scavenge oxidants before cell injures occur, reduce the incidence and size of chemically induced tumors and inhibit the growth of tumor cells. In studies of liver, skin and stomach cancer, chemically induced tumors were shown to decrease in size in mice that were fed with black tea. They may also target and repair DNA aberration caused by oxidants. (Dufresne and Farnworth, 2001; Hakim and Harris, 2001).

Hstological analysis of the lung further highlights its vulnerability to the effect of exhaust fumes on exposure for a long time even with chemoprevantive agent at the dose administered. Supplementation of diet did not protect lungs exposed for 2 h to exhaust fumes against, carbon deposit and generation of product of lipid peroxidation. This is because the lungs receives 100% of the cardiac output and therefore are extensively exposed to toxic substances in the blood and air (Timbrell, 1991) and possibly the induction of antioxidant enzymes threshold cannot cope up with the generation of free radical.

Nevertheless, on comparing the non-exposed control group with exposed-supplemented groups with respect to time, there was no significant decrease in the GST level. This is largely due to the activity of indole-3-carbinol, a powerful inducer of GST (Fong *et al*., 1999) and the other antioxidants capability to increase indigenous antioxidants (Gutteridge and Halliwell, 2002). In rats, the highest level of quercetin was measured in the lungs (De Boer *et al*., 2005).

CONCLUSION

Faced with the perplexing situation of electricity in our country, one cannot afford to do without the use of generator. Furthermore traffic congesting on our roads exacerbates pollution to which populace are exposed. Daily exposure to exhaust fumes for a long period posed serious damage to the lung as it significantly decreases the level of GST. It was observed that these natural antioxidants agents (catechins, quercetin and indol-3-carbinol) have significant chemopreventive effect on lung, as they reduced deposition of carbon soot, MDA and increased the activity of GST. Hence, consumption of combination of these natural chemopreventive supplements may help prevent or minimize the lung damage consequent to fume exposure.

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