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(RESEARCH ARTICLE)

Aqueous stem bark extract of *Rhizophora racemosa* is protective against cardiotoxicity and imbalance in anti-oxidants in normal Wistar rats due to exposure to petrol fumes

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Abstract

The protective potential of aqueous stem bark extract of *Rhizophora racemosa* in normal albino rats against cardio-toxicity and imbalance in anti-oxidants due to exposure to petrol fumes was investigated. A total of 21 albino rats of Wistar strain and of average weight were used in this study. Following exposure to petrol fumes for 1 hour and 3 hours daily, the two test groups were treated with aqueous stem bark extract of *Rhizophora racemosa (400mg/kg)* and vitamin E (200 mg/kg) respectively for 28 days. Blood samples were collected periodically for lactate dehydrogenase and creatine kinase activities determination by spectrophotometric method. On the 28th day of the experiment, all the experimental rats were sacrificed and dissected to obtain the hearts for histological studies and spectrophotometric antioxidant assay. The result obtained showed that exposure to petrol fumes caused increase in the activities of lactate dehydrogenase and creatine kinase when compared to normal control($P<0.05$). Catalase and superoxide dismutase activities were also reduced while malondialdehyde concentration increased($P<0.05$). These conditions were, however, reversed by the administration of the aqueous stem bark extract of *Rhizophora racemosa* and vitamin E. The hearts of the untreated rats showed adverse histological changes which were reversed by the aqueous stem back extract of *Rhizophora racemosa* after 28 days of treatment. The aqueous leave extract of this plant may therefore be useful in preventing cardio-toxicity caused by petrol fumes, thereby conferring protection on the heart.

Keywords: *Rhizophora racemosa;* Petrol; Wistar rat; Lactate dehydrogenase; Creatine kinase; Antioxidants

1. Introduction

Petrol, also referred to as gasoline or premium motor spirit (PMS), is a byproduct of the fractional distillation of petroleum and is frequently employed as an internal combustion engine fuel as well as in industrial settings as a thinner, decorative agent, and solvent. The majority of its composition is an intricate blend of aliphatic and aromatic hydrocarbons. In order to enhance the performance and stability of the hydrocarbon mixture, additives and blending agents are also added to it (Gunathilaka *et al*., 2017).

People are exposed to Petrol fumes as they go about their daily activities; inhalation being the major route of exposure. Due to lack of adequate power supply in Nigeria, many homes, offices and factories are powered by electricity generator sets and people are exposed to petrol fumes during refueling. Occupational exposure to petrol is also very prevalent. This is an important source of health concerns globally (Owumi *et al*., 2020). Road side mechanics for example use petrol to wash their tools, car engine parts and hands after work. Petrol station workers, vehicle drivers and refinery workers are also at high risk of daily exposure to petrol fumes. Inhalation of petrol as a form of recreational drug use is on the rise (NIDA (2020).

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The major constituents of petrol are the volatile organic compounds (VOCs) benzene, toluene, ethylbenzene, xylene (BTEX). These chemicals are considered among the most hazardous compounds for the human health (Lagorio *et al*., 1994; Rashid *et al*., 2017). Studies have revealed that various health problems are linked with occupational exposures to BTEX (Uzma *et al*., 2008). Uzma et al., (2008) also found that exposure to benzene from petrol vapour caused haematotoxicity and diminished pulmonary function that was associated with duration of exposure to petrol vapour among petrol station workers. Industrial exposures to benzene also cause some types of leukaemia and aplastic anaemia (Uzma *et al.,* 2008). Chronic exposure to VOCs including benzene has been linked with cardiac abnormalities (Hunter, 1966; Azeez *et al*., 2015) revealed that inhalation of petroleum products causes increase in arterial blood pressure and heart rate. These effects are as a result of the ability of petrol and other petroleum hydrocarbons to increase the sensitivity of the myocardium to catecholamines and the impairment of its vagal activity (Mill, 2005). Studies also reveal that crude oil and its refined products including petrol, kerosene and diesel cause adverse alterations in biochemical, hematological and histological profile of experimental animals (Nwaogu and Onyeze, 2014). Uboh *et al*. (2009) reported a significant increase in the activities of serum hepatic enzymes AST, ALT and ALP, and total bilirubin in rats fed with petrol contaminated diets. Exposure to Petrol fumes has also been reported to induce nephrotoxicity and alterations in lipid metabolisms in the exposed animals (Uboh *et al*., 2009). Azeez *et al*. (2015) reported that exposure to petrol and other petroleum products elevated serum creatine kinase and caused a degeneration of myocardial tissues in Sprague dawley rats, indicative of myocardial infarction.

Mankind has made use of medicinal plants to treat diseases for ages. Amongst most Africans and in other parts of the world, the use of herbs is steadily gaining acceptance as an alternative to orthodox medicine (Prohp *et al*., 2008). Plants' therapeutic properties are as a result of their content of secondary metabolites that either bring about the desired healings or serve as precursors for the synthesis of useful drugs (Asase *et al*., 2008). These phytochemicals contained in medicinal plants provide them with the ability to also protect the body from oxidative damage (Altemimi *et al*., 2017). *Rhizophora racemosa* is a specie of mangrove tree in the family *Rhizophoraceae* widely distributed along the Atlantic coast of West Africa (Ellison *et al.,* 2010). It covers about 90% of the Nigerian mangrove forests (Abere and Ekeke, 2011) and reaches a height of up to 30 m developing stilt roots and elliptical leaves. Different parts of the plant are believed to provide cure for a variety of diseases such as diabetes, snake bites, skin diseases, throat pains, asthma, rheumatism, diarrhea, fever and intestinal worms. (Bandaranayake 2002; Sur *et al*., 2016). These antioxidants from plant are established radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen extinguishers. They protect the body from various diseases by countering the actions of free radicals (Narayanaswamy and Balakrishnan, 2011). *Rhizophora racemosa* is made up of secondary metabolites such as phenolics, alkaloids, and terpenoids (Wu *et al*., 2008).

The study reported in this paper was aimed at investigating the possible ameliorative effects of aqueous stem bark extract of *Rhizophora racemosa* on petrol induced carditoxicity in wistar albino rats by evaluating the changes in Lactate Dehydrogenase and Creatine kinase enzyme activities.

2. Materials and method

2.1. Experimental Animals

21 male Wistar rats weighing between '150 g' were procured from the animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. They were kept in standard plastic cages in the research laboratory of same department for two weeks to acclimatize.

They were maintained under a 12-hour light/ dark cycle, controlled temperature (20-"25 ℃".

were fed standard feed and water ad libitum. All experimental procedures were carried out in accordance with the guidelines of the Research Ethical Committee of the Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

2.2. Plant materials and preparation of extracts

The stem bark of *Rhizophora racemosa* was obtained from Ogbia town, Ogbia L.G. A, Bayelsa State, Nigeria.The plant was identified by Precious Boukeme Francis, a Botanist and confirmed with the aid of google lens. The stem bark was washed thoroughly with clean water and air-dried for three weeks. The dried sample was blended into smooth powder with an electric milling machine. The ground sample was then macerated with distilled water by soaking 100g/1000ml for 72 hours at room temperature with periodic stirring. The active ingredients of the sample were extracted by filtration using a clean cheese-cloth. A rotary evaporator was used to concentrate the filtrate at 50˚c and 40 rotations per minute. The concentrate was placed in a beaker and taken to the water bath for further drying at same temperature. The extract was stored in a refrigerator until time of use.

2.3. Petrol/Chemical Reagents

The petrol used for this study was obtained from NNPC petrol station Edepie, Yenagoa, Bayelsa State, Nigeria. All the chemical reagents used for this study were of high-quality analytical grades. Enzyme kits used for Lactate dehydrogenase and creatine kinase determination were obtained from Randox™ Laboratories Ltd, Crumlin, Co, Antrim, United Kingdom. All other chemicals used for antioxidant assay were purchased from Loba Chemie PVT LTD India.

2.4. Experimental Procedure

2.4.1. Experimental Design

After acclimatization for two weeks, a baseline test was carried out on the rats. They were fasted overnight and then grouped randomly.

Twenty-one (21) male albino rats of Wistar strain were divided into 7 groups of 3 rats each and treated as follows for 28 days:

- Group 1 (Normal Control)- This group was given pelleted growers mash and distilled water (*ad libitum)* throughout the experiment.
- Group 2 (Positive control 1)- This group was exposed to petrol fumes for one hour daily and was fed with the pelleted growers mash with distilled water (*ad libitum*) throughout the experiment.
- Group 3 (Positive control 2)- This group was exposed to petrol fumes for three hours daily and was fed with the pelleted growers mash with distilled water (*ad libitum*) throughout the experiment.
- Group 4 (Test group 1)- This group was exposed to petrol fumes for one hour daily and aqueous stem bark extract of *Rhizophora racemosa* (400mg/kg body weight) was administered orally using a gavage tube, twice daily for 28 days. They were also fed with pelleted growers mash and distilled water (*ad libitum)* throughout the experiment.
- Group 5 (Test group 2)- This group was exposed to petrol fumes for three hours daily and aqueous stem bark extract of *Rhizophora racemosa* (400mg/kg body weight) was administered orally using a gavage tube, twice daily for 28 days. They were also fed with pelleted growers mash and distilled water (*ad libitum)* throughout the experiment.
- Group 6 (Standard control 1)- This group was exposed to petrol fumes for one hour daily and vitamin E (200mg/kg) was administered orally using gavage tube, twice daily for 28 days. The rats were also fed with pelleted growers mash and distilled water (*ad libitum*) throughout the experiment.
- Group 7 (Standard control 2)- This group was exposed to petrol fumes for three hours daily and vitamin E (200mg/kg) was administered orally using gavage tube, twice daily for 28 days. The rats were also fed with pelleted growers mash and distilled water (*ad libitum*) throughout the experiment.

The dose of the extract used was derived from a study previously conducted to determine the lethal dose (LD50) of the aqueous stem bark extract.

2.5. Exposure to petrol fumes

The animals were exposed to petrol fumes according to the method that was described by Uboh *et al.* (2005) and Owagboriaye *et al.* (2016) with slight modifications. A 1000ml plastic beaker containing 500 ml of petrol was placed in a fume chamber measuring 150 cm × 90 cm × 210 cm 1 hour before the commencement of exposure to ensure that the chamber is saturated with vapour. The animals were transfer into plastic baskets and were placed in the fume chamber to inhale the fumes evaporating from the can for 1 hour and 3 hours according to their groupings, daily, after which the animals were transferred to a fume-free section of the laboratory. The exposure lasted periodic for 28 days.

2.6. Collection of samples

Blood samples were taken from the sub-mandibular vein of the rats after a mild chloroform anaesthesia into lithium heparin bottles for baseline and afterwards on days 0,1,7,14,21, and 28. The blood sample were centrifuged at 2000 rotation per minute for 10 minutes and the supernatant (Plasma) was collected for creatine kinase (CK) and lactate dehydrogenase (LDH) enzyme assay.

On the 28th day, all the animals were sacrificed after anaesthesia using chloroform and their hearts were harvested for antioxidants and histological studies.

2.7. Biochemical Assay

Creatine kinase (CK) and lactate dehydrogenase (LDH) enzyme activities were estimated following the instruction in the appropriate biochemical kits purchased from Randox Laboratories (Crumlin, Co, Antrim, United Kingdom).

2.8. Antioxidant Assay

The heart tissues of the rats were homogenized in 0.1 M Phosphate buffer (pH 7.4) to make 10% homogenate and the homogenate was centrifuged at 3000 x g revolution per minutes (rpm) for 15 minutes at 4°C. The supernatant was collected for the estimation of antioxidant parameters. Superoxide dismutase (SOD) was assayed by the method described by Marklund and Marklund (1974), Catalase (CAT) activity was estimated using hydrogen peroxide as substrate according to the method of Aebi *et al*., (1974) while lipid peroxidation was estimated as malondialdehyde (MDA) according to the method described by Armstrong and Al-Awadi (1991) with slight modifications.

2.9. Histopathological Examination

Portions of the heart from all the experimental groups were fixed in 10% formal-saline, dehydrated in graded alcohol, cleared by xylene, and embedded in paraffin wax. The tissues were then cut into 3- to 4-mm-thick sections by a microtome, fixed on the slides, and stained with Hematoxylin & Eosin. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan), and photomicrographs were taken at x400 magnifications.

2.10. Statistical Analysis

The results obtained were expressed as mean ± SD. The statistical comparisons among the groups were performed using one-way analysis of variance (ANOVA) (SPSS 10.0). Significant difference was analyzed at $P<0.05$ level.

3. Results

The results of the present study are presented in the tables below.

Table 1 Mean plasma activities of Lactate dehydrogenase (U/L) in rats exposed to petrol fumes (1 hour).

	NORMAL CONTROL	POSITIVE CONTROL 1	TEST GROUP 1	STANDARD CONTROL 1	
BASELINE	153.61±9.66 [a]	154.64±3.36 [a]	155.87±5.61	153.23±5.31[a]	
DAY 0	149.46±2.95[a]	148.93±2.26[a]	151.9±4.54[a]	150.99±2.22[a]	
DAY 1	153.1±3.8[a]	153.9±1.2[a]	154.1±2.7[a]	152.8±3.3[a]	
DAY 7	154.93±1.56 [a]	161.64±2.95 [b]	156.61±1.75 [a]	156.44±1.64[a]	
DAY 14	154.75±2.45[a]	179.31±0.01[b]	166.54±1.64[c]	164.6±1.72 [c]	
DAY 21	155.77±2.74[a]	196.61±1.78[b]	171.66±3.85[c]	168.81±3.04[c]	
DAY 28	155.95±3.5[a]	201.62±1.82[b]	176.69±3.83[c]	173.41±3.63[c]	

Data are expressed as the mean ± SD. Means within the same row (in each parameter) carrying different superscripts (a, b, c, d, e) are signficantly different ($p < 0.05$).

Table 1 shows the mean plasma activities of Lactate Dehydrogenase (Baseline, Days 0, 1,7,14 and 28) for animals exposed for 1 hours daily. Exposure to petrol fumes caused a significant ($p<0.05$) increase in the activity of Lactate Dehydrogenase (Positive Control 1) compared to the Normal control group. It also shows that aqueous extract of *Rhizophora racemosa* (Test group 1) and Vitamin E (Standard group 1) significantly ($p<0.05$) reversed the activity of Lactate Dehydrogenase compared to the Petrol fume group (Positive Control 1).

Table 2 Mean plasma activities of Lactate dehydrogenase (U/L) in rats exposed to petrol fumes (3 hours)

	NORMAL CONTROL	POSITIVE CONTROL 2	TEST GROUP2	STANDARD CONTROL2
BASELINE	154.61±9.66 [a]	156.44 ± 3.56 [a]	155.32 ± 4.03 [a]	153.81± 4.85 [a]
DAY 0	151.46 ± 2.95[a]	152 ± 7.20[a]	149.94 ± 1.12[a]	150.99 ± 6.34[a]
DAY 1	153.1 ± 3.8[a]	155.3 ± 4.91[a]	153.1 ± 4.4[a]	158.6 ± 3.72[a]
DAY 7	154.93 ± 1.56[a]	179.2 ± 6.15[b]	167.61 ± 9.71[c]	165.44 ± 8.12[c]
DAY 14	154.75±2.45[a]	213.00 ± 5.25[b]	182.44 ± 6.89[c]	178.5 ± 9.19[c]
DAY 21	155.77±2.74[a]	238.33 ± 6.92[b]	186.19 ± 9.93[c]	178.45 ± 10.9[a]
DAY 28	155.95±3.5[a]	288.56 ± 8.86[b]	198.60 ± 16.73[c]	184.55 ± 8.67[d]

Data are expressed as the mean ± SD. Means within the same row (in each parameter) carrying different superscripts (a, b, c, d, e) are significantly different ($p < 0.05$).

Table 2 shows the mean plasma activities of Lactate Dehydrogenase (Baseline, Days 0, 1,7,14 and 28) for animals exposed for 3 hours daily. Exposure to petrol fumes caused a significant ($p<0.05$) increase in the activity of Lactate Dehydrogenase (Positive Control) compared to the Normal control group. It also shows that aqueous extract of *Rhizophora racemosa* (Test group 2) and Vitamin E (Standard group 2) significantly ($p<0.05$) reversed the activity of Lactate Dehydrogenase compared to the Petrol fume group (Positive Control 2).

Table 3 Mean plasma activities of Creatine Kinase (U/L) in rats exposed to petrol fumes (1hour)

	NORMAL CONTROL	POSITIVE CONTROL 1	TEST GROUP 1	STANDARD CONTROL 1
BASELINE	14.27±0.56 [a]	13.97±0.72 [a]	14.47±0.54 [a]	14.19±0.71[a]
DAY 0	13.91±0.70[a]	13.67±0.31[a]	13.01±0.84[a]	13.92±0.98[a]
DAY 1	14.14±0.15[a]	14.19±0.28[a]	14.64±0.39[a]	14.79±0.17[a]
DAY 7	14.27±0.08[a]	22.88±1.43[b]	18.66±0.63[c]	17.98±0.58[c]
DAY 14	14.61±0.35[a]	29.96±1.01[b]	24.8±1.26[c]	22.87±0.32[a]
DAY 21	15.06±0.98[a]	35.45±0.18[b]	28.67±1.5[c]	27.22±1.31[c]
DAY 28	14.91±0.74[a]	40.32±0.45[b]	33.97±0.75[c]	31.99±1.02[c]

Data are expressed as the mean ± SD. Means within the same row (in each parameter) carrying different superscripts (a, b, c, d, e) are significantly different ($p < 0.05$).

Table 3 shows the mean plasma activity of creatine kinase (Baseline, Days 0, 1,7,14 and 28) at different days of the experimentation period for rats exposed for 1 hours daily. It shows that exposure to petrol fumes caused a significant($p<0.05$) increase in the activity of Creatine Kinase (Positive Control 1) compared to the Normal control group. It also shows that aqueous extract of 400 mg/kg *Rhizophora racemosa* (Test group 1) and 200mg/kg Vitamin E (Standard group 1) significantly ($p<0.05$) reversed the activity of Creatine Kinase compared to the Petrol fume group (Positive Control 1).

Table 4 Mean plasma activities of Creatine Kinase (U/L) in rats exposed to petrol fumes (3 hours)

	NORMAL CONTROL	POSITIVE CONTROL 2	TEST GROUP 2	STANDARD CONTROL 2
BASELINE	14.27±0.56[a]	12.14 ± 1.9[a]	11.57 ± 1.7 [a]	14.57 ± 1.64[a]
DAY 0	13.91±0.70[a]	11.22 ± 2.1[a]	12.68 ± 1.73 [a]	12.77 ± 1.25[a]
DAY 1	14.14±0.15[a]	12.94 ± 1.89 [a]	12.17 ± 2.26 [a]	12.14 ± 1.76[a]
DAY 7	14.27±0.08[a]	42.7 ± 1.6 [b]	36.14 ± 0.98 [c]	34.76 ± 1.95[c]
DAY 14	14.61±0.35[a]	53.74 ± 1.28[b]	43.44 ± 2.08[c]	42.67 ± 1.57[c]
DAY 21	15.06±0.98[a]	79.56 ± 2.14[b]	54.98 ± 1.32[c]	53.45 ± 1.58[c]
DAY 28	14.91±0.74[a]	91.3 ± 1.98 [b]	68.6 ± 1.44 [c]	60.95 ± 1.79[d]

Data are expressed as the mean ± SD. Means within the same row (in each parameter) carrying different superscripts (a, b, c, d, e) are significantly different ($p < 0.05$).

Table 4 shows the mean plasma level of Creatine Kinase (Baseline, Days 0, 1,7,14 and 28) at different days of the experimentation period for rats exposed for 3 hours daily. It shows that exposure to petrol fumes caused a significant($p<0.05$) increase in the activity of Creatine Kinase (Positive Control 2) compared to the Normal control group. It also shows that aqueous stem bark extract of 400 mg/kg *Rhizophora racemosa* (Test group 2) and 200mg/kg Vitamin E (Standard group 2) significantly ($p<0.05$) reversed the activity of Creatine Kinase compared to the Petrol fume group (Positive Control 2).

Table 5 Mean antioxidant activities on Petrol fumes induced cardiotoxicity in wistar rats (1 hour)

	NORMAL CONTROL	POSITIVE CONTROL 1	TEST GROUP 1	STANDARD CONTROL 1
Catalase (U/mg protein)	2.44±0.05 [a]	1.16±0.08 [b]	1.63±0.3[c]	1.64±0.1[c]
SOD (U/mg protein)	6.95±0.24[a]	4.92±0.09[b]	6.87±0.11[c]	5.07±0.09[d]
MDA (U/mg protein)	32.76±1.3[a]	43.5±0.67[b]	26.6±0.95[c]	20.8±0.54[d]

Data are expressed as the mean ± SD. Means within the same row (in each parameter) carrying different superscripts (a, b, c, d, e) are significantly different ($p < 0.05$).

Table 5 shows the mean tissue antioxidant activities at the end of 28 days of the experiment after 1hour daily exposure to petrol fume. Exposure to Petrol fumes decreased the level of tissue Catalase and SOD (Positive Control 1) compared to the Normal Control group; this was reversed by the administration of the extract (Test Group 1) and the Vitamin E (Standard Group 1) Compared to the Petrol fume group (Positive Control 1) Exposure to petrol fumes increased the level of tissue MDA compared to normal group and this was reduced by the administration of the 400 mg/kg extract (Test group 1) and 200 mg/kg vitamin E (Standard group1) compared to the Petrol fume group (Positive control group1).

Table 6 Mean antioxidant activities on Petrol fumes induced cardiotoxicity in wistar rats (1 hour)

	NORMAL CONTROL 2	POSITIVE CONTROL 2	TEST GROUP 2	STANDARD CONTROL 2
CATALASE (U/mg protein)	2.44±0.05[a]	0.81± 1.87[b]	1.26 ± 0.88[c]	1.28 ± 0.79
SOD (U/mg protein)	4.95±0.24[a]	1.92 ± 1.17 [b]	3.86 ± 1.26[c]	3.88 ± 2.93[d]
MDA (U/mg protein)	32.76±1.3[a]	54.54 ± 3.07 [b]	33.6 ± 3.22[c]	25.45 ± 3.08[d]

Data are expressed as the mean ± SD. Means within the same row (in each parameter) carrying different superscripts (a, b, c, d, e) are significantly different ($p < 0.05$).

Table 6 shows the mean tissue antioxidant activities at the end of 28 days of the experiment after 3 hours daily exposure to petrol fumes. Exposure to Petrol fumes significantly ($p<0.05$) decreased the level of tissue Catalase and SOD (Positive Control 2) compared to the Normal Control group. This was reversed by the administration of the extract (Test Group

2) and the Vitamin E (Standard control 2) Compared to the untreated Petrol fumes exposed group (Positive Control 2). Exposure to petrol fumes significantly ($p<0.05$) increased the level of tissue MDA compared to the normal group and this was reduced by the administration of the extract (Test group 2) and vitamin E 200mg/kg (Standard control 2) compared to the Petrol fume group (Positive control 2).

3.1. Histological Analysis

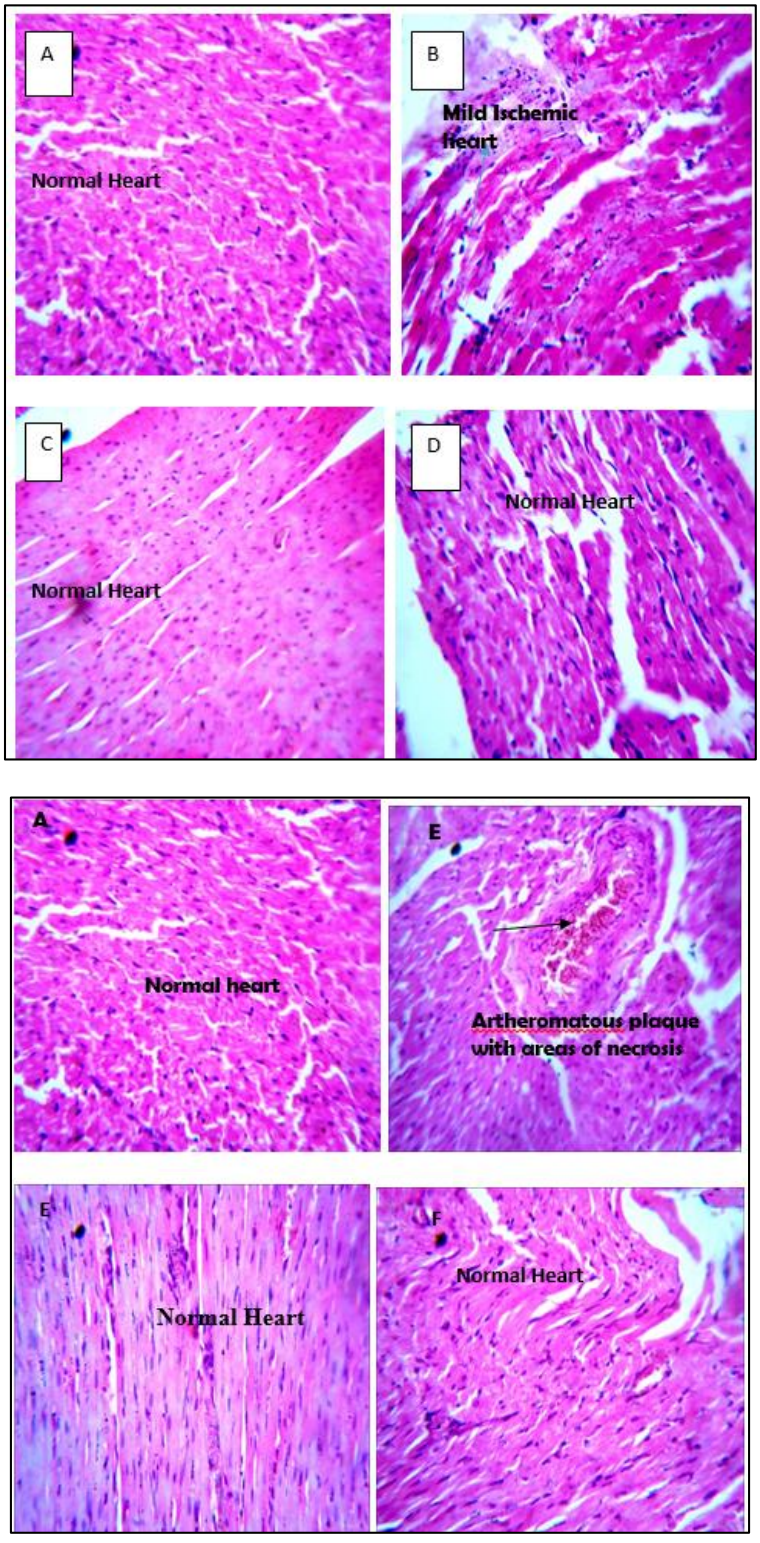

Figure 1 Photomicrograph of cardiac tissues

- (A)Normal control: animal shows smooth bundles of myocardiocytes with normochromic oval shape nuclei, consistent with normal histology of the heart.
- (B)Positive control 1: Petrol fumes exposed animal (1 hours) shows areas mild ischemic necrosis with anisionucleosis consistent with abnormal heart tissue.
- (c)Test Group 1: Petrol fumes exposed animal (1 hours) with 400mg/kg aqueous stem bark extract of *Rhizophora racemosa* treatment shows a lesser ischemic necrosis compared to Positive Control.
- (D)Standard Group 1: Petrol fumes exposed animal (1 hours) with 200mg/kg Vitamin E treatment shows smooth bundles of myocardiocytes with normochromic oval shape nuclei consistent with normal histology of the heart.
- (E)Postive control 2: Petrol fume exposed animal (3 hours) shows severe areas of ischemic necrosis and artheromatous plaque consistent with abnormal heart tissue.
- (F) Petrol fume exposed animal (3 hours) with 400mg/kg aqueous stem bark extract of *Rhizophora racemose treatment* shows smooth bundles of myocardiocytes with normochromic oval shape nuclei consistent with normal histology of the heart.
- (G) Petrol fume exposed animal (3 hours) with 200mg/kg Vitamin E treatment shows smooth bundles of myocardiocytes with normochromic oval shape nuclei consistent with normal histology of the heart.

Histological analysis of the heart tissue of the experimental animals in (Fig. 1) shows that frequent exposure to petrol fumes affects the heart cells, it implies that the heart is one of the major organ that is affected by petrol fumes induced toxicity.

4. Discussion

All around the world, petrol is a common fuel source. It has been commonly noted that exposure to petrol can be toxic. Many toxicological consequences linked to exposure to petrol can be attributed to its constituent volatile organic compounds (VOCs), which include benzene, toluene, ethylbenzene, and xylene (BTEX) (Kirchstetter, *et al*., 1999). Even minute amounts of hydrocarbons in the bloodstream can impair the central nervous system's (CNS) performance and harm many organs, including the liver and heart (Owagboriaye *et al*., 2018). Humans can be exposed to petroleum hydrocarbons through consuming contaminated food, drinking contaminated water, coming into contact with them (dermal exposure), inhaling polluted vapour, or breathing in airborne dirt (Streicher *et al*., 1981; Azeez, et al., 2012).

The usefulness of medicinal plants to man has been enormous. It has also served as a source for the development of va rious medications in addition to being utilized to treat numerous disorders. According to Asase *et al*. (2008) and Salme rónManzano *et al*. (2020), plants' medicinal capabilities are a result of their chemical compositions, which either suppl y the necessary therapy or act as precursors for the manufacture of beneficial pharmaceuticals. Phytochemicals contained in medicinal plants provide them with the ability to also protect the body from oxidative damage (Altemimi *et al*., 2017). *Rhizophora racemosa* has been shown to be abundant in phenolics, alkaloids, and terpenoids (Wu *et al.,* 2008). It has been discovered to have anti-fungal, anti-cancer, anti-inflammatory, and anti-malaria effects (Sachithanandam, 2019).

Creatine kinase and lactate dehydrogenase are biomarkers of cardiac damage. The study's findings demonstrated that, as compared to the normal control group, exposure to gasoline fumes significantly (p 0.05) increased the activities of lactate dehydrogenase and creatine kinase (positive control groups). This may be due to oxidative stress, which may have caused the necrosis of the tissues, which may have caused the enzymes to seep from the tissues into the blood stream (Aulbach *et al*., 2017). However, when compared to the untreated petrol fumes exposed groups, administration of 400 mg/kg aqueous stem bark extract of *Rhizophora racemosa* (Test groups) and vitamin E (Standard groups) significantly (p0.05) reversed the effects of petrol fumes on the activities of lactate dehydrogenase and creatine kinase (Positive Controls).

Excess reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anion radicals, hydrogen peroxide, singlet oxygen, nitric oxide radicals, hypochlorite radicals, etc., lead to oxidative stress. The production of free radicals and antioxidants in the human body are in equilibrium and a distortion in this equilibrium leads to oxidative stress (Mau *et al*., 2002; Adebayo *et al*., 2012). The antioxidant defense system's essential enzymes, Superoxide Dismutase (SOD) and catalase, work to counteract the effects of free radicals in the body. The study's findings demonstrated that whereas malondialdehyde content increased significantly following exposure to petrol fumes, catalase and superoxide dismutase

anti-oxidant activity was significantly lowered (P0.05). The injection of vitamin E and the aqueous stem bark extract of *Rhizophora racemosa*, however, restored these conditions.

The findings of this study make it abundantly evident that the level of toxicity is in direct proportion with the amount of time spent inhaling the petrol fumes. Rats treated for three hours had higher levels of lactate dehydrogenase and creatine kinase activity than rats exposed for one hour. Malondialdehyde levels were also noticeably higher in the three-hour group of rats compared to the one-hour group. Rats treated for three hours had considerably lower levels of super oxide dismutase and catalase than rats exposed for one hour. Creatine kinase and lactate dehydrogenase levels started to rise on the seventh day.

The results of the histological examination of the heart tissues, which showed that exposure to petrol fumes caused a severe ischemic necrosis and atheromatous plaque consistent with abnormal heart tissue in the rats exposed for three hours, and a mild ischemia in the rats exposed for one hour, corroborate the findings of this study (Figure 3.1). *Rhizophora racemosa* and vitamin E both have protective effects on the structure of the heart tissue, as evidenced by the smooth bundles of myocardiocytes that were produced after the administration of 400 mg/kg of an aqueous stem bark extract of the plant and 200 mg/kg of vitamin E. This is consistent with normal histology of the heart. This is in line with earlier discoveries made by Azeez *et al* (2015).

5. Conclusion

The results of this study indicate that inhaling petrol fumes may damage cardiac cells by raising serum lipid peroxidation and decreasing the activity of antioxidant enzymes like catalase and super oxide dismutase. Therefore, it is crucial that people, especially those whose everyday activities expose them to petrol fumes, are well-informed and prepared to avoid its inhalation. Findings also indicate that *Rhizophora racemosa's* aqueous stem bark extract has the potential to counteract the cardiotoxicity that had been noticed, protecting the heart from the cardiotoxicity caused by gasoline. Hence, this aqueous extract may be helpful in reducing cardiotoxicity brought on by petrol fumes.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest.

Statement of ethical approval

Ethical approval was obtained from the College Ethics Committee, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

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