

# Assessment of some biochemical markers in Plantain(Bole) rosters in Port Harcourt, Rivers State, Nigeria

## ABSTRACT

**Aim:** To assess some biochemical parameters (AST and ALT) and acute phase reactants (ESR and CRP) of plantain rosters in Port-Harcourt.

**Study design:** Cross-sectional study.

**Place and Duration of Study:** Eni-Yimini Laboratories Ltd Address Yenezue-Gene, Epie, Yenagoa, Bayelsa State between August and October 2022.

**Methodology:** A cross-sectional observational study was used in which a convenient sample size of 100 subjects between the ages of 20-50 years was used; out of which 50 subjects were plantain (Bole) rosters (test subjects), while the remaining 50 subjects were not plantain (Bole) rosters (control subjects). The test and control subjects were recruited from Agip, Rumuokoro, Rumuagholu, Igwuruta, Choba, and Aluu axes of Port-Harcourt. Oral consent was obtained from the subjects, and a well-structured questionnaire was used to gather relevant information. Also, blood samples were collected from interested persons, and were dispensed into plain bottles and EDTA-anticoagulated bottles; the blood samples in the plain bottles were used for the analysis of AST, ALT using the ELITech Selectra Pro S method, and CRP using the latex particle-enhanced immunoturbidometry method, while the blood samples in the EDTA-anticoagulated bottles were used to assay for ESR using the Westergren method. GraphPad Prism version 9.0.4 of Apple Macintosh HD Big Sur (version 11.0) statistical package was used for the data analysis, and  $p < 0.05$  was used and considered statistically significant.

**Results:** The results showed no significant difference in the levels of CRP ( $p=0.0548$ ), ESR ( $p=0.1207$ ), AST ( $p=0.2970$ ) and ALT ( $p=0.0848$ ) when the control and test subjects were compared. The test subjects within the age range of 20-29 years had a significant decrease in CRP ( $p=0.0035$ ) compared to the control subjects within the same age range. Also, the test subjects within the age range of 40-49 years had a significant increase in ESR ( $p=0.0094$ ) compared to the control subjects within the same age range. Furthermore, there was a significant positive correlation between age and ESR ( $p=0.0006$ ), between duration of exposure and ALT ( $p=0.0008$ ), and between duration of exposure and ESR ( $p=0.0026$ ). These results may imply that occupational exposure to burning woods when roasting plantain (Bole) did not trigger any inflammation or hepatocellular injury; however, an increase in age in the exposed individuals resulted in an increase in ESR, and an increase in the duration of exposure to wood smoke resulted in an increase in ALT and ESR.

**Conclusion:** Exposure to burning woods while roasting plantain (Bole) did not trigger any inflammation or hepatocellular injury. However, robust studies establishing the molecular basis for cause and effect are recommended.

*Keywords: Assessment, biochemical markers, Plantain rosters (Bole), Port Harcourt, Rivers State, Nigeria*

## 1. INTRODUCTION

Numerous toxicological studies have revealed that exposure to smoke emissions adversely affects human health [1,2]. Cooking smoke affects the health of millions of individuals on

earth. Smoke from cooking activities is so dangerous and harmful as evidenced in various studies. The World Health Organization (WHO) estimated that 3.8 million individuals have died prematurely from air pollution associated with inefficient cooking methods [3]. Again, studies have shown that approximately 3 billion people in developing countries rely on firewood or charcoal for their daily cooking purposes [1]. According to the World Health Organization (WHO), smoke-induced diseases are responsible for the death of 4.3 million people every year, which exceeds the number of deaths when compared with fatal cases of malaria or tuberculosis [3]. The largest burden of mortality is borne by women and young children [3]. Among the 4.3 million who die from the consequences of smoke emission each year, 500,000 are children under five that die due to acute respiratory infections [4].

Researchers affiliated with the World health Organization reported that approximately 396,000 deaths occurred in year 2002 because of indoor smoke pollution in sub-Sahara Africa [5]. Scientists have demonstrated that Inefficient cooking practices can lead to deaths associated with stroke, pneumonia, heart disease, chronic obstructive and lung cancer amongst others [5]. Chronic exposure to elements such as barium and titanium can result to the proliferation of cancer cells [5]. Several studies have demonstrated that wood-burning, roasting and fireplaces as well as agricultural fires can produce significant quantities of hazardous pollutants, including polycyclic aromatic hydrocarbons, benzene, aldehydes, respirable particulate matter, carbon monoxide, nitrogen oxides, and other free radicals [6,7]. Roasting of food with firewood releases smoke which is a mixture of chemicals and particles produced by incomplete combustion of carbon materials. All smoke contains particulate matter (soot), carbon dioxide and carbon monoxide. Smoke which can be produced through roasting using charcoal, firewood amongst others also consists of sulfur dioxide, acid gases, aldehydes, metals, styrene, benzene, dioxins, and polycyclic aromatic hydrocarbons [6]. The soluble fractions of smoke can cross to the extra-pulmonary circulations which can lead to the production of harmful effects in extra-pulmonary organs such as liver kidney and heart [7]. Inhaling carbon monoxide decreases the body's oxygen supply. It has been postulated that decrease in blood supply can lead to decrease in ESR linked with the amount of oxygen carrying red blood cells [7].

Carbon monoxide which is one of the main gaseous pollutants in wood smoke can bind competitively to hemoglobin which can stimulate hypoxia and necrosis as tissues receive less oxygen. Wood smoke can result to changes in immune mediated actions such as oxidative stress response [8]. The age at which cooking activities started and the availability of ventilation hood is related to the increase in health risks [9].

Liver function tests are blood tests that provide information about the functional state of the liver. These tests include the liver transaminases aspartate transaminase (AST) and alanine transaminases (ALT) which are useful indicators of liver injury [10]. Biochemical tests are important in management of individuals with liver dysfunction. These tests can be used to diagnose, distinguish, and monitor liver diseases. The erythrocyte sedimentation rate is the rate at which red blood cells descend in a calibrated tube over a period of one hour. The red blood cells are present in anticoagulated whole blood, The ESR is a hematological test and is useful as a non-specific measure of inflammation. However, C-reactive protein which is an acute phase protein is a better marker for acute phase reaction than ESR [10].

A high percentage of previous studies on the effect of smoke on human health focuses on the cardiovascular and respiratory systems. Also, there is paucity of research on the effects of smoke on plantain roasters especially in Port Harcourt metropolis. There is need for these roasters and the society to be informed of the effects of the smoke from roasting plantain and other food items. Port Harcourt is uniquely known for its roasted plantain recipes and roasting is one of the frequently practiced methods of preparing plantain. Also, there is an

increase in the number of plantain roasters in Port Harcourt which could be due to the relative increase in consumption of the products by the residents [10].

## **2. MATERIALS AND METHODS**

### **2.1 Study Area**

This study was carried out in Port-Harcourt, Nigeria. Port-Harcourt is the capital of Rivers State which is (with 23 local government areas) in the Southern part of Nigeria; it lies along Bonny River in the Niger Delta region of Nigeria. Port Harcourt is a metropolis that is considered the commercial center of the Nigeria oil Industry with an estimated population of 1,865,000. It is a major industrial Centre as it has many multinational firms as well as other industrial concerns, particularly business related to the petroleum industry. It is the chief oil-refining city in Nigeria and has two main oil refineries located at Eleme Local Government Area.

### **2.2 Study Population**

A case-controlled observational study was used in which a convenient sample size of 100 subjects between the ages of 20-50 years was used; out of which 50 subjects were plantain (Bole) roasters used as the test subjects, while the remaining 50 subjects were not plantain (Bole) roasters, used as the control subjects. The subjects were recruited from Agip, Rumuokoro, Rumuagholu, Igwuruta, Choba, and Aluu axes of Port-Harcourt. They were intimated about the study, and oral consent was obtained from interested persons. Also, a well-structured questionnaire was used to gather relevant information (such as age, sex, duration of the occupation, other occupational or residential exposures, smoking status, use of medication, alcohol status, medical history, etc) from each subject.

### **2.3 Study Design**

This was a case-controlled observational study aimed at assessing some biochemical markers (AST, ALT, ESR and CRP) in plantain (Bole) roasters and comparing it with those who are not plantain roasters.

### **2.4 Eligibility Criteria**

#### **2.4.1 Inclusion Criteria**

- Individuals between the ages of 20 and 50 years were included in the study.
- Individuals who have been roasting plantain (Bole) routinely as their occupation for at least the past 2 months were recruited as test subjects.
- Individuals who do not roast plantain (Bole) were recruited as control subjects.

#### **2.4.2 Exclusion Criteria**

- Subjects, who are routinely exposed to other forms of smoke not resulting from plantain roasting were excluded for the study.
- Subjects, who are routinely exposed to fuel fossils or hydrocarbons, wood dust, cement dust or paint chemicals, were excluded from the study.

- Subjects who are smokers and/or alcoholics were excluded for the study.
- Subjects with health conditions such as heart diseases, kidney diseases, diabetes mellitus or other forms of serious medical conditions were excluded from the study.

## **2.5 Blood Sample Collection**

With the aid of sterile vacutainer specimen bottles, about 10 ml of whole blood was collected from each subject via venepuncture. About 5 ml was collected using a plain vacutainer; the serum was obtained from the plain vacutainer after centrifugation at 3500 rpm for 5 minutes, and was transferred into a plain container and stored in the freezing compartment of the refrigerator at -40°C until time for analysis, where it was used for the determination of AST, ALT and CRP. The remaining 5 ml was collected using a vacutainer containing 0.5 ml of 1.2 mg/ml K<sub>2</sub>-EDTA (dipotassium ethylenediaminetetraacetic acid) and was used for the determination of the ESR.

## **2.6 Sample Analysis**

### **2.6.1 Determination of Erythrocyte Sedimentation Rate (ESR) [11]**

*2.6.1.1 Method:* Westergren Method

*2.6.1.2 Principle:* The erythrocyte sedimentation rate (expressed in mm per hour) is the rate at which red blood cells settle when an anticoagulant blood is allowed to stand in a narrow tube (Westergren). It is measured by the height of the column of clear plasma at the end of one hour.

### **2.6.2 Determination of Serum Levels of C - reactive protein (CRP)**

*2.6.2.1 Method:* Latex Particle-enhanced Immunoturbidometry

*2.6.2.2 Principle:* Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidometry.

### **2.6.3 Determination of Serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT)**

*2.6.3.1 Method:* ELITechSelectra Pro S

*2.6.3.2 Principle:* It is based on colorimetric principle which relates the absorption of light to the properties of the material through which light is traveling.

**AST:** AST catalyzes the reversible transfer of amino group of L-aspartate and  $\alpha$ -ketoglutarate to oxaloacetate and L-glutamate. In the presence of malate dehydrogenase, the produced oxaloacetate is further reduced to malate, while NADH is oxidized to NAD. The resulting decrease in the absorbance at 340nm is directly proportional to the activity of AST in serum.

**Principle for ALT:** ALT catalyzes the reversible transfer of amino group of L-alanine and  $\alpha$ -ketoglutarate to pyruvate and L-glutamate. In the presence of lactate dehydrogenase, the produced pyruvate is further reduced to lactate, while NADH was oxidized to NAD. The

resulting decrease in the absorbance at 340nm is directly proportional to the activity of ALT in serum.

### 3.7 Statistical Analysis

GraphPad Prism version 9.0.4 of Apple Macintosh HD Big Sur (version 11.0) statistical package was used for data analysis. Descriptive statistical tools such as mean & standard deviation (SD) were used. ANOVA was used to compare means of more than two groups for inferential evaluation, with Tukey's multiple comparison test to check for mean difference between multiple groups. Spearman's correlation was used to check for the linear relationships between variables. The probability (p) value less than 0.05 ( $P < 0.05$ ) was used and considered statistically significant.

### 3. RESULTS AND DISCUSSION

**Table 1: Sex Distribution of the Test Subjects**

Subjects	Number	Percentage (%)
Male	49	98
Female	1	2

**Table 2: Mean Values of CRP and ESR for Control and Test Subjects Compared**

Groups/Parameters	CRP (mg/l)	ESR (mm/hour)
Control (N=50)	9.725±4.49	20.24±22.62
Test (N=50)	7.65±6.034	27.42±23.25
P-value	0.0548	0.1207
Remark	NS	NS

**Key:** CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate, NS = Not significant

**Table 3: Mean Levels of AST and ALT for Control and Test Subjects Compared**

Groups/Parameters	AST (U/l)	ALT (U/l)
Control	29.28±11.30	31.14±13.86
Test	26.32±16.45	24.44±23.42
P-value	0.2970	0.0848
Remark	NS	NS

**Key:** AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, NS = Not significant

**Table 4: Mean Levels of AST, ALT, CRP and ESR Based on Age Range of 20-29 years for Control and Test Subjects Compared**

Groups/Parameters	AST (U/l)	ALT (U/l)	CRP (mg/l)	ESR (mm/hr)
Control (N=10)	24.0±10.83	28.80±10.84	11.73±3.961	12.0±6.498
Test (N=9)	24.56±8.974	23.0±10.69	5.947±3.409	17.56±10.11
P-value	0.9052	0.2573	0.0035	0.1680
Remark	NS	NS	S	NS

**Key:** CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate, NS = Not significant, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, NS = Not significant, S = Significant

**Table 5: Mean Levels of AST, ALT, CRP and ESR Based on Age Range of 30-39 years for Control and Test Subjects Compared**

Groups/Parameters	AST (U/l)	ALT (U/l)	CRP (mg/l)	ESR (mm/hr)
Control (N=18)	28.11±11.65	27.67±17.22	9.909±4.609	18.83±16.32
Test (N= 9)	24.53±12.70	21.16±28.59	7.209±6.192	22.26±14.25
P-value	0.3778	0.4105	0.1432	0.4997
Remark	NS	NS	NS	NS

**Key:** CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate, NS = Not significant, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, NS = Not significant

**Table 6: Mean Levels of AST, ALT, CRP and ESR Based on Age Range of 40-49 years for Control and Test Subjects Compared**

Groups/Parameters	AST (U/l)	ALT (U/l)	CRP (mg/l)	ESR (mm/hr)
Control (N=14)	30.57±7.593	33.43±11.90	9.711±4.701	17.46±11.55
Test (N=11)	26.27±11.34	26.09±15.83	5.658±5.974	24.62±15.35
P-value	0.2684	0.1983	0.0700	0.0094
Remark	NS	NS	NS	S

**Key:** CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate, NS = Not significant, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, NS = Not significant, S = Significant

**Table 7: Mean Levels of AST, ALT, CRP and ESR Based on Age Range of 50-59 years for Control and Test Subjects Compared**

Groups/Parameters	AST (U/l)	ALT (U/l)	CRP (mg/l)	ESR (mm/hr)
Control (N=8)	36.25±14.32	37.88±10.45	6.829±3.659	21.0±8.701
Test (N= 8)	37.0±31.53	36.25±31.38	11.54±5.348	49.75±44.08
P-value	0.9520	0.8914	0.0587	0.0919
Remark	NS	NS	NS	NS

**Key:** CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate, NS = Not significant, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, NS = Not significant

**Table 8: Correlation between Age and Parameters**

	Age (years) vs. CRP (mg/l)	Age (years) vs. ALT (u/l)	Age (years) vs. AST (u/l)	Age (years) vs. ESR (mm/hr)
Pearson r	0.08799	0.2518	0.2324	0.4708
P value	0.5434	0.0777	0.1044	0.0006
Remark	NS	NS	NS	S

**Key:** CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate, NS = Not significant, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, NS = Not significant, S = Significant

**Table 9: Correlation between Duration of Exposure and Parameters**

	Duration (Years) vs. ALT (u/l)	Duration (Years) vs. AST	Duration (Years) vs. ESR (mm/hr)	Duration (Years) vs. CRP (mg/l)
Pearson r	0.4609	0.1843	0.4175	0.1103
P value	0.0008	0.2002	0.0026	0.4457
Remark	S	NS	S	NS

**Key:** CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate, NS = Not significant, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, NS = Not significant, S = Significant

The study was aimed at assessing two liver enzymes (AST and ALT) and some acute phase reactants (ESR and CRP) of plantain rosters in Port-Harcourt. There was no significant difference in the levels of CRP and ESR when values for the control and test subjects were compared. The ESR and CRP are used for non-specific measures of inflammation; therefore, the report obtained from this study may be attributed to the fact that exposure to burning woods while roasting plantain (Bole) probably did not trigger any inflammation. **This report is not in accordance with that of Ghio et al. [12]** which recorded inflammation in healthy volunteers who were exposed to wood smoke particles generated by heating an oak log on an electric element.

A non-significant difference in the hepatic enzymes (AST and ALT) was reported when the control and test subjects were compared. The AST and ALT are biomarkers of hepatocellular injury, therefore, the report obtained from this study may be attributed to the fact that inhalation of the wood smoke while roasting plantain (Bole) did not trigger hepatocellular injury. This report disagrees with that of Dadzie et al. [13] which recorded increased AST levels triggered by exposure of wistar rats to wood smoke carried out in a locally constructed smoke chamber. The report obtained from this study also disagrees with that of Dike et al. [14] which recorded increased ALT levels because of exposure of wistar rats to wood smoke carried out in a locally constructed smoke chamber.

Based on the age range of 20-29 years on Table 4, the hepatic enzymes and inflammatory markers showed no significant difference when the control and test subjects were compared, except for CRP that was significantly lower ( $p=0.0035$ ) in the test subjects compared to the control subjects. A decrease in CRP is achieved through exercise, weight loss and diet modification [15]. Thus, this decreased CRP in the test subjects may be suggestive of the fact that the test subjects within the specified age range may have been actively involved in exercise which could have consequently led to weight loss. Similarly, based on the age range of 30-39 years, the hepatic enzymes and inflammatory markers showed no significant differences (ESR,  $p=0.1432$ ; CRP,  $p=0.4997$ ) when the control and test subjects were compared, and this could be due to the fact that the wood smoke exposure may not have induced hepatocellular injury and inflammation, owing to possibly sufficient endogenous anti-oxidants capable of preventing oxidative stress [16].

Based on the age range of 40-49 years, the hepatic enzymes and inflammatory markers showed no significant difference when the values for control and test subjects were compared, except for ESR that was significantly higher in the test subjects compared to the control subjects. The ESR indicates and monitors an increase in inflammatory activity within the body [16]. Also, wood smoke contains toxic pollutants such as carbon monoxide, respirable particulate matter, nitrogen dioxide, benzene and other free radicals that can cause cancer and other health hazards including inflammation through the production of free radicals [17]. Therefore, this elevated ESR in the test subjects may be attributed to the fact that exposure to wood smoke while roasting plantain (Bole) may have triggered oxidative stress, which in turn, may have resulted in inflammation in the test subjects within the specified age range. Similar to the age range of 30-39, the age range of 50-59 years also had no significant difference in the hepatic enzymes and inflammatory markers when the control and test subjects were compared, and this also, may be attributed to the fact that the wood smoke exposure may not have induced hepatocellular injury and inflammation, which may in turn, be attributed to sufficient endogenous anti-oxidants capable of preventing oxidative stress.

From this study also, there was a non-significant positive correlation between age and the hepatic enzymes **in the test subjects** (AST and ALT). However, a significant positive

correlation was recorded between age and ESR, whereby, as the age of the subjects increased, the ESR also increased. This result of increased ESR due to increasing age (Table 8) may be attributed to a decline in the functional capacity of the tissues, organs, and cells of humans during old age. However, the result from this study agrees with the report of Alende-Castro et al. [18] which confirms that ESR increases steadily with an increase in age.

Moreover, there was a non-significant positive correlation between duration of exposure to wood smoke from Bole roasting and AST, and between duration of exposure to wood smoke from Bole roasting and CRP. On the other hand, a significant positive correlation was recorded between duration of exposure and ALT, and between duration of exposure and ESR. This may imply, that an increase in the duration of exposure resulted in increased ALT level and ESR. This significantly elevated ALT levels (and not AST levels) may have been triggered by other non-alcoholic fatty liver disease such as hepatic steatosis (in which ALT level is usually higher with normal AST level) which may have been induced by the wood smoke during bole roasting, while the elevated ESR may be attributed to the presence of a possible inflammation.

#### **4. CONCLUSION**

It can be deduced from this study, that exposure to burning woods while roasting plantain (Bole) did not trigger any inflammation or hepatocellular injury. Based on age bracket, plantain (Bole) roasters within the age range of 20-29 years did not develop inflammation or hepatocellular injury. Similarly, plantain (Bole) roasters within the age range of 30-39 years did not develop hepatocellular injury or inflammation. However, those subjects within the age range of 40-49 years did not develop hepatocellular injury, but rather developed some form of inflammation. Also, those subjects within the age range of 50-59 years did not develop hepatocellular injury and inflammation. Also, an increase in age resulted in an increased ESR which may be due to inflammation. Also, an increase in the duration of exposure to the wood smoke resulted in increased ALT level and ESR.

#### **Ethical Approval**

Ethical Approval for this study was obtained from the Ethical Committee of the Rivers State Ministry of Health, Port Harcourt, Nigeria.

#### **CONSENT**

Both oral and written consents were obtained from the participants before they were recruited into the study.

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