



# REVERSAL OF HAEMORRHAGIC MYOCARDIUM USING OCIMUM GRATISSIMUM

## ABSTRACT

Effects of *Ocimum gratissimum* on haemorrhagic myocardium in albino rats exposed to high volumes of Bonny light crude oil was investigated. Male and female rats weighing 180-220g were placed in control and test groups consisting of four(4) subgroups with each subgroup consisting of five (5) animals and used to test the effect of crude oil on the myocardium; the middle muscular layer of the heart. The animals were divided into control and test groups. Control group rats were administered unpolluted “normal” feed and water ad libitum (as desired). While the test groups animals were administered crude oil polluted feed in the ratio of 5kg feed mixed with 500ml. Bonny light crude oil and water for 21(twenty one) days. Histological examination on animals given crude oil feed and water showed observable haemorrhage of the myocardium. Findings from previous crude oil toxicity tests indicate that the haemorrhagic myocardium was possibly due to the noxious effects of the crude oil on the myocardial tissue. Successive studies were carried out incorporating *Ocimum gratissimum* (African basil) into the feed and water of experimental animals in the ratio of 5kg feed mixed with 450ml water and 50ml *Ocimum gratissimum* solution. Experimental animals (rats) that were not “sacrificed” from the previous experiment were used in the second study. On day 21 of the second experiment, the test and control animals were sacrificed and histological studies carried out again on the myocardial tissue with observations of no blood being present in the myocardial tissue of both control and test animals. With this discovery, conclusions were made that cold water extracts of *Ocimum gratissimum* (African basil) reversed haemorrhage of the myocardial tissue.

## INTRODUCTION

### Pollution

The term pollution refers to any substance that negatively impacts the environment or organisms that live within the affected environment. Addition of the substance is done at rates faster than can be dispersed, diluted, decomposed, recycled or stored in some harmless form

### Oil spillage (Pollution)

Oil spillage can be defined as the release of crude oil hydrocarbons into the environment. It is an important environmental disaster of global concern that usually occurs accidentally or intentionally mostly resulting from everyday human activities that release crude oil into coastal waters and land. Environmental pollution arising from oil prospecting and exploration in the Niger Delta regions of Nigeria has definitely impacted negatively on the biodiversity of the affected regions. The major problems arise from leakage of crude oil, gas flaring and the escape of other chemicals used in the production process.

Records of oil spills in Nigeria only became evident in the late 1970's due to occasional spills caused by corrosion and ageing facilities than through sabotage. An estimated 240,000 barrels of crude oil is spilled in the Niger Delta of Nigeria every year polluting waterways, contaminating crops and releasing toxic chemicals.

The Nigerian National Petroleum Corporation places the quantity of crude spilled into the environment yearly at 2,300 cubic meters with an average of 300 individuals of 300 individual spills annually. Oil spills have become a common event in Nigeria with half of all the spills occurring due to pipeline and tanker accidents.

Other causes include sabotage, oil production operations and inadequate or non-functional production equipments. Sabotage and theft through oil siphoning has become a major issue in the Niger Delta states as well as contributing to degradations.

In December 2006, more than 200 people were killed in Lagos state, Nigeria in an oil-line explosion.

## CONSEQUENCES OF SPILLS

Oil spills have a major effect on the ecosystem into which spilled crude is released and may therefore constitute ecocide. Ecocide is criminalized human activity that violates the principles of environmental justice such as causing extensive damage, destroying ecosystems or harming the health and well being of species humans inclusive.

The mangrove forests are highly susceptible to oil. The oil is stored in the soil and released annually during inundations. An estimated 5-10% of Nigerian mangrove ecosystems have been wiped out.

The rainforest that previously occupied about 7,400km<sup>2</sup> of land has disappeared. In populated areas, spills often spread out over a wide area destroying crops and aquacultures through contamination of the ground water and soil. Reports of death of fish and other aquatic forms that are of great economic importance are high.

The consumption of dissolved oxygen by bacteria feeding on the spilled hydrocarbons also contributes to the death of fish. Agriculture is also affected and some agricultural communities experience losses in food production. The effects of an oil spill will depend on a variety of factors including the quality and type of oil spilled and how it interacts with the marine environment. Prevailing weather conditions also influence the oil's physical characteristics and behaviour.

Other factors include biological and ecological attributes of the area; ecological significance of key species and their sensitivity to oil pollution as well as the time of year.

## CRUDE OIL COMPOSITION

Crude oil is a mixture of comparatively volatile liquid hydrocarbon compounds composed mainly of hydrogen and carbon. It also contains some nitrogen, sulphur and oxygen. The elements form diverse groups of complex molecular structures with some not being readily identified. Variations notwithstanding, almost all crude oil range from 82-87% carbon by weight. Customarily, classification is done by the type of hydrocarbon compound that is most prevalent in them.

These are paraffins, naphthenes and aromatics, with paraffin being the most common hydrocarbon found in crude oil. Certain liquid paraffins are the major constituents of gasoline and are therefore of very great importance. Naphthenes are a crucial part of all liquid refinery products and also form some of the heavy asphalt like residues of refinery processes. The hydrocarbon structures found in oil include saturates, aromatics and polar compounds which include resins and asphaltenes.

Resins and asphaltenes are largely refractory in the environment. They evaporate, dissolve and degrade poorly and end up accumulating as residues especially after a crude oil spill. The percentage of saturates and aromatics called the light compounds in comparison to the heavy residue forming resins and asphaltenes vary with type of crude oil.

Aromatics generally constitute only a small percentage of most crude with the most common aromatic in crude oil being benzene, a popular building block in the petrochemical industry. Because crude oil is a mixture of such widely varying constituents and proportions, its physical properties also vary widely. In appearance, crude oil ranges from

colourless to “black”. The most important physical property probably is specific gravity; which is the ratio of the weight of equal volumes of the crude oil and pure water at standard conditions.

In laboratory measurements of specific gravity, it is customary to assign pure water a measurement of one (1). Substances lighter than water such as crude oil receive a measurement less than one (1). The petrochemical industry however uses the American Petroleum Institute (API) gravity scale in which pure water has been arbitrarily assigned an API gravity of  $10^0$ .

Liquids lighter than water such as oil have API gravities numerically greater than 10. On the basis of API gravities, crude oil can be classified as heavy, medium and light as follows;

Heavy 10 – 20<sup>0</sup> API gravity

Medium 20 – 25<sup>0</sup> API gravity

Light above 25<sup>0</sup> API gravity

Crude oil is also categorized as “sweet” or “sour” depending on the level of sulphur which occurs whether as elemental sulphur or in compounds such as hydrogen sulphide. Sweet crudes have sulphur contents of 0.5% or less by weight while “sour” crudes have sulphur contents of 1% or more by weight.

Generally, the heavier the crude oil, the more its sulphur content. Excess sulphur is often removed from crude oil during refining because sulphur oxides released to the atmosphere during combustion of oil is a major pollutant. Petroleum in Nigeria is often classified as “light” and “sweet” since the oil is largely free of sulphur.

Most crude oils are named according to export terminals; for example there is Bonny light, Qua iboe, Escravos blend, Brass river, Forcados and Pennington Anfan. The major and minor classes of crude oil come from various export terminals in Nigeria. Bonny light was so named because its export terminal is located in the city of Bonny in Rivers State, South-South Nigeria.

Another classification into light, medium and heavy oil is based on their densities and toxicity levels which also depend on their volatilities. The purification of crude oil focuses first on the use of industrialized fractional distillation to separate crude oil into primary products which is followed by different cracking and refining processes to generate secondary products from the primary products whose purification is outsmarted by their utilization locally or whose utilization by the Nigerian populace is well reduced as compared to their level of production. Crude oil is not a single indistinguishably homogenous substance without differences that are unique. There are many different types of crude oil.

In its natural unrefined state, crude oil ranges in density and consistency from very thin light weight and volatile fluidity to an extremely thick semi-solid heavy weight oil. There also exists a gradation in colour that the oil extracted from the ground exhibits ranging from a light golden yellow to the very deepest black.

## **TYPES OF CRUDE OIL**

### **BRENT BLEND**

This blend is actually a mixture of different oils. Its API gravity is 38.8<sup>0</sup> which makes it light crude oil. It also contains about 0.37% sulphur and this makes it a “sweet” crude oil.

Brent blend is excellent in making gasoline and middle distillates, it remains a major benchmark for other crude oils in Europe and Africa.

## OPEC BASKET

The OPEC basket is a collection of seven (7) different crude oils from Algeria, Saudi Arabia, Indonesia, Nigeria, Dubai, Venezuela and the Mexican Isthmus. Because OPEC oil has a much higher percentage of sulphur within its natural make up and therefore is not nearly as “sweet” as Brent blend it is also not naturally as light as Brent blend.

The bulk of the compounds present in crude oil are hydrocarbons. Crude oil generally contains classes of hydrocarbons and other compounds.

## HYDROCARBON COMPOUNDS

### Alkanes (Paraffins)

Alkanes are straight chain normal alkanes and branched iso-alkanes with general formula  $C_nH_{2n+2}$ . The major paraffinic components of most crude oils are in the range  $C_1 - C_{35}$ , although small quantities of alkanes up to  $C_{60}$  or higher may be present. Crude oils vary widely in alkane content.

### CYCLOALKANES (Naphthenes)

Cycloalkanes, cycloparaffins or naphthenes are saturated hydrocarbons containing structures with carbon atoms linked in a ring. The cycloalkane composition in crude oil worldwide typically varies from 30-60%.

The predominant monocycloalkanes in crude oil are in the cyclopentane series having 5(five) carbon atoms in the ring and cyclohexane having a six (6) membered ring.

## **AROMATIC HYDROCARBONS**

Aromatic hydrocarbons are those which contain one or more benzene rings. The name of the class comes from the fact that many of them have strong pungent aromas. The most common aromatic compounds in crude oil are benzene. The concentration of benzene in crude oil is estimated to be between 0.01% and 1%, Aromatic hydrocarbons also include benzene active metabolites.

## **ALIPHATIC HYDROCARBONS**

Aliphatic stems from the Greek word meaning fat or oil. It refers to hydrocarbons that are straight chained, branched or non aromatic. Hydrocarbons which do not contain a benzene ring are called aliphatic hydrocarbons. Some of the most common flammable and volatile compounds are aliphatic hydrocarbons. They can either be saturated or unsaturated.

## **NON HYDROCARBON COMPOUNDS**

Vanadium, nickel, nitrogen and sulphur are known to be the most significant non hydrocarbon constituents of crude oil. Non hydrocarbon compounds provide fundamental answers in petroleum geo-chemistry.

They may be small in quantity but some have considerable influence on the quality of products. They have harmful effects in most cases and must be removed or converted to less harmful compounds during the refining process. The most common occurring non-hydrocarbons are sulphur, nitrogen and oxygen.



## SULPHUR COMPOUNDS

Sulphur is common in crude oil but varies from 0.2-6% by weight. There are corrosive and non corrosive sulphur compounds. One corrosive compound is hydrogen sulphide ( $H_2S$ ). Fortunately, sulphides have highly obnoxious smell that gives some warning of their danger.

If one hydrogen atom is replaced by a hydrocarbon group, the compound is called a Mercaptan or thiol. Such compounds are formed during the distillation of crude oils. They can cause severe corrosion of the processing units and the addition of chemicals; proper temperature control and the use of special alloys in refinery equipment are required to control them. If both of the two hydrogen atoms are replaced by hydrocarbon groups, the compound is called a sulphide or thio-ether. An example is thiophene ( $C_4H_4S$ ). Thiophenes have a relatively pleasant odour.

## NITROGEN COMPOUNDS

Nitrogen compounds in crude oil are complex. They may cause discolouration in gasoline and kerosene or may produce a lacquer quality there by reducing the effectiveness of lubricating oils. Almost all crude oils contain nitrogen usually in quantities less than 0.1% by weight.

## OXYGEN COMPOUNDS

Some crude oils contain oxygen compounds. Their structures have not been established but on distillation of the crude, the oxygen compounds decompose to form ring compounds with a carboxylic acid group in the side chain. The oxygen content of crude oil is usually less than 2% in weight with heavier oils containing the most oxygen.

## OTHER COMPOUNDS

Several other organic and inorganic compounds occur in crude oil. An example is the metal vanadium which is an important component in the manufacture of specialty steels and can be recovered from the residue of the refining process. Many metallic elements are found in crude oils including most of those that occur in sea water probably because of the close association between sea water and the organic forms from which oil is generated.

Vanadium is the most common metallic element in oil followed by nickel. They probably occur in organic combinations as they do in living plants and animals. Crude oil also contains a small amount of decay resistant organic remains such as siliceous skeletal fragments, wood, spores, resins, coal and various other remnants of former life.



### CONSTITUENTS OF NIGERIA CRUDE OIL

The following are found in Nigeria crude oil;

1. Methane  $\text{CH}_4$
2. Ethane  $\text{C}_2\text{H}_6$
3. Propane  $\text{C}_3\text{H}_8$
4. Iso-butane I- $\text{C}_4\text{H}_{10}$
5. N-butane N- $\text{C}_4\text{H}_{10}$
6. Pentanes  $\text{C}_5\text{H}_{12}$
7. Hexanes  $\text{C}_6\text{H}_{14}$
8. Octanes  $\text{C}_8\text{H}_{18}$
9. Decanes  $\text{C}_{10}\text{H}_{22}$
10. Tetradecanes  $\text{C}_{14}\text{H}_{30}$
11. Hexadecanes  $\text{C}_{16}\text{H}_{34}$

12. Triacontane  $C_{30}H_{62}$

13. Tetracontane  $C_{40}H_{82}$

14. Asphaltane  $C_{80}H_{162}$

There are hydrocarbons of simple, short chains, long chains and branched chains. These hydrocarbons are also called simple paraffins (alkanes), branched paraffins (cycloalkanes), Naphthenes, aromatics, benzene, naphthalene and alkyl derivatives, polynuclear aromatic hydrocarbons (PAHs) which contain two or more fused rings. Essentially, some of these paraffins are gasoline and kerosene.

Crude oil also contains small but significant amounts of impurities such as formation sand and water, sediments, trace metals especially lead (Pb), iron (Fe), copper (Cu), asphalt (As) and Vanadium (V). Compounds of sulphur(s), chlorine(Cl), oxygen(O) and Nitrogen(N) are present as mercaptans, salts, phenols and anilines respectively.

In addition to the constituent compounds that are derived from the crude which result from the interaction of crude oil constituents and living cells also exist when crude oil is spilled or taken into a living cell.

The compounds are;

1. 3, 4 benzo(a) pyrene
2. 20 methylcholanthrene (2methylcholanthrene)
3. 7, 12 dimethyl benzanthracene
4. Polycyclic aromatic hydrocarbons
5. Dibenz (a, b) acridine
6. 5-methyl chrysene
7. Dibenz (a, b) anthracene

## 8. Toluene

A significant effect resulting from crude oil spillage is the emission into the atmosphere some of the gases of non-metallic origin and discharge into the air of volatile hydrocarbons. The emission of these gases constitutes air pollution.

The direct consequences of air pollution is the oxidation of the non metallic gases to form gaseous compounds which produce harmful effects in the environment. The gases include;

1. Hydrogen sulphide
2. Nitrogen oxides
3. Carbon monoxide
4. Ozone
5. Sulphur oxides
6. Particulate sulphates

## **TOXIC EFFECTS OF THE CHEMICALS IN THE NIGERIAN PETROLEUM CRUDE OIL.**

1. **ALIPHATIC HYDROCARBONS:** The  $C_1$ - $C_4$  aliphatic hydrocarbons are simple asphyxiants and their effects are observed when they are highly concentrated in air leading to reduction in the amount of oxygen
2. The  $C_5$ - $C_8$  aliphatic hydrocarbons depress the central nervous system causing dizziness and in-coordination.

### **Paraffin, gasoline and Kerosene**

These contain aliphatic, aromatic and a variety of branched chain and unsaturated hydrocarbons. Ingestion or exposure of humans to these agents produce unpleasant signs and symptoms which include in-coordination, restlessness, excitement, confusion,

disorientation, ataxia, delirium and coma. Paraffinic vapour sensitizes the myocardium such that small amounts of circulating epinephrine may precipitate ventricular fibrillations.

High concentrations of paraffinic vapour may lead to rapid depression of the central nervous system and finally death from respiratory insufficiency. Poisoning from these hydrocarbons result either from inhalation of the vapours or from ingestion of the liquid. Ingestion is known to be more hazardous. Ingestion of the substances can be easily aspirated into the respiratory tract by vomiting or eructation.

Chemical pneumonitis complicated by secondary bacterial pneumonia and pulmonary edema is the most serious sequel to aspiration. Death usually occurs by haemorrhagic pulmonary edema within 16-18 hours.

## **AROMATIC HYDROCARBONS**

Aromatic hydrocarbons example benzene is very toxic. After acute exposure to large amounts to benzene either by ingestion or breathing concentrated vapours, the major toxic effects is on the central nervous system (CNS). Symptoms from mild exposure include headache, nausea, vomiting, tightness in the chest and staggering. With more severe exposures, symptoms progress to blurred vision, tremors, shallow and rapid respiration, ventricular irregularities, paralysis and unconsciousness. Chronic exposure to benzene is usually due to inhalation of vapour.

Signs and symptoms include effects on the central nervous system and the gastrointestinal tract which include nervousness, headache, and loss of appetite, drowsiness and palor. Aplastic anaemia is the major manifestation of toxicity. The most sensitive to benzene are bone marrow cells in early stages of development with arrest of maturation leading to gradual depletion of circulating cells.

## GENOTOXICITY OF AROMATIC HYDROCARBON DERIVATIVES

### BENZENE

The major effect of benzene from long term exposure is on the blood. Long term exposure could be a year or more. Benzene causes harmful effects on the bone marrow causing a decrease in red blood cells thereby leading to anaemia. Epidemiological studies demonstrate that benzene is a human leukenogen. Benzene has also been shown to be a multi organ carcinogen in animals, high levels of benzene can cause eye, skin and respiratory irritation, difficulty breathing, cardiovascular effects such as ventricular fibrillations, gastritis, kidney congestion and neurological effects such as distal neuropathy, abnormality in nerve conduction velocity, difficulty sleeping and memory loss.

At high levels of exposure, confusion, convulsive movements, paralysis and death can occur. Intermediate and chronic exposure to benzene cause a variety of pathological states which include cytopenia (anaemia, leukopenia or thrombocytopenia).

Central nervous system effects such as headache, dizziness, fatigue, anorexia, visual disturbances, hearing loss and respiratory irritation evidenced by difficulty in breathing. Benzene has also been shown to be an immune suppressive agent. Critical effect of chronic exposure to benzene is an increased risk of cancer.

Benzene can also cause chromosomal aberrations in humans. Chromosomal analyses have been used in investigations of benzene exposures.

### ARENE OXIDE

Arene oxide is a metabolite of benzene and two important families of arene oxides are benzene oxides and naphthalene oxides which are intermediates in the oxidative degradation of benzene and naphthalene which are two common pollutants.

Arene oxide causes leukemia and its threshold value is set at 1ppm (parts per million).

Other metabolites of aromatic hydrocarbons such as benzo (a) pyrene, 3 methycholanthrene, benza anthracene dibenz, anthracene and toilene are carcinogenic.

## OZONE AND NITROGEN DIOXIDE

### OZONE

Ozone is found in very high amounts during a prolonged spillage of crude petroleum. It is a lung irritant capable of causing death from respiratory edema. Long term exposure to ozone may cause thickening of the terminal respiratory bronchioles, chronic bronchitis, fibrosis and emphysematous changes are observed in a variety of species exposed to ozone at concentrations slightly above 1ppm.

The ozone layer or ozone shield is a region of the earth's stratosphere that absorbs most of the sun's ultraviolet radiation. It contains high concentration of ozone relative to other parts of the atmosphere.

The ozone layer contains less than 10parts per million of ozone while the average ozone concentration in earth's atmosphere as a whole is only about 0.3 parts per million. The ozone layer absorbs 97-99% of the sun's medium frequency ultra violet light from (about 200nm – 500nm) wave length which otherwise would potentially damage exposed life from near the surface.

The ozone molecule is unstable and is created in the earth's stratosphere by ultra violet light striking ordinary oxygen molecules containing two oxygen atoms (atomic oxygen). It then combines with unbroken oxygen to form ozone, the molecule is unstable and when ultra violet light hits ozone it splits into a molecule of  $O_2$  and an individual atom of  $O_2$  (oxygen) in a process known as the ozone-oxygen cycle.

The ozone-oxygen cycle is the process by which ozone is continually regenerated in earth's stratosphere, converting ultraviolet radiation (UV) into heat. Sydney Chapman resolved the chemistry involved in 1930. The process is commonly called the Chapman cycle. He discovered the photochemical mechanisms that give rise to the ozone layer.

Ozone is also known tri-oxygen is composed of 3(three) oxygen atoms. That is to say one molecule of ozone is made up of 3 oxygen atoms and averages 3 molecules of ozone for every 10million air molecules. The ozone layer was discovered by Charles Faby and Henri Buisson in 1913. The British meteorologist G.M.B Dobson developed a simple spectrophotometer (the Dobson meter) for use in measuring atmospheric ozone. September 16<sup>th</sup> has been designated for the preservation of the ozone layer by the United States general assembly.

### **NITROGEN OXIDE**

Nitrogen dioxide is a pollutant that is capable of causing pulmonary edema and is a particular risk to farmers. Chronic exposure results in emphysematous changes. Nitrogen dioxide can irritate the lungs and also lower resistance to respiratory infections such as influenza.

Effects are not clear with short term exposures but frequent exposure to concentrations that are typically much higher than what is found in ambient air may likely cause increased incidence of acute respiratory illness in children.

### **CARBON MONOXIDE**

Carbon monoxide is a major pollutant that results from crude oil spillage because its natural source is atmospheric oxidation of methane, a gas that is found in abundance in petroleum crude oil.



It reduces the oxygen carrying capacity of blood. Signs and symptoms of carbon monoxide poisoning include headache, weakness, dizziness, nausea, vomiting, syncope, increased respiration and pulse, depressed cardiac function, respiratory failure, coma and death.

The pathology of acute carbon monoxide poisoning indicates that the tissues most affected are the brain and heart and lesions are predominantly haemorrhagic. During prolonged and low level exposure to carbon monoxide, the heart is highly susceptible since there is a shift in metabolism from aerobic to anaerobic. Foetuses have been observed to be extremely susceptible to effects of carbon monoxide during prolonged exposure with the gas readily crossing the placenta. Women that survived short term exposures to high concentrations of the gas while pregnant delivered infants that often displayed neurological sequelae with possibilities of gross damage to the brain.

Polycythemia develops with long term exposures to carbon monoxide. Poisoning occurs after much inhalation of the gas. It is a toxic gas but since it is colourless, odourless and tasteless and initially non-irritating, it is very difficult to detect.

Carbon monoxide is a product of incomplete combustion of organic matter due to insufficient oxygen supply to enable a complete oxidation to carbon dioxide. Carbon monoxide is frequently produced in domestic or industrial settings by motor vehicles that run on gasoline, diesel, methane or other carbon based fuels. Poisoning occurs when carbon monoxide builds up in the blood stream.

When there is too much carbon monoxide in the air, the body replaces the oxygen in the red blood cells with carbon monoxide which can lead to serious tissue damage or death.

## **SULPHUR DIOXIDE AND SULPHURIC ACID**

Because crude oil contains sulphur, oxidation may occur from sulphur oxides and sulphuric acid during a spill of great magnitude. Oxides and sulphates of sulphur primarily cause bronchial constriction and increase in airway resistance which leads to a decrease in pulmonary function particularly with sulphur dioxide. Particulate sulphates also have similar effects to those of sulphur oxide and sulphuric acid. Sulphur dioxide is a gas with a suffocating odour that is similar to a just struck match. It has an acidic taste and is a liquid when under pressure.

Sulphur dioxide is formed when fuels containing sulphur such as coal and oil are burned. The chemical symbol for sulphur dioxide is  $\text{SO}_2$ . Most sulphur dioxide in the air comes from burning of coal and oil at electric power plants. Other sources of sulphur dioxide in the air are industrial facilities that use coal or oil, petroleum refineries, cement manufacturing, metal processing, paper pulp manufacturing and copper smelting, trains, large ships and some equipments burn high sulphur fuel which releases sulphur dioxide into the air. Volcanic eruptions also release sulphur.

### **Signs and symptoms of poisoning**

Breathing difficulties and obstruction of airways especially for patients with lung disease. Long term exposures to persistent levels of sulphur dioxide can cause chronic bronchitis, emphysema and respiratory illness.

It can also exacerbate existing heart disease. Short term exposure can also cause stomach pain, menstrual disorders, watery eyes, inhibition of thyroid function, nausea, vomiting, fever, convulsions and dizziness. Prolonged industrial exposure to sulphur dioxide may decrease fertility in men and women.

Breathing sulphur dioxide can irritate the nose, throat and lungs and cause coughing and shortening of breath.

## SULPHURIC ACID

Sulphuric acid is a highly corrosive, strong mineral with a molecular formula  $H_2SO_4$  and molecular weight 98.079g/mol. It is a pungent ethereal colourless to slightly, yellow viscous liquid which is soluble in water at all concentrations. It is sometimes dyed dark brown during production to alert people to its hazards. Sulphuric acid shows different properties depending on its concentration.

It has strong dehydrating and oxidizing properties. At high concentrations, sulphuric acid can cause very serious damage upon contact since not only does it cause chemical burns via hydrolysis, it also causes secondary thermal burns through dehydration. It can lead to permanent blindness if splashed into the eyes and irreversible damage if swallowed. It is hygroscopic readily absorbing water vapour from the air.

Applications of sulphuric acid include: Domestic acidic drain cleaner, electrolyte in lead acid batteries.

## OCIMUM GRATISSIMUM

### Scientific classification

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Asterids
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum

Species O. gratissimum

### **Binomial name – *Ocimum gratissimum***

*Ocimum gratissimum* is a common culinary herb in West Africa, it has many local names; Efirin in Yoruba, Nchanwu/Arimu in Igbo, Ntong in Ibibio/Efik Ekeni in Kalabari. It is known colloquially as scent leaf.

### **PHARMACOLOGY**

The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity. The essential oil has potential for use as a food preservative.

*Ocimum gratissimum* is an aromatic perennial plant native to Africa, Madagascar and South Asia. It grows up to a meter in height and has multiple branches that arise from its base. The leaves are elliptic and lanceolate with a characteristic tapering of the leaves at both ends. It also has long pale, white to pinkish flowers or spikes.

### **CONSTITUENTS**

There are at least 6 (six) chemotypes mainly eugenol, thymol, citral, ethyl cinnamate, geraniol and linalool. Eugenol has been identified to be the most economically important. Recent studies have shown that phenyl propanoids are the major constituents of *Ocimum gratissimum*.

Scientific family is Lamniaceae (mint).

## EUGENOL

Eugenol is an allyl chain-substituted guaiacol, a member of the allyl benzene class of chemical compounds. It is a colourless to pale yellow aromatic oily liquid extracted from certain essential oils especially from clove oil, nutmeg, cinnamon, basil and bay leaf.

Eugenol has a pleasant, spicy, clove like scent and the name is derived from *Eugenia caryophyllata* which is the former Linnean nomenclature term for cloves.

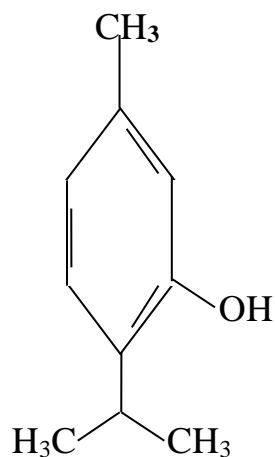
Eugenol is one of the most powerful anti-oxidants. It is an antibacterial and antimicrobial agent. It also has anti-inflammatory properties. Although cloves are the richest plant sources of eugenol, it is also found in significant amounts in bay, rum, nutmeg, turmeric, bay leaf and many other medicinal plants.

## THYMOL

Thymol also known as 2-isopropyl 5-methylphenol (IPMP) is a natural monoterpenoid phenol derivate of cymene;  $C_{10}H_{14}O$ . It is found in oil of thyme and extracted from *thymus vulgaris* the common thyme.

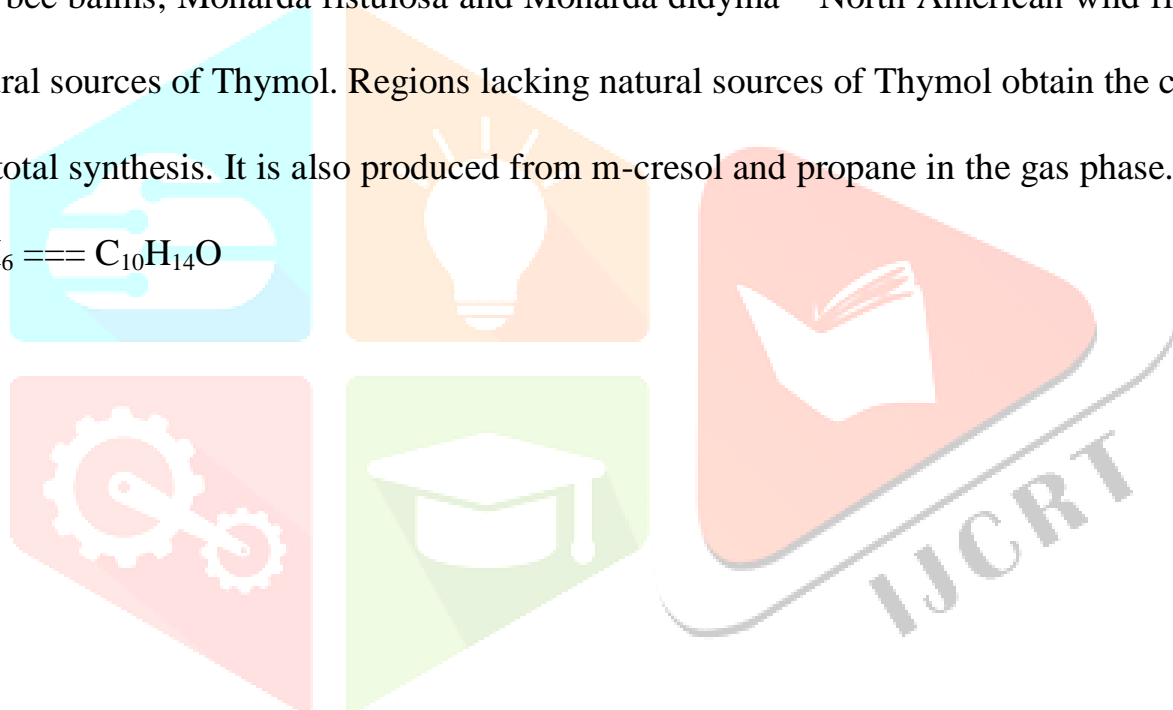
Thymol is isomeric with carvacrol is useful as an antifungal agent an antibacterial and is also the active antiseptic ingredient in some tooth pastes. It is used to reduce plaque and gingivitis, as a medicinal disinfectant and a general purpose disinfectant. It is a pesticide that offers a safe alternative to other more persistent chemical pesticides that can be dispersed in runoff and produce subsequent contamination.

Thymol also provides the distinctive strong flavour of the culinary herb thyme.



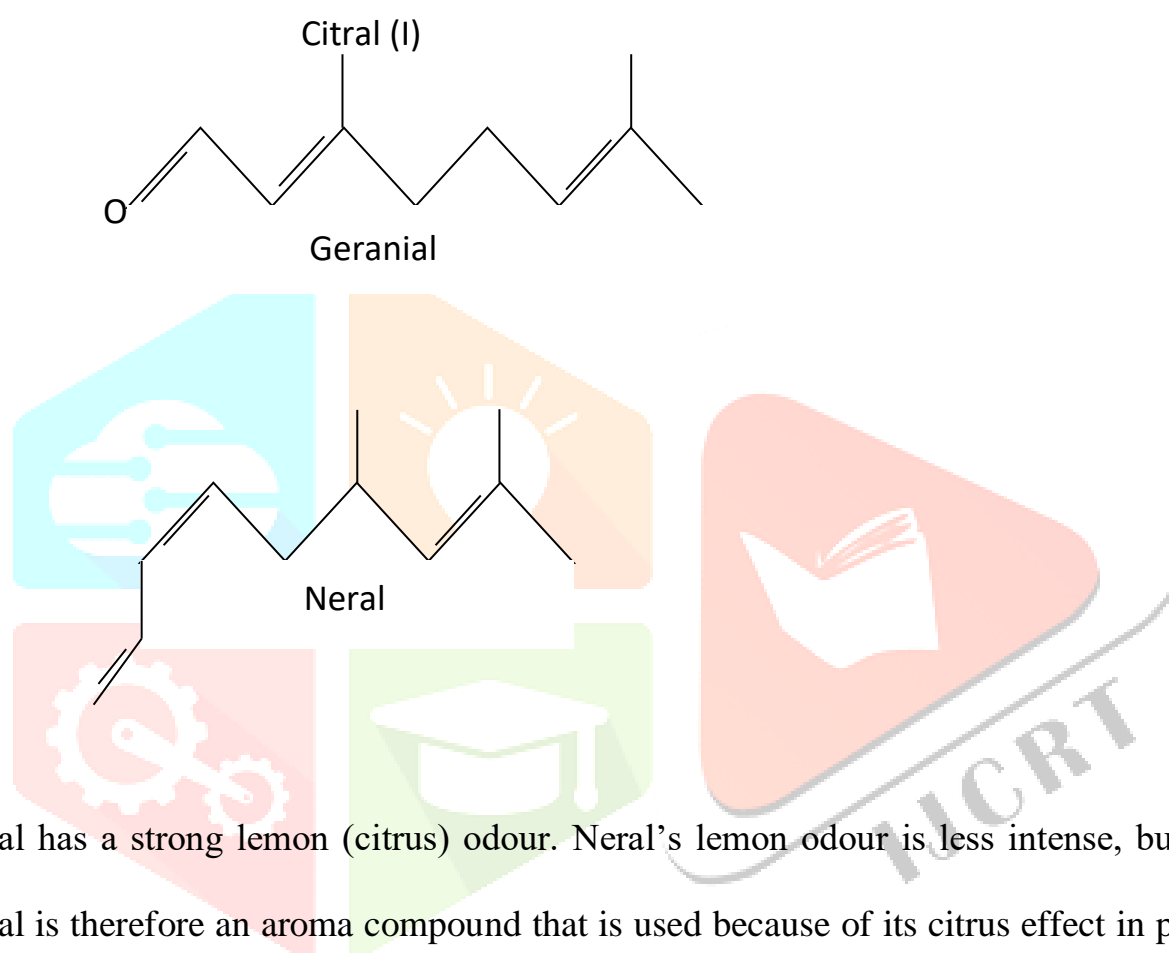
**THYMOL**

The bee balms; *Monarda fistulosa* and *Monarda didyma* – North American wild flowers are natural sources of Thymol. Regions lacking natural sources of Thymol obtain the compound via total synthesis. It is also produced from m-cresol and propane in the gas phase.  $C_7H_8O + C_3H_6 \rightleftharpoons C_{10}H_{14}O$



## CITRAL

Citral or 3, 7-dimethyl-2, 6-Octadienal or lemonal is either a pair or a mixture of terpenoids with the molecular formula  $C_{10}H_{16}O$ . The two compounds are double bond isomers, the E-isomer is known as geranial or citral A while the Z-isomer is known as neral or citral B.

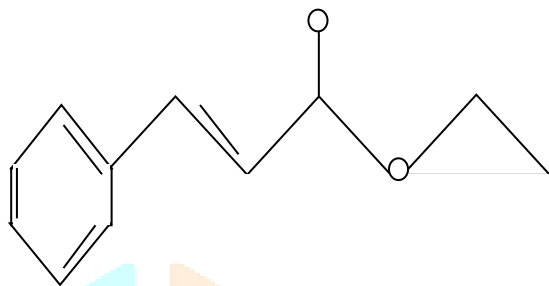


Citral has a strong lemon (citrus) odour. Neral's lemon odour is less intense, but sweeter. Citral is therefore an aroma compound that is used because of its citrus effect in perfumery. It has strong antimicrobial qualities and pheromonal effects in acari and insects.

Citral is used in the synthesis of vitamin A, lycopene, ionone and methyl ionone to mask the smell of smoke. It is strongly sensitizing to allergies it is present in the oils of several plants including lemon myrtle, lemon tea tree, Lindera, citriodora, lime, lemon iron bark etc. Citral can be synthesized from myrcene.

## ETHYL CINNAMATE

Ethyl cinnamate also known as B-phenyl acrylate and 3-phenyl propionate is the ester of cinnamic acid and ethanol. In its pure form, it has fruity and balsamic odour that is similar to cinnamon.



Ethyl (2E)-3-phenylprop-2-enoate

Other name

Ethyl cinnamate

Ethyl cinnamate appears as a colour less oily liquid at room temperature. It is an important kind of fragrance and spice intermediate. It can be obtained through the esterification, between cinnamic acid and ethanol in the presence of sulphuric acid with a yield of about 60%.

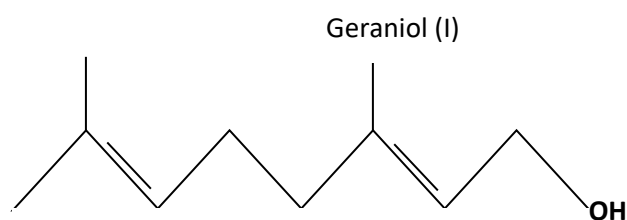
It can also be derived through the esterification between benzaldehyde and ethyl acetate in the presence of sodium metal. It can be used for the formulation of edible fragrance of almond, vanilla, cinnamon, honey, berries, mixed sweet drinks, grape and cherry flavours.

Owing to its high boiling point and stability, it is appropriate to be used for baked goods. Ethyl cinnamate has a P-methoxy derivative which has been reported to be a monoamine oxidase inhibitor. Ethyl cinnamate is present in the essential oil of cinnamon. An example of a plant that contains ethyl cinnamate is *Kaempferia galanga*.



## GERANIOL

Geraniol is a monoterpene and an alcohol. It is a colourless oil but some commercial samples appear yellow.



Its chemical formula is  $C_{10}H_{18}O$

Geraniol is the primary component of rose oil, Palma Rosa oil and citronella oil. It also occurs in small quantities in geranium, lemon and many other essential oils.

It is produced in the scent glands of honey bees to mark nectar bearing flavours and locate the entrances to their hives. It is used in flavours such as peach, raspberry, grape fruit, red apple, plum, orange, lemon, pineapple and blueberry.

Geraniol is important in the biosynthesis of other terpenes. It has been shown to sensitize tumour cells to commonly used chemotherapies including fluorouracil and docetaxel represents a promising cancer chemotherapeutic agent.

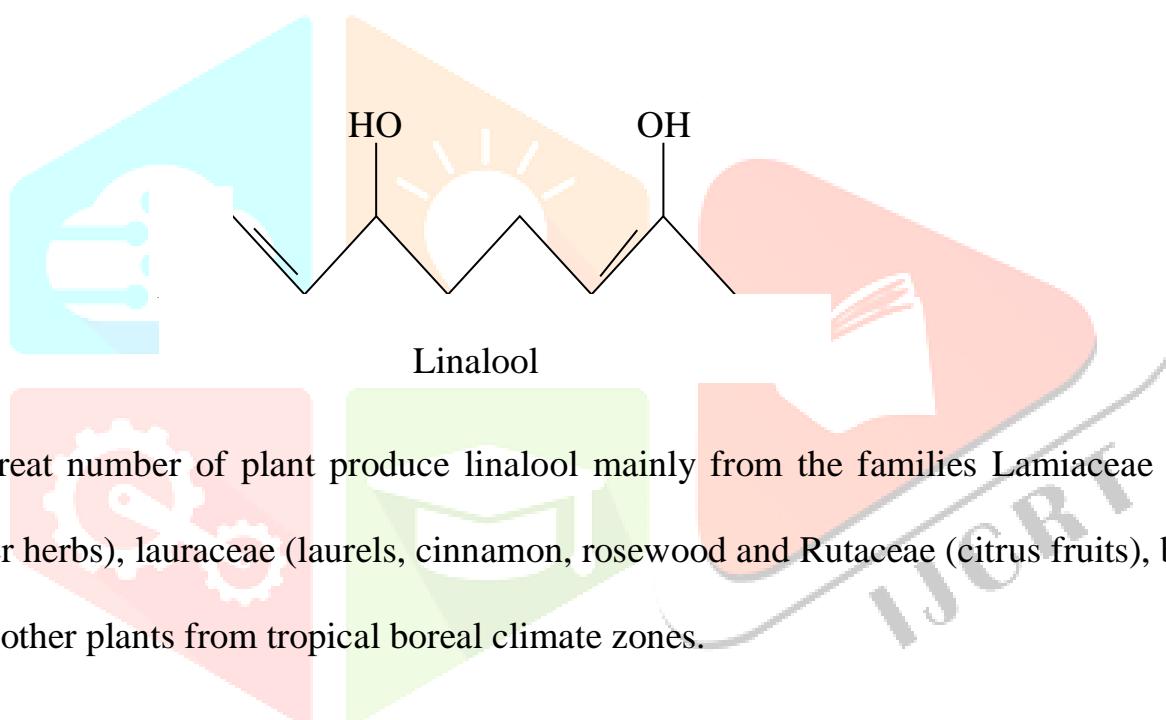
Geraniol has demonstrated a wide spectrum of pharmacological activities including anti microbial, anti inflammatory, anti-oxidant, anti cancer and neuro protective functions. It is commonly used by food, fragrance and cosmetic industry.

Geraniol is found within many essential oils of fruits, vegetables and herbs, lavender and other aromatic plants. It is approved for use within allergenic epicutaneous patch tests which are indicated for use as an aid in the diagnosis of allergic contact dermatitis (ACD) in person's 6 years of age and above.

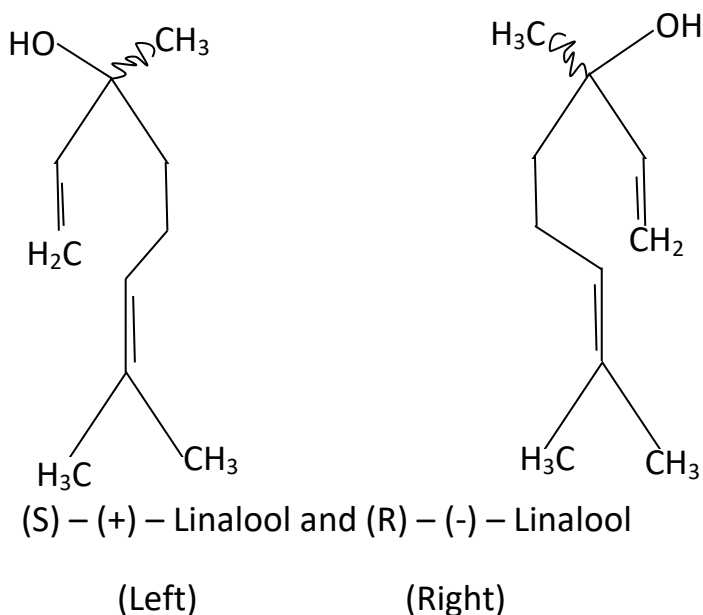
It is stable in human whole blood whereas, following intravenous (IV) administration; geraniol is eliminated from the blood stream within a relatively short half life of about 12minutes.

## LINALOOL

Linalool refers to two (2) enantiomers of naturally occurring terpene alcohol found in many flowers and spice plants. It has other names such as B-linalool, linalyl alcohol, linaloyl oxide, allo-ocimenol and 3,7-dimethyl -1,6 octadien-3-ol.



A great number of plant produce linalool mainly from the families Lamiaceae (mint and other herbs), lauraceae (laurels, cinnamon, rosewood and Rutaceae (citrus fruits), birch trees and other plants from tropical boreal climate zones.



Both enantiomeric forms are found in nature (s) – linalool is found for example as a major constituent of the essential oils of coriander (*Coriandrum sativum*), sweet orange (*Citrus sinensis*) flowers. (R)-linalool is present in lavender (*Lavandula officinalis*), bay laurel (*Laurus nobilis*) and sweet basil (*Ocimum basilicum*) among others. Linalool is used as a scent in 60-80% of perfumed hygiene products and cleaning products/agents including soaps, detergents, shampoos and lotions.

Linalool is also used as a chemical intermediate. Some plants that contain linalool include;

- a. *Ocimum basilicum*
- b. *Cannabis sativa*
- c. *Cannabis indica*
- d. *Artemisia vulgaris*

### **PHENYLPROPANOIDS (Phenolic Compounds)**

Phenylpropanoids are a large family of organic compounds that are synthesized by plants from the amino acids phenylalanine and tyrosine. Their name is derived from the 6-carbon aromatic phenyl group and the 3-carbon propane tail of coumaric acid, which is the central intermediate in phenyl propanoid biosynthesis.

Phenylpropanoids can be categorized into five (5) different groups which include flavonoids, lignins, Phenolic acids, stilbenes and coumarins. Ethyl cinnamate is a common example of Phenylpropanoids. Phenylpropanoids are found throughout the plant kingdom where they serve as essential components of a number of structural polymers, provide protection from ultra violet light, defend against herbivores and pathogens and also mediate plant-pollinator interactions as floral pigments and scent components.

Medicinal benefits of consuming phenylpropanoids include; antihypertensive, anti-inflammatory, anti aging, insulin sensitizing activities, anti retroviral and the reduction of the risk of a range of chronic diseases including cardiovascular disease, cancer and osteoporosis. Phenolic compounds are the most widespread dietary anti oxidants, they also play an important role in the removal of harmful by-products (reactive oxygen species).

### **OBJECTIVE OF THE STUDY**

On August 17<sup>th</sup> 2016 an oil spill traced to a crude oil trunk line from the pipeline and products marketing company (PPMC) the products marketing and distribution subsidiary of the NNPC. 10(ten) communities which are Tebujor/Okepele-Ama, Ikpokpo, Okerenkoko-Gbere, Opuedebubor, Opuede, Opuendezion, Atanba, Oto-Gbene, Meke Ama communities in Gbaramatu kingdom along the Escravos river in Warri South-West Local government area of Delta State were affected.

The spill had devastating effects on agricultural capacity and also threatened the local ecosystem. Toxicity was expressed in the inhabitant's example man, aquatic animals and plants of Gbaramatu Kingdom. Subsequently in our laboratory in Bayelsa State, Nigeria rats were fed with crude oil at concentrations below that at the oil spill sites. Yet again, the rats (experimental animals) showed signs and symptoms of toxicity similar to those in humans. These signs and symptoms include loss of appetite, reduced ability to respond to external stimuli, reduced ability to mate, loss of pregnancy and eventually death.

Furthermore, 21days after the crude oil exposure to the rats, extracts of *Ocimum gratissimum* was administered to the experimental animals and it was observed that there was recovery of the rats.

With the observations made, it was pertinent to carryout studies into the pathology of the affected organs in the rats which resulted in their death during repeated exposures to Nigerian crude oil which inspired this present study.

## HISTOLOGY STUDY

The organs obtained from the rats were cut into sections and dehydrated with a range of concentrations of ethyl alcohol and later cleared with xylem and embedded in molten paraffin wax.

The embedded tissue blocks were sectioned with a microtone and slides were prepared with the sections. Staining of the tissues was done using Ehrlich's haematoxylin and eosin blue applying Lillie's method.

## METHODOLOGY

### Materials I

Twenty albino rats where obtained from Daniel Okwudiri's farm in Omoku, Rivers State.

The rats were fed ad libitum (as desired). They were acclimatized to the environment in which the test was carried out for 7days. Five (5) rats were kept in each cage. Petroleum crude oil was obtained from Nigerian National Petroleum Cooperation (NNPC) Port Harcourt.

## PREPARATION OF FOOD AND WATER

The crude oil polluted food and water were prepared by adding 250ml of crude oil to 500ml of tap water and stirred vigorously for 2hours. The crude was later decanted and the water used as the animals source of drinking water.

The other part of the decanted crude oil was mixed with the animal feed in the ratio of 3kg of feed per 500ml of decanted crude oil. The feed and crude mixture was done thoroughly and manually.

### METHOD 1

Male and Female albino rats weighing 180-200g were used for the experiment. The study consisted of two groups which were; (a) the control and (b) the test groups. Each test group consisted of two males and 3 females, the control group also consisted of two male and 3 female rats. The control group received normal food (unpolluted) and water ad libitum, while the test group were given crude oil polluted food and water for 21days.

The animals were weighed during this period at 7days intervals. Appetite was measured by how much quantity of food per body weight was consumed individually and collectively and was scored from 0 which means absence of eating to 4 which means consumption of all the food.

Strength was measured by animals ability to cross graded bars, mating and pregnancy were assumed to have taken place by the presence of sperm in the vaginal fluid and the development of a permanent diestrus which was later accompanied by the observation of physiological bleeding on day 14. Observations on different organs were made with a highly visible haemorrhage in the animals' myocardium.

## METHODOLOGY II

### Materials

The rats that were fed crude oil polluted food and water were used.

Leaves of *Ocimum gratissimum*, plastic mortar and pestle, plastic bowl, water and sieve.

### PREPARATION OF OCIMUM GRATISSIMUM EXTRACT

5kg of fresh leaves of *Ocimum gratissimum* (African basil) was purchased from the Swali market in Yenegoa, Bayelsa State, Nigeria.

The leaves were confirmed to be *Ocimum gratissimum* by a botanist in the department of biological sciences Niger Delta University (N.D.U), Amassoma, Bayelsa State, Nigeria. The leaves were plucked from the stem, washed and placed in the plastic mortar, pounded into a pulpy mass, placed in a basin and 3 litres of clean water was used in soaking the *Ocimum* pulp.

Three hours later, the contents were drained using the sieve and the liquid saved for use as water for the experimental animals. Like the first experiment 5kg of feed was mixed with 450ml water and 50ml *Ocimum gratissimum*.

On day 21 of the second experiment, the animals were “sacrificed” again using the same methods and histological process. After obtaining the samples and viewing under the microscope, it was discovered that the animals had normal myocardium with no signs of haemorrhage.

The control group also showed no histological change, meaning the myocardium of control and test organisms was devoid of blood.

## **EFFECTS OF NIGERIAN CRUDE OIL**

### **RESULTS**

Effects of Nigerian Crude oil on myocardial tissue was reported, the first experiment showed the presence of haemorrhage (blood) in the myocardium; the middle muscular layer of the heart in the test animals while the control group animals had normal myocardial tissue.

In the second experiment incorporating *Ocimum gratissimum*, the test animals had normal myocardial tissue with absence of blood just like the control group rats. This means with incorporation of *Ocimum gratissimum*, the haemorrhage (blood) was stopped.

### **DISCUSSION**

The study provides evidence that exposure of animals to Nigerian crude oil as that observed during massive crude oil spillage is highly toxic to animals (rats). This finding is in agreement with earlier findings (Nwankwoala, 2000): Effects of exposure of Nigerian crude oil on rats and guinea pigs.

The study on the effect on the myocardium showed that animals (rats) had haemorrhagic myocardium once they were placed on crude oil polluted food and water this effect is sufficient in causing the death of the rats.

The toxic manifestations observed on the myocardium are similar to those observed on the effects of crude oil spillage on inhabitants of the affected crude oil spillage sites. The results of this study are also in accordance with other reports of crude oil toxicity. Ziwontin B, O.A. Gerogewill and R.N.P Nwankwoala Jan. 2006: Histopathological effects of Nigerian crude oil on rats.



In the second experiment it was observed that incorporation of *Ocimum gratissimum* in the food and water of the experimental (test group) animals resulted in the abolishment of myocardial haemorrhage probably due to the bioactive and phytochemical properties of *Ocimum gratissimum*.

*Ocimum gratissimum* contains chemotypes mainly eugenol, Thymol, citral, ethyl cinnamate, geraniol and linalool. *Ocimum gratissimum* is made up basically of phenylpropanoids.

*Ocimum gratissimum* contains terpenoids which are useful for the following properties such as anti microbial, anti fungal, anti viral, anti hyperglycaemic, anti inflammatory, anti oxidants, anti parasitic, immunomodulatory and skin permeation enhancer. All these have many healing properties.

The seed and leaves of *Ocimum gratissimum* help in detoxifying the system and in this experiment, cold water extracts of *Ocimum gratissimum* was incorporated into the food and water of test animals. The anti haemorrhagic effects observed in this experiment may be as a result of *Ocimum gratissimum*'s effects on the reduction of the risk of cardiovascular diseases.

In summary, exposure of rats to crude oil led to haemorrhage (bleeding) in the myocardial tissue which was stopped by extracts of *Ocimum gratissimum*.

**REFERENCES**

- Abbot A; 2005: Animal testing more than a cosmetic change PDF Nature 438 (7065): 144-146
- Amdur M.O; 1989: Health effects of air pollutants; Sulphuric acid, the old and the new. Environmental health.
- Boyer J; R.H. Liu 2004; Apple phytochemicals and their benefits Nutr J. 3:1-15
- Casarete and Doull's Toxicology (2019) the basic science of poisons 9<sup>th</sup> edition.
- Croteau R, Kutchan T.M, Lewis N.G 2000 Natural products (secondary metabolites) in: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants American Society of plant Physiologists. Rockville pp 1250-1252
- George F. Kalf and Carroll A. Snyder; Recent advances in metabolism and toxicity of benzene, pages 141 -143.
- Harvard medical school: Jan 2019, Harvard health publishing carbon-monoxide poisoning.
- <https://www.researchgate.net>: 2008 Effects of *Ocimum gratissimum* leaf extracts on cardiovascular and renal function in rats.
- John A. Donald; 2016 In: handbook of carbon monoxide.
- Lillie, R.D; 1965; Histopathologic techniques and practical histochemistry.
- Menzel D.B.: 1984, Ozone: an overview of its toxicity in man and animals; Journal of toxicol. Environ Health, 13; 183-204.
- National research council 2003, Biological effects of oil releases; oil in the sea II
- Nwankwoala R.N.P 2000: effects of Nigerian crude oil on inhabitants of Asitubo Gbanraun.
- Nwankwoala R.N.P and Georgewill O.A. 2000: Reversibility of crude oil effects to rats.
- Nweze E.I: Eze E.E. 2009 BMC complementary and alternative medicine 9(37) Article number 1472.
- Oboh F.O.J, Madsodge, H.I, Enabulele S.A 2009 Nutritional and antimicrobial properties of *Ocimum gratissimum* leaves. Journal of Biological sciences 9:4 (377-380)
- Robert Snyder, Gisela Witz and Benard D. Goldstein 1993; the toxicology of benzene, environmental health perspectives vol.100, pp. 293-295
- Van der Muellen, J.H 1982: some conclusions regarding long term biological effects of some major oil spills. Phil. Trans. Roy. Soc. London B29, 7:335-351

- Zhang et al 2002: the nature of chromosomal aberrations detected in humans exposed to benzene.
- Ziworitin B, Georgewill O.A. and Nwankwoala R.N.P 2006: Histopathological effects of prolonged exposure of Nigerian Crude oil to rats. African A.P.C Environ. Biol. 8: 73-79.

