

Research Article

## Changes in erythrocyte membrane properties following exposure to premium motor spirit (petrol vapour) and modulatory effects of *moringa oleifera* and vitamin c in wistar rats

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**Keywords:**

Erythrocyte osmotic  
Fragility, Erythrocyte  
Sedimentation Rate,  
*Moringa oleifera*,  
*Ascorbic acid*

**ABSTRACT**

**Background:** The present study was carried out to evaluate the effect of petrol vapour on properties of Erythrocyte: osmotic fragility (EOF), erythrocyte sedimentation rate (ESR) and red cell indices as well as the comparative beneficial effect of *Moringa oleifera* and *Ascorbic acid* (vitamin C) on the values. **Methods:** Forty adult male Wistar rats randomly divided to four with ten rats in a group. Group 1: control, was given water and feed *ad-libitum* without any treatment. Group 2 (petrol only) were exposed to petrol vapour only without any pretreatment. Group 3 (Vit C group) pretreated with *Ascorbic acid* (100mg/kg) before exposure to petrol vapour; and group 4 were (*Moringa* group) were pretreated with *Moringa oleifera* extract (40mg/kg) before exposure to petrol vapour. The exposure to petrol vapour was done 10 minutes every day for eight weeks. Exposure to petrol fume was generated by using human compressor nebulizer adopted for rats and connected to fume chamber where the rats were kept. The pretreatment was effected by gavage using the oral cannula, 30 minutes before exposure. At the end of the exposure, period 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 to evaluate erythrocyte osmotic fragility (EOF). RBC, PCV and Hb were analyzed from heparinised blood sample. The red cell indices were thereafter calculated. 1.2ml of blood kept inside sodium oxalate bottle was suspended in the Westergren tube for one hour. **Results:** There was significant increase in the fragility of the group exposed to petrol vapour only compared with control and the pre-treated groups. There was zero sedimentation with little or no rouleaux of the erythrocyte in the petrol only group compared with control. There was sedimentation with rouleaux formation in the control, *Moringa* and vitamin C groups, the difference was however not significant ( $p > 0.05$ ). **Conclusion:** The study concluded that Erythrocytes membrane became more fragile on exposure to petrol vapour, The degree of amelioration shown by pretreatment with *Moringa oleifera* was significantly ( $p < 0.05$ ) higher than that of *Ascorbic acid*. *Moringa oleifera* was found to be more effective in protecting the erythrocyte properties following exposure to petrol vapour than vitamin C.

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**INTRODUCTION**

Premium motor spirit (Petrol) is widely used as fuel for automobile, electric generators, grinding machines. The liquid form is very volatile with many organic and inorganic constituent. When it evaporates the vapour

constitutes environmental pollution (Uboh et al, 2009). Some of the constituents of the petrol vapour (such as alkanes, benzenes tetraethyl lead and xylene) have been reported to be heamatotoxic to human and experimental animals (D'Azevedo et al 1996; Rothmans et al, 1996, Synda and Hedli 1996, Uboh et al, 2009). Exposure to petrol vapour had also been found to cause increase in blood pressure and heart rate, cardiotoxicity as well as baroreflex disability (Azeez et al, 2012, 2013, 2015). Growth depression,

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weight loss and hematotoxicity have been reported to be associated with exposure to gasoline vapour (Uboh et al, 2008; Abubakar et al, 2014). In our environment, it is a regular event to be exposed to petrol through self refueling using hose and kegs at petrol stations, on the road at home and at our work place. Several studies have reported that significant exposure to benzene occurs during self-refueling of cars at service stations (Egeghy et al 2000; Pandey et al, 2008). Treatment of petrochemical station workers with tablets containing antioxidants such as vitamins, micro-elements, and flavonoids was shown to improve the oxidative stress associated with gasoline vapor exposure (Georgieva *et al.*, 2002). Several experimental animal studies have also demonstrated that supplementation with antioxidants produced reduction in toxic effects of petrol vapour exposure. Treatment of rats exposed to petrol vapours with both water and lipid soluble vitamins (A, E, and C) have been shown to reduce the toxicities associated with the petrol vapour exposures. Administration of vitamin A ameliorated the toxic effects of gasoline exposure on Hb, PCV, RBC, and weight of both male and female rats exposed to gasoline (Uboh *et al.*, 2008). Honey has also been used to prevent and or promote recovery from toxicity effects of inhalation of gasoline constituents on haematological indices and bone marrow (Abubakar et al, 2015).

Therefore, this research is looking at ways by which the populace can be helped with Pharmacological intervention through supplementation with locally available remedy to ameliorate the life threatening toxic effect that is reducing life expectancy of the populace. *Moringa* (*Moringa oleifera* Lam.) is native to the Indian subcontinent and has become naturalized in the tropical and subtropical areas around the world. "Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and the protein quality of Moringa leaves rivals that of milk and eggs. In fact, the nutritional properties of Moringa are now so well known that there seems to be little doubt of the substantial health benefit to be realized by consumption of Moringa leaf powder in situations where starvation is imminent. (Fuglie, 1999).

L-ascorbic acid ( $C_6H_8O_6$ ) is the trivial name of Vitamin C. Most plants and animals synthesize ascorbic acid from D-glucose or D-galactose. A majority of animals produce relatively high levels of ascorbic acid from glucose in liver. (Naidu, 2003); however, guinea pigs, fruit eating bats, apes and humans cannot synthesize ascorbic acid due to the absence of the enzyme L-gulonolactone oxidase. In humans, ascorbic acid has to be supplemented through food and or as tablets.

Ascorbic acid is a potent antioxidant with increasingly diverse uses in health promotion and disease prevention. It is a versatile, water soluble donor antioxidant which protects low density lipoproteins from oxidation, reduces harmful oxidants in the stomach and promotes iron absorption (Carr & Frei, 1999a; May, 1999; Padayatty *et al.*, 2003).

Osmotic fragility index is a measure of the resistance of red blood cells to lysis by osmotic stress (Oyewale et al, 2011; Oyewale and Ajibade 1990). The test is generally useful to ascertain the level of stability and functionality of plasma membrane (Krogmeier et al., 1993).

The erythrocyte sedimentation rate (ESR), also called a sedimentation rate or Westergren ESR, is the rate at which red blood cells sediment in a period of one hour (reported in mm/hr). The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called 'rouleaux,' which settle faster, due to their increased density. The ESR is increased in inflammation, pregnancy, anemia, autoimmune disorders and infections, The ESR is decreased in polycythemia, hyperviscosity, sickle cell anemia, leukemia, low plasma protein (due to liver or kidney disease) and congestive heart failure (Westergren, 1957). In 1967 it was confirmed that ESR values tend to rise with age and to be generally higher in women (Böttiger and Svedberg, 1967). Values are increased in states of anemia (Kanfer and Nicol, 1997). and in black populations (Gillum, 1993). Uboh and colleagues has showed that inhalational exposure to petrol vapours caused decreased packed cell volume (PCV), Hemoglobin (Hb), and red blood cells (RBC) count, as well as increased white blood cells (WBC) counts in Wister rats (Uboh et al., 2008).

Red cell indices MCV, MCH and MCHC are calculated from hemoglobin, hematocrit, and red blood cell count, Red cell indices are valuable in the morphologic classification of anaemias (Bessman et al, 1983). Mean corpuscular volume (MCV) is the average volume of red cells in a specimen is expressed as femtoliters ( $10^{-15}$ ; fl) or as cubic microns ( $\mu m^3$ ). Low MCV indicates microcytic (small average RBC size), normal MCV indicates normocytic (normal average RBC size), and high MCV indicates macrocytic (large average RBC size) (Ravi Sarma, 1990). (Mean cell hemoglobin concentration (MCHC) refers to the average concentration of hemoglobin in the RBCs contained within the sample When Erythrocytes

contain normal amount of hemoglobin are called normochromic (normal MCHC units is g/dL), abnormally low MCHC are called hypochromic and abnormally high, hyperchromic. Mean cell hemoglobin (MCH) refers to the average weight of hemoglobin in the RBCs in the sample (units is picograms (pg/cell); There are no hyperchromic anemias (Ravi Sarma, 1990).

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 is aimed at studying the comparative beneficial effect of *Moringa oleifera* and *Ascorbic acid* (vit c) on the toxicologic effect of petrol vapour. It also aimed at elucidating the mechanism mediating the anaemia reported by previous researchers.

## MATERIALS AND METHODS

### *Animals*

Twenty adult male Wister rats (120- 200 g by weight) were purchased from the animal house of the Faculty of Veterinary Medicine, University of Ilorin, Ilorin. The rats were maintained on a standard and balanced rat's diet and were allowed free access to food and water *ad-libitum*. The rats were allowed to adapt to the room conditions for seven days before commencement of the experiment. These rats were kept in a standard cage with bedding in a well-ventilated animal room. The rats were randomly grouped to 4 with 5 rats in a group. 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 (NIH publication No 85-23, revised 1996).

### *Moringa leaves authentication and preparation*

The *Moringa* leaves were collected from the University farm and authenticated by the Department of Plant Biology, University of Ilorin with herbarium number-UIH-001/1011. The leaves were harvested green, air-dried under shade and milled into powder using the method of Busani Moyo et al, 2011. They were stored in well-dried black plastic containers inside the storeroom at room temperature of 25°C. The powder was made into aqueous extract by using suxcler extractor, concentrated on rotary evaporator (Buchi, Flavil, Switzerland) at 40°C, then dried and kept at room temperature till used for the assay. The extract was giving to the rats using the oral cannula at the rate of 40 mg/kg.

### *Petrol vapour generation and exposure*

The petrol was purchased from Total petrol station close to the 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 only) exposed to petrol vapour only. Group 3(vit c group) were pretreated with vit c (100mg/kg) while Group 4 (*Moringa* group) were pretreated with *Moringa oleifera* extract (40mg/kg), All the pretreatment were given using the oral cannula 30 minutes before each group were placed in the fume chamber. The fume chamber was 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 the nebulizer was 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 experimental room. This was done for all the exposed groups except control. The room condition was monitored and maintained at temperature at  $25 \pm 2$  °C. The average dosage exposure was  $0.005\text{cm}^3/\text{min}/\text{rat}$ .

### *Evaluation of erythrocyte osmotic fragility(EOF)*

Freshly collected blood from each rat into heparinized sample bottles were analyzed for erythrocyte osmotic fragility using the method described by Faulkner and King (1970) and modified by Oyewale (2011). The blood was pipetted into a set of test tubes containing phosphate buffer saline solution graded as follows- 0.1, 0.2, 0.3...0.9 g/dl of NaCl (pH 7.4), and thereafter carefully mixed and incubated for 30 min at room temperature ( $25\pm 2^\circ\text{C}$ ). The test tubes were centrifuged at 3000 rpm for 10 min using a centrifuge model IEC HN-SII (Damon/IEC Division, UK). The supernatant carefully withdrawn into a glass cuvette and the absorbance of the supernatant read colorimetrically using Spectrometer 20 (Bausch and Lomb, USA) at a wavelength of 540 nm. The percentage haemolysis for each sample was calculated using the formula below by Faulkner and King (1970):

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

### *Erythrocyte sedimentation Rate (ESR) Determination*

The ESR determination was done using the Westergren method; 1.6 ml of a mixture of blood was mixed with 0.4 mL of 3.8% sodium citrate solution in a graduated transparent tube with an internal diameter of 2.5 mm. The zero mark of the tube was at the upper extremity, 200 mm from pipette tip. The tube was then placed in a vertical position for one hour. A reading was made of the height of the plasma column that formed at the upper part of the pipette, expressed as millimeters per hour (mm/h) using the method of Santos et al, (2000); Kalayanarooj and Nimmannitya, 1989).

**Determination of red blood indices**

Red blood indices was calculated from RBC, PCV and Hb measured according to Uboh et al, 2008.

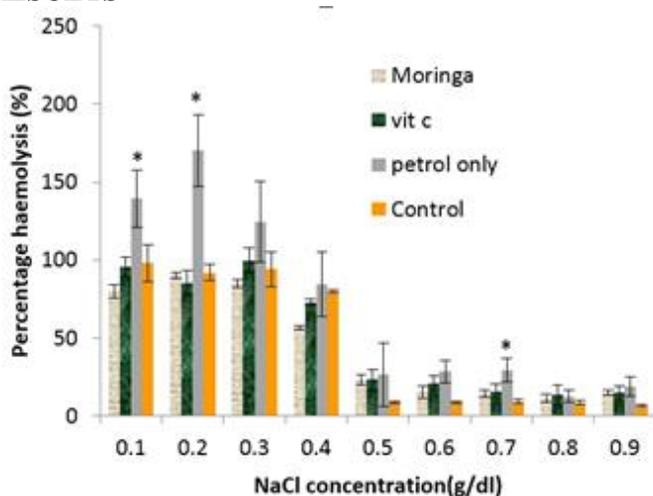
$$MCV = \frac{PCV(\%)}{RBC (10^6/ml)} \quad \text{femtoliters } (10^{-15}; \text{fl}) \text{ or } \mu\text{m}^3$$

$$MCH = \frac{Hb (g/dl)}{RBC (10^6/ml)} \quad \text{Pg/cell}$$

**Statistical analysis**

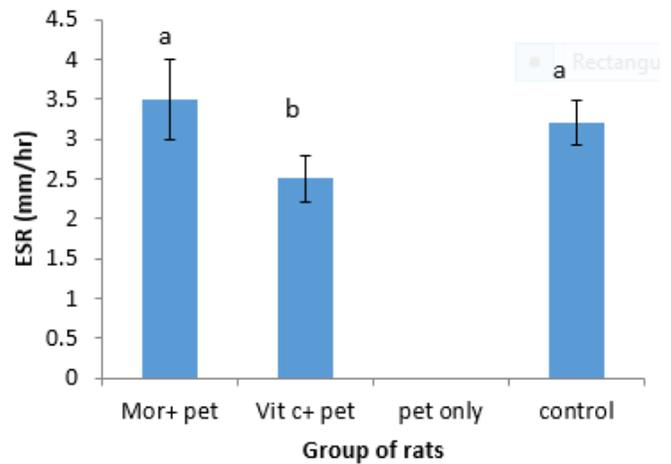
Results were expressed as mean ± standard error of the mean (Mean ± SEM) and subjected to one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison *post-hoc* test to compare differences between the means obtained from the control and tested rats, using GraphPad Prism version 5.3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

**RESULTS**



**Fig.1:** EOF in Wistar Rats exposed to petrol vapour for 10 minutes every day for eight weeks (petrol only, vit c+ petrol and Moringa + petrol) and control. n=5 \* = significant difference.

The petrol only group showed haemolysis of about 25-30% at 0.9g/dl of the buffered solution which is isotonic. The haemolysis was significantly higher compared with control, but not with the pretreated groups. This can be deduced from the fact that the pretreated groups were also exposed to the petrol vapour. The result at 0.4g/dl already started showing the ameliorative effect of the *Moringa oleifera* group which was significantly reduced compared with that of vit c. The reduction in the vit c group was statistically insignificant when compared with the petrol only group.



**Fig. 2:** Effect of inhaling petrol vapour on Erythrocyte sedimentation rate (ESR) in different rat groups (control, petrol only, Moringa+petrol and vit c+ petrol). Bars carrying different letters are significantly different; n=5, there is significant difference ( $p < 0.05$ ) between ESR of Moringa group and vit c; and no significant difference ( $p > 0.05$ ) between ESR of Moringa and control. There is significant difference between ESR of Moringa and vit c groups

From the above figure, there was no rouleaux formation and no sedimentation in the petrol only group over the period of one hour. The sedimentation rate in Moringa group was insignificantly higher than that of vit c and control groups in one hour.

**DISCUSSION**

This study showed that inhalation of petrol vapour 10 minutes every day for eight weeks caused increased fragility of the erythrocyte to normotonic solution (0.9) and hypotonic solutions. The increased EOF observed indicates the ability of the petroleum hydrocarbon to compromise the integrity of the erythrocyte membrane increased oxidative damage to the erythrocyte membrane (Wagner et al, 1986) which may result in anemia. Anemia has been recorded by other workers (Uboh et al, 2005). This anemia recorded by these authors was as a result of haemolysis caused by increased EOF by the hydrocarbon component of the petrol. The hydrocarbon component acted as xenobiotics on the lipid bilayer with a consequent increase in the membrane instability leading to osmotic fragility. There may also be membrane fluidity and ultimate destruction of the bilayer integrity of erythrocyte membrane (Girotti, 1985). There was alteration of the Na-K pump mechanism that maintains low level of sodium ion concentration inside the cell by the hydrocarbon component of petrol. The lipid peroxidative alteration in the structural and functional c

**Table 1:** Total Red Blood Cell counts (RBC), Packed cell volume (PCV), Haemoglobin content (Hb) and red cell indices responses (Mean ± SEM) of male Wister rats in control, petrol only and pretreated groups following exposure to petrol vapour

Groups	Red cell indices					
	RBC(106 µL)	PCV (%)	Hb (g/dL)	MCV(fL)	MCH(pg)	MCHC (g dL-1)
Control	7.25±0.41	43.24±0.97	14.22±0.32	79.52±0.80	20.05±0.42	30.28±0.56
Petrol only	5.96±0.36*	39.81±0.26*	11.46±0.21*	87.13±1.42*	18.20±0.51*	28.01±0.43*
Vit c+ petrol	7.22±0.21	40.94±0.76	12.83±0.34	76.10±1.22*	19.62±0.35	29.84±0.42
Moringa + petrol	7.31±0.44	41.91±0.35	14.32±0.56	76.45±0.61*	19.94± 0.33	29.96±0.38

n=5, \* significant difference n=5, \* significant increase, † Significant decrease RBC, PCV and Hb were significantly reduced in the petrol only group compared with control and other groups. The MCV was significantly increased while MCH and MCHC were reduced significantly compared with control and other groups. From this result, we suspected macrocytic hypochromic anemia

components of the erythrocyte membrane may have caused perturbations in the membrane integrity resulting in increased EOF. In this study pretreatment with vit c and *Moringa oleifera* ameliorated the EOF. It has been previously reported that administration of vitamins A C, and E ameliorated the toxicity effects of petrol exposure on haematological parameters (Uboh et al, 2008). L-ascorbic acid (vit c) is a co-factor for hydroxylases and monooxygenase enzymes involved in the synthesis of collagen, carnitine and neurotransmitters. Ascorbic acid plays an important role in the maintenance of collagen which represents about one third of the total body protein. This property of ascorbic acid was used to repair the disrupted protein component of the erythrocyte membrane (Naidu 2003). According to Fugilie, 1999; *Moringa oleifera* contains more Vitamin C than oranges, and more potassium than bananas,” and that the protein quality of *Moringa* leaves rivals that of milk and eggs. *Moringa oleifera* and vit c were able to ameliorate the structural and functional alteration in the erythrocyte membrane. However *Moringa oleifera* showed statistically higher amelioration than that of vit c.

#### Erythrocyte Sedimentation Rate

ESR was zero in the petrol only group. The hydrocarbon component of petrol altered the negative charge on the erythrocyte membrane (zeta potential) which facilitate its binding together in the presence of fibrinogen. *In vitro* studies have shown that after isolation of erythrocytes from healthy volunteers, the ESR increased when albumin was added to a mixture of fibrinogen and immunoglobulin. In clinical situations in which the albumin is normal, the ESR will only be increased by fibrinogen (Reinhart and Nagy, 1995). *Moringa oleifera* and vit c ameliorated the sedimentation as in figure 2 above, although there

amelioration in the *Moringa* group was significantly higher than that of vit c when compared with control. Addition of *Moringa oleifera* and vit c differently. Previous workers have found reduced total protein and fibrinogen in plasma of rats expose to petrol vapour (Azeez et al, 2013). This suggested, the non-production of rouleux and inability of erythrocyte to sediment.

#### The red cell indices

This study showed that MCV increased significantly (<0.05), in the petrol only group compared with control and the groups pretreated with *Moringa oleifera* and vit c. Increased MCV is an indication of macrocytic anaemia characterized by large red cell often with insufficient haemoglobin per cell and increased red cell membrane surface area to finally result in a total blood hemoglobin concentration that is less than normal (i.e., anemia). Many researchers have found anaemia in association with exposure to petroleum product in experimental animals , auto mechanic and petrol Attendants (Uboh et al, 2005; Ajugwo et al, 2014; Okonkwo et al, 2016). This study is in line with the findings of Okoro et al, (2006). The group found significant(p<0.001) reduction in RBC, PCV, Hb, MCH, MCHC. However MCV was also significantly reduced in their finding. Non-megaloblastic macrocytic hypochromic anaemia was suspected in our findings. The anaemia that had been reported by previous researchers were not tracked down to the specific type. Abubakar et al, (2014) observed high percentage of abnormal megakaryocytes in the marrow of rat exposed petrol vapour. According to Abubakar et al, (2014), the reasonably large size of the megakaryocytes helped considerably in identifying the nuclear abnormalities associated with the inhalation of constituents of petrol in the exposed rat models. The significant reduction in peripheral RBC count reported was due to adverse

changes in the bone marrow, although no significant alteration was observed in the cell lineage and the myeloid/ erythroid ratio. Future studies is recommended to further look into alteration in cell lineage and features of the peripheral RBC in relation the anaemia.

## CONCLUSION

The populace is continuously exposed to petrol vapour (at home, at work on the road and more) keeping the harmful effects without knowing the significance. *Moringa oleifera* is locally produced and has great prophylactic potentials against the adverse effects of continuous exposure to petrol vapour.

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