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Possible *Moringa oleifera* Amelioration of Atrial Fibrillation Induced by exposure to petrol Vapour in Wistar Rats

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Abstract

The present study was carried out to investigate the actions of *Moringa oleifera* aqeous extract in ameliorating the effect of petrol vapour on atrial functions of Winstar rats to that of standard anti hypertensive drugs (captopril and candesartan cilexetil). Twenty five adult male Winstar rats were divided into five groups with five rats in each group. Animals in group 1 (control) were not exposed to petrol fume. Group 2 was exposed to petrol vapour only. Groups 3, 4,and 5 were pretreated with aqueous extract of *Moringa oleifera* (40mg/kg), captopril (25 mg/kg) and *candesartan cilexetil* (16mg/kg) respectively before exposure to petrol vapour for10 minutes daily for eight weeks. Exposure to petrol vapour was generated by using human compressor nebulizer adapted for rats and connected to fume chamber where the rats were kept. The pretreatment were administered by gavage using the oral cannula. At the end of the experimental period, rats were anesthetized with 1% chloralose and 25% urethane intraperitoneally; the electrocardiography was done using EDAN 10. The study showed that exposure to petrol vapour resulted in atrial fibrillation and this was ameliorated by ageous *Moringa oleifera* extract. The result obtained was comparable to that of candesartan. The study concluded that the since the action of *Moringa oleifera* resembled that of candesartan, *Moriga oleifera* is suggested to be have an Angiotensin II receptor blocker effects.

Introduction

It has been reported that oil drillers, refinery workers petro-chemical workers, refuel station attendants and motor mechanics suffer greater risk of chronic exposure to petrol pollutants (Wong, 1996). Increased blood pressure, heart rate and baroreflex sensitivity have been reported in experimental animals (Azeez *et al.,* 2012) following exposure to petrol, kerosene and diesel. Emeji *et al.,* (2015) in their work found a significant increase electrolyte concentration in fuel pump attendant. Humans and animals are faced with hazard of the effect of the hydrocarbon on almost all the body systems including cardiovascular functions. There is erratic problem of petroleum products distribution in Nigeria which results to the use of different unconventional mode of filling vehicle tanks with use of funnels and hoses. Vandalization, black marketing, storing fuel privately are other challenges. Deliberate inhalation of hydrocarbons as a form of recreational drug use, has become a significant health issue affecting children and adolescents (Azeez *et al.,* 2012). The large number of motor vehicles exhaust typically

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found in our urban cities can be a source of many hydrocarbon chemicals, many of these substances are a mixture of hydrocarbons (Murphy *et al.,* 2003).

At every beat, the heart is depolarized to trigger its contraction. This electrical activity transmitted throughout the body, can be picked up at the skin and displayed graphically. The Sinoatrial node (SAN) is the pacemaker of the heart. The wave of depolarization spreads across the atrioventricular node (AVN). The impulse is displayed briefly at the AVN for atrial contraction to be completed. Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. Atrial fibrillation (AF) contributes to significant cardiovascular morbidity and mortality (Benjamin *et al.,* 1998).

Despite the availability of numerous therapeutic agents, the available treatments have significant limitations (Wyse *et al.,* 2002; Natel and Opie, 2006) and AF continues to be a clinical challenge. Many episodes of AF are asymptomatic or minimally symptomatic. The basic mechanisms underlying AF can be seen by Allessie *et al.,* (1977); Nishida *et al.,* (2009) in their separate studies as follows: Rapid ectopic foci arise by abnormal automaticity originating in regions other than the sinus node, or as a result of early after-depolarizations (EADs) or delayed after-depolarizations (DADs). EADs involve the reactivation of L-type Ca^{2+} channels during prolonged repolarization, whereas DADs appear when Ca^{2+} is released from the sarcoplasmic reticulum (SR) during diastole. Diastolic Ca^{2+} rises activate the Na^{+}–Ca^{2+} exchanger (NCX), which carries three Na^{+} ions into the cell in exchange for each Ca^{2+} transported out, causing net inward movement of one positively charged ion per cycle and depolarizing the cell. Atrial stretch (volume overload) makes the atrium to be vulnerable to fibrillation In a cohort study by Magnani *et al.,* (2016) and Roberts *et al.,* (2016): Black individuals were found to be less likely to have atrial fibrillation than their white counterparts, they are rather at higher risk of adverse cardiovascular outcomes (stoke hypertension and heart failure). Roberts *et al.,* (2016) in their findings suggested that some heritability related to multiple low-level genetic variants that cannot be uncovered by current approaches or by gene-gene and/or gene-environment interactions are major cause of AF in the black. The traveling electrical impulse of a premature atrial beat can encounter areas of refractoriness, return to its origin in a retrograde way, and through reentry initiate and maintain afrial fibrillation.

The adverse health implications of exposure to petroleum products (crude oil) remain a public health concern since these compounds form a basic part of our lives. Human exposure to the hydrocarbons contained in these products can occur through ingestion of contaminated food, drinking contaminated water and soil residues (Azeez *et al.,* 2012).

Moringa oleifera leaves have been reported to be a valuable source of both macro- and micronutrients, rich source of β-carotene, protein, vitamin C, calcium, and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods (Dillard and German, 2000). Fruit (pod)/drum sticks and leaves have been used to combat malnutrition, especially among infants and nursing mothers for enhancing milk production (Dillard and German, 2000; Estrella) and also regulate thyroid hormone imbalance (Pal *et al.*, 1995; Tahiliani and Kar, 2000). Candesartan cilexetil is an angiotensin II receptor blocker (ARBs) used to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. It works by relaxing blood vessels so blood can flow more easily. In a study carried out by Julius *et al.,* (2006), to see if candesartan can prevent hypertension, candesartan significantly reduced the risk of hypertension, by more than 15%.

Captopril is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of congestive heart failure. Captopril was the first ACE inhibitor developed and was considered a breakthrough both because of its novel mechanism of action and also

because of the revolutionary development process. Used to treat hypertension, cardiac conditions such as congestive heart failure and after myocardial infarction 3). Preservation of kidney function in diabetic nephropathy, it is marketed by Bristol-Myers Squibb under the trade name Capoten (Bryan and Jenny, 2009). In our previous work, it has been found that exposure to petrol vapour caused changes in cardiovascular function and erythrocyte osmotic fragility which was ameliorated by extract of *Moringa oleifera.* (Azeez *et al.,* 2017).

This work was therefore aimed at comparing the ameliorative effects of *Moringa oleifera* aqueous extract on the atrial dysfunction associated with exposure to petrol vapour with that of captopril and candesartan cilexetil that are used to treat hypertension.

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 fed on standard rat chow and tap water *ad' libitum.* They were acclimatized for 7 days before the experimention 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 and National research Council (1996) Guide for the Care and Use of Laboratory Animals. 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 2 was exposed to petrol fume only, while groups 3, 4 and 5 were pre- treated with *Moringa oleifera* leaf extract, Captopril and Candesertan cilexetil respectively.

Preparation of Plant Material and other drugs

The leaves of *Moringa oleifera* Lam were collected from the University Teaching and Research Farm. It was identified and authenticated in the Department of Plant Biology, University of Ilorin with herbarium number- UIH-001/1011. The leaves were separated from their stems, air-dried under shade, without exposure to direct sunlight and thereafter grinded to powder. Aqueous extract was prepared by using Soxhlet extractor, concentrated on rotary evaporator (Buchi, Flavil, Switzerland) at 40°C, then dried and kept at room temperature till used for the assay. The captopril (25mg /kg) and candesartan cilexcetil (16mg /kg) were prepared by mixing with distilled water

Exposure to petrol vapour and dosage

The petrol was purchased from retail 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) 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 animals in each group was placed in the fume chamber. Human compressor nebulizer was used to convert liquid to vapour inhalant by human. This was adapted to convert the liquid petrol to vapour inside a 20 litre fume chamber 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 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 experimental room. This was done for all the exposed groups. The room condition was monitored and maintained at temperature at 28 ± 2°C. The average dosage exposure was 0.008cm^3/min/rat.

Measurement of Electrocardiography with EDAN 10

At the end of the eight weeks exposure to petrol vapour, EDAN 10 Veterinary electrocardiography (ECG) made by Ronseda Electronics CO, LTD China; was used to measure electrical activities of the heart. Each rat was anaesthesized with 1% chloralose and 25% urethane intraperitoneally; once the rat was anaesthesized, 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.

Statistical Analysis

Results were expressed as mean ± standard deviation 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 (www.graphpad.com).

Differences were considered significance at $P<0.05$.

Results

Table 1: ECG Recording showing Heart rate (bpm), PR (ms) and QRS (ms) the pre-treated groups (Moringa, candesartan and captopril) and control

	Heart Rate (BPM)	PR (ms)	QRS (ms)
Control	298±51.27	61± 6.29	19.0±4.06
Petrol only	497±10.69*	42± 18.77†	15.6±1.52†
Moringa/petrol	294±23.04a	58.4±20.27a	17.2±3.03a
Candesartan/petrol	286±29.65a	54.6±12.46a	16.6±0.89a
Captopril/petrol	328±19.04	71.0±17.92	19.8±0.84

* significant increase
† significant reduction. significant difference is at $P<0.05$

From Table 1, Heart rate was significantly high in the petrol only (497±10.69) group compared with control and other pretreated groups. PR interval was significantly reduced in the petrol only group compared with control (61 ± 6.29ms) and other pretreated groups. QRS complex in the petrol only group (15.6 ± 1.52ms) was significantly narrower compared with control and other pretreated groups. There was a significant decrease in the QT interval of the petrol only group compared with the control but the difference is not significant when compared with other groups

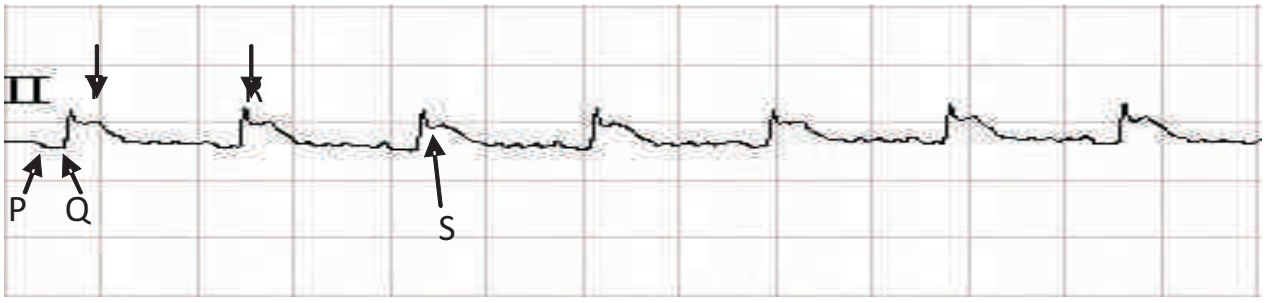

Figure 1: ECG result of control group showing normal P, QRS complex and T waves

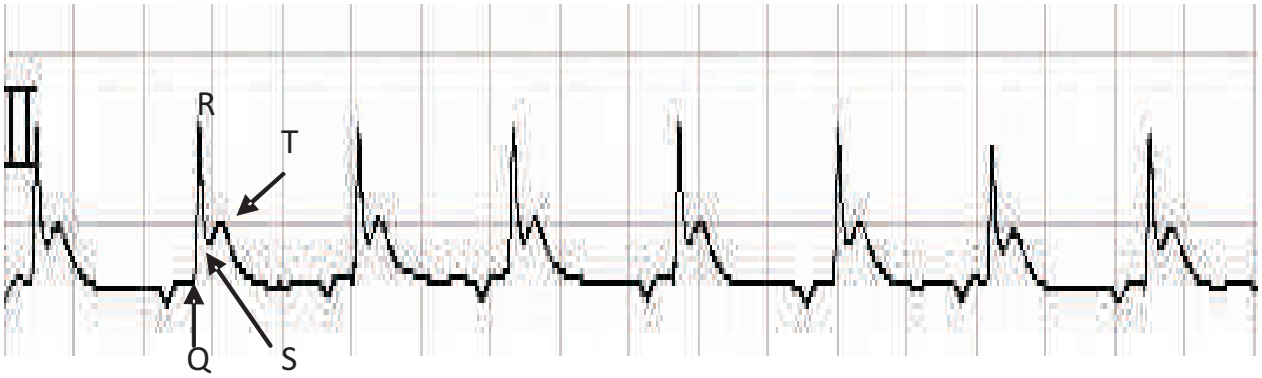

Figure 2: ECG result of petrol only group showing no P, longer R narrower QRS complex shorter QT. Irregular rhythm

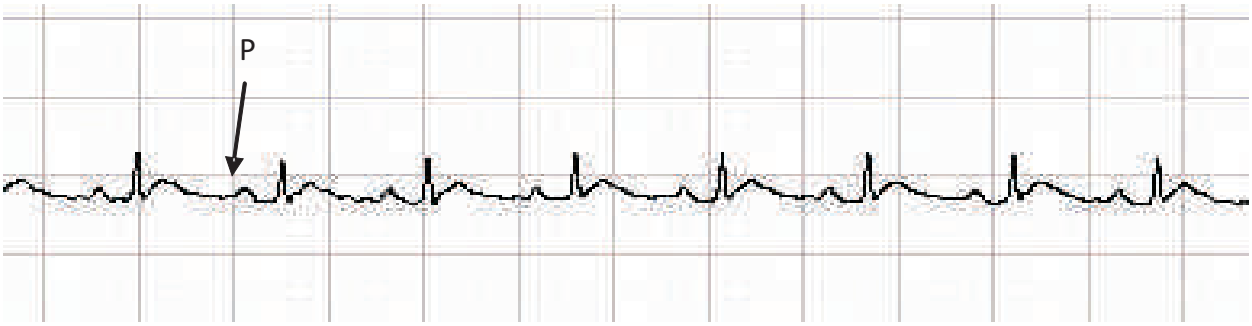

Figure 3: ECG result of Moringa/petrol group showing P, QRS and T with reappearance P wave

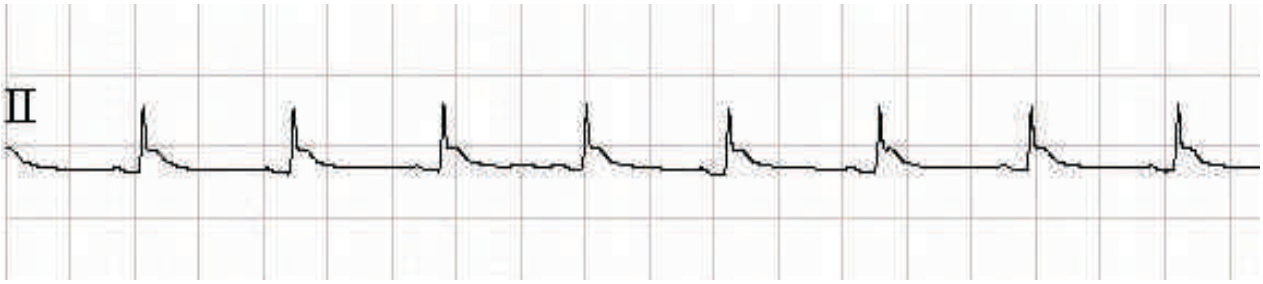

Figure 4: ECG result of Candesartan group showing very small P, QRS and T

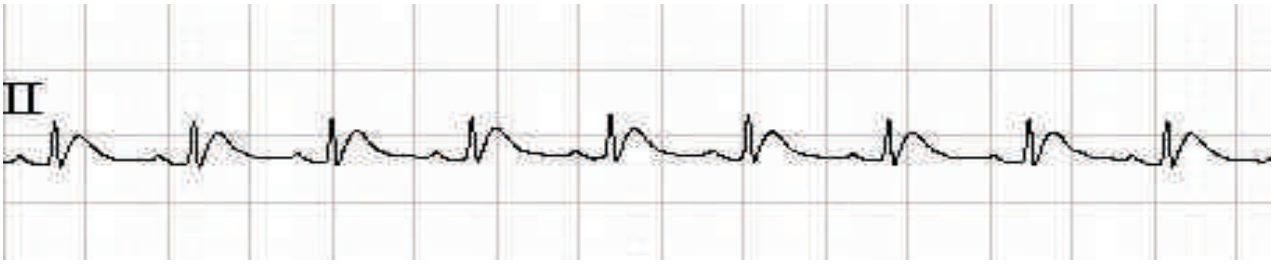

Figure 5: ECG result of Captopril group showing P, QRS and T

From figures 1-5, the ECG recording (ECG lead II) of group 2 (rats exposed to petrol only) showed no clearly detectable P-wave, very narrow and long QRS complexes and very high heart rate, when compared with control. The ECG recording in group 3-Candesartan cilexcetil (an AT II receptor blocker) was comparable with the recording of *Moringa oleifera* group (Azeez *et al*., 2017).

Discussion

Exposure to petrol vapour (Figure 2) resulted in disappearance of sinus rhythm (no P wave), the QRS complexes appear narrow with tall R wave. Irregular R-R intervals and the baseline appeared noisy which are major hallmark of atrial fibrillation. The concave ST elevation seen is a sign of pericarditis as explained by Ashley and Niebauer, (2004).

Pretreatment with candesartan resulted in restoring of the P wave with reduced PR interval. R-R rhythm became more regular and the concave elevation of T reduced as in the Moringa pretreated group. However the height of R reduced insignificantly compared with that of the petrol group.

Pretreatment with captopril restored the P, unable to reduce pericarditis as described by Ashley and Niebauer (2004) which is concave elevation of T.
Pretreatment with *Moringa oleifera* extracts appeared to have restored the P wave, reduce the noisy baseline and reduced the pericarditis. The increased height of R was also reduced; R-R rhythm became more regular. The significant reduction in the heart rate in Moringa group showed that Moringa could serve as rate- control as well as rhythm control for regulating the Atria fibrillation as a result of exposure to petrol vapour. In the work done by Wyse *et al*., (2002) rate-control drug was regarded as more life saving and effective in treating Atrial fibrillation. Stroke is probably the most serious direct clinical consequence of atrial fibrillation (Wolf *et al*., 1991, 1987, and 1987). The rates of ischemic stroke were low, at approximately 1 percent per year.

The possible mechanism for our observation of the following conditions in the rats exposed to petrol are very important to be noted

Significantly shorter duration of the QRS complex;

Abnormally high amplitude of the R wave ("tall QRS") with a steep upstroke of the initial portion;

Irregular R-R interval; and
Non-specific fluctuating ST-T abnormalities

The ECGs presented here could be the early manifestation of left ventricular hypertrophy. The ST segment elevation at rest can be interpreted as an early repolarization comparable to the findings of Haissaguerre *et al*., (2007) and Bouineau, (2007). They mentioned that in some cases of early repolarization the QRS complex can be abbreviated and the R wave amplitude is increased. The narrow (and tall) QRS Syndrome might correspond to a fast initial depolarisation (dV/dt) and fluctuating repolarization changes in terms of ST segment elevation. Could be attributed to enhanced intercellular communication and/or altered distribution of the His-Purkinje network.

Conclusion

This work showed that *Moringa oleifera* extract was capable of ameliorating the atria fibrillation that ensued as a result of long time exposure to petrol vapour in our environment. More work will still have to be done on the mechanism of the action. This effect displayed by Moringa on atrial fibrillation was comparable with that of candesartan which is an Angiotensin II receptor blocker. The work thus gave scientific base of use of Moringa Leaves in herbal and pharmaceutical drug development.

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