



The ameliorative effects of *Moringa oleifera* leaf extract on cardiovascular functions and osmotic fragility of wistar rats exposed to petrol vapour

OM Azeez*, SA Adah, FH Olaifa, A Basiru & R Abdulbaki

University of Ilorin, Ilorin, Nigeria

*Correspondence: Tel.: +2348033858362; E-mail: misibisol@yahoo.com

Abstract

The present study was aimed at evaluating the ameliorating effects of *Moringa oleifera* extract compared to captopril and candesartan cilexetil on cardiovascular functions and osmotic fragility of rats exposed to petrol vapour. Twenty five adult male Wistar rats (130g-200g) were randomly grouped to five with five rats in a group. Group 1 (control) was not exposed to petrol fume. Groups 2 (petrol only), was exposed to petrol fume only. Groups 3, 4 and 5 were pretreated with *Moringa oleifera* extract (40mg/kg), captopril (25 mg/kg) and candesartan (16mg/kg), respectively before exposure to petrol vapour, 10 minutes every day for eight weeks. All groups were given feed and water *ad-libitum*. Petrol vapour was generated by using human compressor nebulizer adopted for rats and connected to fume chamber where the rats were kept. The pretreatment were administered by oral cannula. At the end of the exposure, 0.2ml of blood samples obtained from individual rat in each group were suspended in separate sets of Phosphate buffer saline (PBS) solution of decreasing concentrations. Erythrocyte osmotic fragility (EOF) was determined by spectrophotometer. Electrocardiography was done using EDAN 10. There was significant increase ($p < 0.05$) in EOF of the rats exposed to petrol vapour only. However, *Moringa oleifera*, captopril and candesartan cilexetil significantly ameliorated this effect. There was no significant difference in the amelioration of *Moringa oleifera* and candesartan cilexetil. There was absence of p-wave and significant increase in heart rate observed in the electrocardiogram of petrol only group, this was significantly restored in the *Moringa oleifera*, captopril and the candesartan cilexetil group. The results showed that exposure to petrol vapour elevated EOF, resulted in atria arrhythmia and increased heart rate. These effects were ameliorated by pretreatment with *Moringa oleifera*, captopril and candesartan cilexetil. The amelioration in *Moringa oleifera* was comparable with that of candesartan cilexetil suggesting that *Moringa oleifera* may be an Angiotensin II receptor blocker.

Keywords: Candesartan cilexetil, Captopril, *Moringa oleifera*, Osmotic fragility, Petrol vapour

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Introduction

Premium motor spirit (PMS) is a mixture of aliphatic and aromatic hydrocarbons. It is commonly used as fuel for internal combustion engines. Human beings and animals continued to be exposed to petrol vapour in the environment, occupationally by petroleum attendants, refinery workers, at home and on the roads. The hazardous effects of exposure to PMS are believed to be caused by the volatile organic compound (VOC)

(Kirchstetter *et al.*, 1999). Occupationally exposed individuals as well as those residing in a heavy traffic area constitute the population at greater risk of frequent and long term exposure to constituents of petrol (Uboh *et al.*, 2010). A number of previous studies on exposure to petrol vapour have shown the occurrences of various health issues including increased blood pressure and heart rate (Azeez *et al.*, 2012; Azeez *et al.*, 2013).

Pharmacological intervention through supplementation with a suitable combination of vitamins complex and microelements could be potentially used to ameliorate the adverse effects of gasoline vapor exposure among petrochemical station workers (Georgieva *et al.*, 2002). However, there is paucity of information on the mechanism through which the substances carry out the ameliorative effects.

Moringa oleifera (The Miracle Tree) has been found to have a range of medicinal uses with high nutritional value throughout the world (Suaib *et al.*, 2012). Studies have reported the presence of various phytoconstituents in the leaves of *M. oleifera* extracts using various solvents (Roopalatha & Vijayama, 2013). Different parts of this plant contain a profile of important minerals, and a good source of protein, vitamin, carotene, amino acids and various phenolics (Farooq *et al.*, 2007). The *Moringa* plant provides a rich and rare combination of zeatin, quercetin and alkaloids that have been found to have some ameliorative effects against the Petroleum hydrocarbons-linked health hazards in our past studies (Azeez *et al.*, 2015).

Candesartan is a potent, long-acting, non-peptide tetrazole derivative angiotensin II antagonist that selectively blocks the binding of angiotensin II to the AT₁ receptors in tissues such as vascular smooth muscle and the adrenal gland. Candesartan cilexetil is marketed by AstraZeneca and Takeda Pharmaceuticals, commonly under the trade names of Blopress®, Atacand®, Amias® and Ratacand® (Husain *et al.*, 2011).

Captopril (captopen) is a potent competitive inhibitor of angiotensin-converting enzyme (ACE), It prevents the conversion of angiotensin I to angiotensin II by inhibition of ACE, a peptidyl dipeptide carboxy hydrolase. This inhibition has been demonstrated in both healthy human subjects and in animals by showing that the elevation of blood pressure caused by exogenously administered angiotensin I was attenuated or abolished by captopril. In animal studies, captopril did not alter the pressor responses to a number of other agents, including angiotensin II and norepinephrine, indicating specificity of action (Mittra & Singh, 1998).

Erythrocyte osmotic fragility index is a measure of the resistance of red blood cells to lysis by osmotic stress (Oyewale *et al.*, 2011; Oyewale & Ajibade, 1990). The test is generally useful to ascertain the level of stability and functionality of plasma membrane (Krogmeier *et al.*, 1993), Erythrocyte Mean Cell Volume (MCV) and Surface Area-to-Volume Ratio (SAVR) are diagnosis of hereditary spherocytosis. There has been no study carried out to assess the benefit of *Moringa oleifera* extract on effect of exposure to petrol vapour. Therefore, this study was

aimed at studying the mechanism through which the beneficial effect is carried out.

Materials and Methods

Animals used

A total of 25 male adult Wistar rats [(130-200 g) *Rattus norvegicus*] were purchased from the animal house of Faculty of Veterinary Medicine, University of Ilorin, Ilorin. They were housed in well-ventilated cages maintained at 28 ± 2°C. Rats were on standard rat chow and tap water *ad libitum*. They were acclimatized for two weeks before the experimental period (Azeez *et al.*, 2012). Procedures involving animals and their care were performed in accordance with the National Institutes of Health (NIH) guideline for the care and use of animals (NRC, 1996). Rats were randomly assigned to one of five groups with five rats in a group. Group 1 Control was exposed to ambient air daily, group two was exposed to petrol fume only, while groups 3, 4 and 5 were pre-treated with *Moringa oleifera* leaf extract, Captopril and Candesartan cilexetil respectively.

Preparation of plant material and other drugs

The plant materials were identified and authenticated in the department of Plant Biology, University of Ilorin Nigeria; with herbarium number-UIH-001/1011. The leaves were separated from their stems, washed in tap water and air-dried under shade, without exposure to direct sunlight. The dried leaves were reduced into fine powder by grinding. The aqueous extract was prepared by using Soxhlet extractor, concentrated in rotary evaporator (Buchi, Flavil, Switzerland) at 40°C, then dried and kept at room temperature till used for the assay. The extract was given to the rats at the rate of 40 mg/kg using the oral cannula. The captopril (25mg/kg) and candesartan cilexetil (16mg/kg) were prepared by mixing with distilled water using the instruction in attached literature

Exposure to petrol and dosage

The petrol used in this experiment was purchased from PMS station, close to University of Ilorin gate. Rats in group 1(control) were kept in a petrol-vapour-free section of the animal house. Rats in group 2 (petrol) were exposed to petrol vapour only. The rats in groups 3, 4, and 5 were pretreated with *Moringa oleifera* extract (40mg/kg) captopril (25mg/kg) and candesartan cilexetil (16mg/kg) respectively 30 minutes before each group will be placed in the fume chamber. The fume chamber is a 20-liter bucket with very tight lid. During the

exposure period, rats from each group were placed in the chamber, the nebulizer cup was filled with petrol and the liquid petrol turned to vapour as soon as the nebulizer is switched on. They were allowed to stay in the fume chamber for 10 minutes and removed back to their cages in the vapour free section of the experimentation room. This was done for all the exposed groups every day for eight weeks. The room condition was monitored and maintained at temperature of 28 ± 2 °C. The average dosage exposure was $0.008\text{cm}^3/\text{min}/\text{rat}$.

Measurement of electrocardiography with EDAN 10

At the end of the eight weeks, each rat was anaesthetized with 1% chloralose and 25% urethane intraperitoneally; once the rat was anaesthetized, the rat was placed on a white board, the EDAN electrode clips for right arm, left arm, right leg, left leg and the heart were put in place after rubbing the site with adequate quantity of gel. The EDAN was connected to the laptop, information about each rat was recorded and saved. This was followed by ECG recording for one minute and saved until it was done for rats in all groups.

Determination of weight changes

Body weight was measured once a week using Sartorius AG, Germany digital scale. The body weight as weekly percentage weight gain was calculated from = $\frac{\text{FBW}-\text{IBW}}{\text{IBW}} \times 100$,

where, FBW = final body weight
IBW = initial body weight

Evaluation of erythrocyte osmotic fragility

Blood samples were collected from each rat into well labeled heparinized sample bottles. Blood was analyzed for erythrocyte osmotic fragility using the method described by Faulkner & King (1970) and modified by Oyewale *et al.* (2011). The blood (0.2 ml) was pipetted into a set of test tubes

containing graded phosphate buffer saline solution (pH 7.4), and thereafter carefully mixed and incubated for 30 minutes at room temperature (25 ± 2 °C). The test tubes were centrifuged at 3000 rpm for 10 minutes using a bench centrifuge (model Anxiom Medical LTD, UK). The supernatant carefully withdrawn into a glass cuvette and the absorbance read colorimetrically using UV/VIS spectrophotometer (UV 752 PEC Medical USA) at a wavelength of 540 nm. The percentage haemolysis for each sample was calculated using the formula below by Faulkner & King (1970):

$$\% \text{ haemolysis} = \frac{\text{optical density of test solution} \times 100}{\text{optical density of standard solution}}$$

Statistical analysis

Results were expressed as mean \pm standard deviation (Mean \pm SD) and subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test to compare differences between the means obtained from the control and tested rats, using GraphPad Prism version 5.3 for windows from GraphPad Software, San Diego, CA, USA. Differences were considered significant at $P < 0.05$.

Results

Changes in cardiovascular function

Result of the ECG recording as seen in Tables 1 and 2 showed a significant increase ($p < 0.05$) in heart rate of the rats exposed to petrol only (577.00 beats/min) compared to control (331.80 beats/ min), the Moringa group (292.4 beats/min), captopril (350.2 beats/min) and candesartan cilexetil (279.0 beats/min) groups. From Figures 1-5 the QRS complex was narrow significantly $p < 0.05$ in the petrol only group compared to control, *Moringa oleifera*, captopril and the candesartan cilexetil group. However there was no significant difference $p > 0.05$ in the QRS complex of the control and the pre-treated groups (Moringa, captopril and candesartan groups) as in Figures 1-5.

Table 1: Recording showing heart rate (bpm) in control, rats exposed to petrol vapour and the pre-treated groups

Group	Mean	SD	N
Control	331.8	8.29	5
Petrol only	577.0	24.47	5
Moringa+petrol	292.4	22.03	5
Captopril+petrol	350.2	22.15	5
Candesartan+petrol	279.0	10.19	5

Ameliorative effects of Moringa oleifera, captopril and candesartan cilexetil on erythrocyte osmotic fragility following exposure to petrol vapour by Wistar rats

The result in Figure 6 showed significant haemolysis in the petrol only group at 0.08g/dl and 0.05g/dl of the phosphate buffered saline (NaCl). From 0.5 g/dl

of NaCl the haemolysis increased upwardly until it became 100% at 0.1 g/dl. There was significant decrease ($p < 0.05$) in haemolysis of the *Moringa oleifera*, captopril and candesartan cilexetil treated groups when compared with the petrol only group. On the other hand there was no significant difference ($p > 0.05$) in the haemolysis seen in

Table 2: ECG Recording showing QRS(ms) in control, rats exposed to petrol vapour and the pre-treated groups

	Mean	SD	N
Control	16.5	2.0	5
Petrol only	12.1	0.71	5
Moringa + petrol	17.0	0.89	5
Captopril + petrol	19.4	1.14	5
Candesartan + petrol	17.0	0.00	5

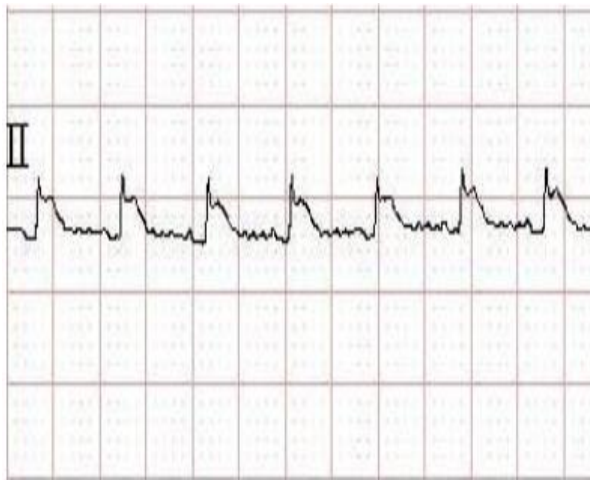


Figure 1: ECG recording of rats showing normal P, QRS and T



Figure 2: ECG recording of rats groups that were exposed to petrol vapour only showing very narrow QRS complex, longer R and no P

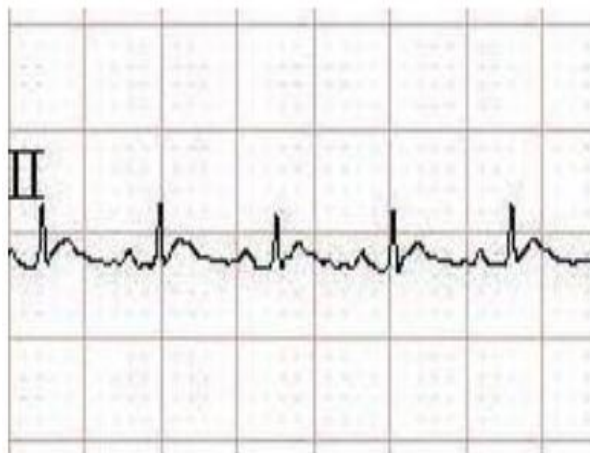


Figure 3: ECG recording of the rat groups that were pretreated with *Moringa oleifera* before exposure to petrol vapour showing wider QRS complex than the petrol group

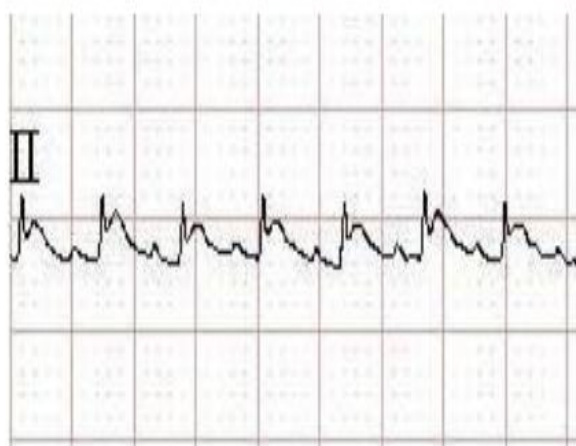


Figure 4: ECG recording of rat groups that were pretreated with captopril before exposure to petrol vapour showing wider QRS complex, and presence of P

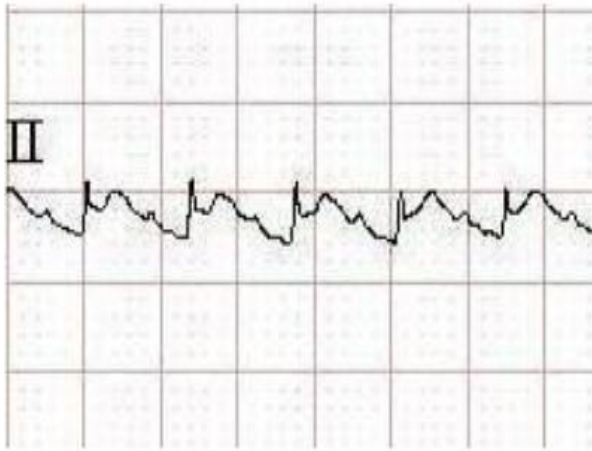


Figure 5: ECG recording of rat groups that were pretreated with candesartan before exposure to petrol vapour showing wider QRS complex, shorter R and P

Moringa oleifera, candesartan cilexetil and control groups at 0.3, 0.2 and 0.1g/dl NaCl. The ameliorative ability of *Moringa oleifera* was comparable with that of candesartan cilexetil which is an AT II receptor blocker.

Figure 7 showed that there was steady increase in percentage weight gain (PWG) from week 1 to the 5th week. However, there was significant reduction in the rate of weight gain in the petrol only group compared with control and the pretreated groups (*Moringa*, captopril and candesartan). This suggested that exposure to petrol vapours significantly alters body weight gain. Moreover, there was no significant difference ($p > 0.01$) in PWG of the *Moringa oleifera*, captopril, the candesartan treated groups as well as control. This showed that the percentage weight loss has been ameliorated in the pretreated groups. However, no significant difference ($p > 0.05$) was observed in pre-treated (*Moringa*, captopril and candesartan) and the control groups. (*Moringa*, captopril and candesartan).

Discussion

No mortality was recorded in the study, indicating That exposure to petrol vapour may not be associated with mortality. This study showed that inhalation of petrol vapour 10 minutes every day for eight weeks caused a very significantly reduced rate of percentage weight gain (PWG) compared with control (unexposed group) and the groups pretreated before exposure. The mean weekly PWG of exposure to petrol in other animals and human may occasionally be associated with severe and life threatening weight loss, as encountered

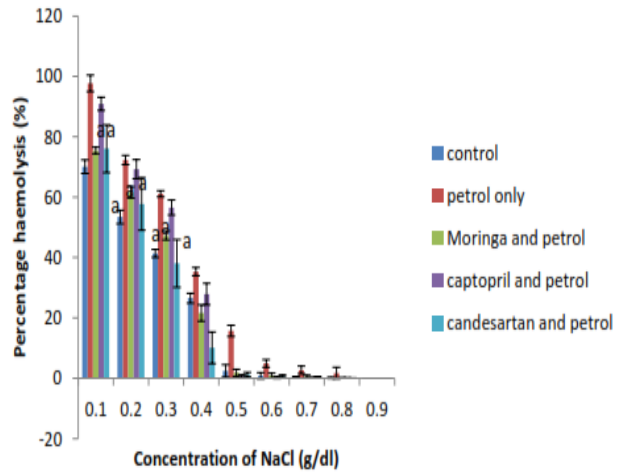


Figure 6: Percentage erythrocyte Osmotic fragility in rats exposed to petrol vapour and those pretreated with *Moringa oleifera* extract (40mg/kg), captopril(25mg/kg) and candesartan cilexetil (16mg/kg)as well as the control

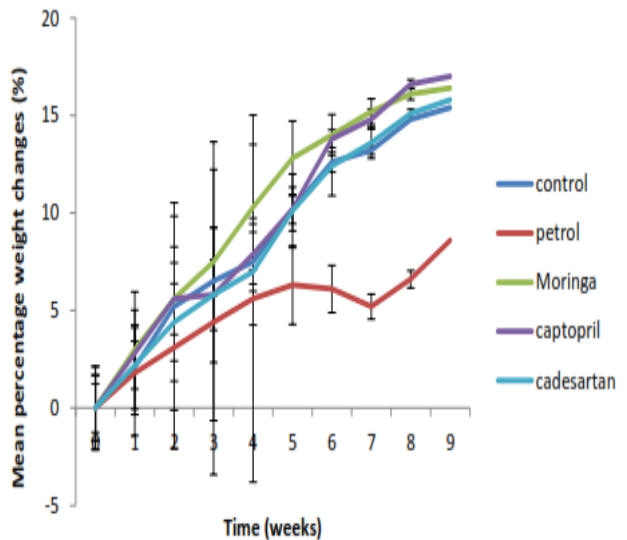


Figure: 7 shows the effect of petrol vapour and pre-treatment on the weekly percentage weight gain of Wistar rats

in this study. This is in consistence with previous study by Uboh *et al.* (2010); Abubakar *et al.* (2015). Some studies have demonstrated that benzene which is a major component of petrol metabolites are capable of covalent interaction with cellular macromolecules, such as DNA, as well as protein inhibition and RNA synthesis. This could result in reduced PWG. The other possible mechanism could be due to the ability of benzene metabolites to induce oxidative stress with a

consequent alteration in the DNA structure (Snyder & Hedli, 1996). However, *Moringa oleifera*, has been found to moderate this reduction in weight gain and growth rate. Exposure to petrol vapour 10 minutes every day for eight weeks increased fragility of the erythrocyte cell membrane. The component of petrol acted as xenobiotics which interfered with the redox status of red blood cells. The cytoplasm of the erythrocyte normally has no mitochondria and hence no oxidative phosphorylation occurs in it. The hydrocarbon component of the petrol interferes with the anaerobic glycolysis leading to alteration of the Embden Meyerhof pathway of glucose metabolism, which generates ATP and NADH. It also interfered with glucose-6-phosphate dehydrogenase activity that are required for membrane integrity (Mayes, 1983; Champe *et al.*, 2005; Ojo *et al.*, 2006). Although erythrocytes are well equipped with several biological mechanisms to defend against intracellular oxidative stress (Prasanthi *et al.*, 2005) they can be oxidatively damaged due to toxic chemicals and environmental pollutants (Uchendu *et al.*, 2014). Another view of the mechanism of fragility is that the hydrocarbon component also interfered with the membrane sodium pump mechanism that maintains a low level of sodium ion concentration inside the cell. This interference made the membrane to become very fragile and readily disrupted by osmotic changes. *Moringa oleifera* seemed to have protected the membrane integrity of the erythrocytes thereby stabilizing the cells and made them osmotically resistant to the redox effect of petrol. So also was captopril and candesartan but there is no significant difference in the amelioration by *Moringa oleifera* and candesartan cilexetil (Azeez *et al.*, 2015).

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- The ECG recording showed that exposure to petrol vapour had negatively affected atria contraction and reduce the time of ventricular contraction and ventricular filling. This effect was modulated by *Moringa oleifera* extract, captopril, and candesartan cilexetil. The hydrocarbon component of petrol might have inactivated the sodium current and thus interfere with proper firing of the pace setter cells and the myocytes. *Moringa oleifera* contains alkaloids that have some cardiovascular property and as such able to revert the damage done by petroleum hydrocarbon. *Moringa oleifera* appeared to have carried out the ameliorative effect as an ATII receptor blocker considering the similarity in ameliorative action to that of candesartan cilexetil.
- In conclusion, human and animals are exposed to petroleum product regularly in our environment, losing red blood cells faster than normal, losing weight unnoticed and having cardiovascular challenges as found out in this research are imminent. From the result it could be concluded that *Moringa oleifera* leaf extract ameliorated erythrocyte osmotic fragility in Wistar rats exposed to petrol vapour in similar manner with candesartan cilexetil which is an Angiotensin II receptor blocker. *Moringa oleifera* could be described as Angiotensin II receptor blocker. *Moringa oleifera* is locally planted and cheap to get compared with other cardiovascular drugs around. *Moringa oleifera* is easily available remedy when food animals and the animals raised extensively are exposed to water contaminated with petroleum products. Azeez *et al.* (2012) and Azeez *et al.* 2015 suspected exposure to petroleum products to be one of the major causes of cardiovascular derangement and sudden death in our environment.
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