EFFECT OF METHANOLIC AND AQUEOUS EXTRACTS OF *Annona muricata* STEM BARK ON HAEMATOLOGICAL PARAMETERS IN WISTAR ALBINO RATS

BY

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JUNE, 2021.

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DEPARTMENT OF BIOCHEMISTRY
FACULTY OF LIFE SCIENCES
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JUNE, 2021.

CERTIFICATION

This is to certify that this project work was carried out by **ADEBAYO EMMANUEL GBENGA** with matriculation number **LSC1705024** of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This research work is dedicated to God almighty, for his grace, love and favour throughout my training in University of Benin and may His name be praised forever.

Also to my parents, Mr. and Mrs. ADEBAYO for their immense support physically, financially, emotionally and otherwise as well as everyone who in one way or the other has contributed to my success this far.

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ABBREVIATIONS

AMC	*Annona muricta*
DV	Daily value
FBC	Full blood count
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
NOAEL	No observed adverse effect level
PCT	Plateletcrit
PDW	Platelet distribution width
PI	Platelet indices
PLT	Platelets
RBC	Red blood cell
RDW	Red cell distribution width
WBC	White blood cell
WHO	World Health Organization

ABSTRACT

A wide array of ethno-medicinal values has been attributed to the different parts of *Annona muricata*, and indigenous communities in Africa and South America extensively use this plant to augment conventional drugs.

Acute and subchronic toxicity studies were carried out on the stem bark extract of *Annona muricata Linn.* The aim of this study was to determine the effect of *A. muricata* on hematological parameters in wistar albino rats.

A total of Sixty (60) male albino rats were divided into 12 groups of five (5) rats each. They were given grower pellets and water. Groups one (control), two, three, four, five, six were administered 0, 200, 500, 1000, 3000 and 5000 mg/kg body weight of the extract per day respectively for a period of 28days.Weekly measurements of body weight were carried out. After 30 days, the rats were subjected to overnight fast after which they were sacrificed, with their blood and organ samples collected into sterile containers with anticoagulant.

Hematological profiles showed increased levels of red blood cells and its related indices for both methanolic and aqueous extract of *Annona muricta*. The investigation into the sub-chronic toxicity of methanol and aqueous extract of the stem bark of *Annona muricata* revealed no significant effect on the immunity of wistar albino rats.

Methanolic and aqueous stem extract of *A. muricta* at 3000 mg/kg b.wt and 1000mg/kg body weight respectively may have effect on thrombopoiesis and morphology of blood platelets establishing the acclaimed wound-healing properties of *A. muricata.* We posit that the results justify the use of the stem bark of *Ann6ona muricata* as a hematinic with hemomodulatory properties and for treatment of wounds by traditional medical practitioners showing its relative safety. Further experiments are needed to evaluate its safety.

CHAPTER ONE

1.0 INTRODUCTION

The World Health Organization consultative group defined a medicinal plant as one which a single or more of its part contains substances that can be used for therapeutic purposes, or which are precursors of useful drugs (Sofowora, 1982).

Medicinal and herbal plants are considered as the basis for health preservation and care around the world. Natural products, especially phytochemicals, have been used by mankind to sustain health since the dawn of medicine. The first herbal records date back to 2838–2698 B.C., when the Chinese emperor Shen Nung cataloged 365 medicinal herbs. In 1500 B.C., the Egyptian manuscript "Ebers Papyrus" recorded information on 811 prescriptions and 700 drugs (Firmo et al., 2011).

Phytotherapy has provided solutions for ailments to the present day (Peter, Bosze and Horvath, 2017). Non-communucable and degenerative diseases (diabetes, cancer etc.) have reached epidemic proportions and are considered as serious health challanges. Therefore, treatments of these diseases are of clinical importance (WHO, 2005). However, most herbal products have not been subjected to standardization or clinical studies to prove their safety and effectiveness in humans.

In a pharmaceutical landscape, plants with a long history of use in ethno medicine are a rich source of active phytoconstituents that provide medicinal or health benefits against various ailments and diseases. A plant with ernomous traditional use is *Annona muricata*.

Annona muricata is a member of the Annonaceae family and is a fruit tree with a long history of traditional use. *A. muricata*, also known as soursop, graviola and guanabana, is an evergreen plant that is distributed mainly in tropical and subtropical regions of the world. The Annonaceae family has attracted attention due to its bioactivity and toxicity (Pinto *et al.,* 2005).

A wide range of ethnomedicinal activities is contributed to different parts of *A. muricata* and indegenious communities in Africa and South America extensively use this plant as traditional medicine (Moghadamtousi *et al.,* 2015).

Phytochemical studies reveal that alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterols, terpenoids, proteins, essential oils, and annonaceous acetogenins are the major constituents of *A. muricata*

Ethnobotanical studies have indicated that *A. muricata* has been used as pesticides (Idoko and Ileke,2020) and parasiticide (Ana V. *et al.,* 2016). Fruit juice and infusions of leaves or branches have been used against fever (Banne *et al.,* 2017), sedative (Defilippis et al., 2004),respiratory illness(Beyra et al., 2004), malaria (Voravuth *et al.,* 2016), gastrointestinal problems (Atawodi, 2011) liver, heart and kidney affections (Badrie and Schauss, 2009). *Annona muricata* has also been used in hypoglcemic, hypotensive and cancer treatments(Mishra *et al.,* 2013; Adewole and Caxton-Martins, 2006).

1.1 JUSTIFICATION OF STUDY

Annona muricata has been reported in regions of the world where this this plant is grown due to its several health benefits like its stem bark hot water decoction to treat diabetes.

Most research on the subchronic effect of *Annona muricata i*n wistar rats in recent times have been on the subchronic effects of the aqueous and methanol extracts of the leaves and fruits of *A.*

muricata. In essence, there is very little data available on the subchronic effects of the aqueous and methanol extract of the stem-bark of *A. muricata*.

The importance of this study is to investigate the toxic effects that *Annona muricata* stem bark extract may have on the body by analyzing some biological parameters in Wistar rats after administration of aqueous and methanolic extracts of the stem bark of *Annona muricata.*

1.2 AIM

This study is aimed at perusing the effect of aqueous and methanolic extracts of *Annona muricata* on the haematoloical parameters of wistar albino rats.

1.3 OBJECTIVES

The following were the objectives of the study:

1. To assess the short-term toxic effect of *A.muricata* stem bark.

2. Determination of the effect A.*muricata* stem bark on:

   (i) Hematological parameters (WBCS, RBCs, LYM, GRAN, HCT, HGB, MCV, MCH, MCHC, PLT, PCT)

1.4 LITERATURE REVIEW

1.4.1 Sour Sop…… (*Annona muricata)*

1.4.2 TAXONOMIC HIERARCHY

Kingdom: Plantae (Plants)

Phylum: Tracheophyta (Vascular Plants)

Class: Spermatopsida

Order: Magnoliales

Family: Annonaceae (Custard Apple Family)

Genus: Annona L.

Species: A. muricata. L

Binomial name: Annona muricata. Linn.

1.4.3 DESCRIPTION

Annona muricata is of the genus Annona of Annonaceae family, a slender, evergreen tree which is the most widely grown among over 70 species of the genus Annona (Mishra *et al.,* 2013). The plant is 5-10 m in height and 15-83cm in diameter (Orwa et al., 2009). Straight trunk, smooth bark, dull grey, rough and fissured with age. The inner bark is pinkish and tasteless, branches first ascending with the crown forming an inverted cone but later spreading. Crown at maturity is spherical due to lack of apical dominance; twigs are brown or grey bearing minute raised dots (lenticels); root system extensive and superficial, spreading beyond the diameter of the crown although shallow rooted; juvenile plants have a taproot that is eventually lost.

Leaves are 7.6-15.2 cm long, 2.5-7.6 cm wide, leathery, obovate to elliptic, glossy on top, glabrous on underside, simple; stipules are absent; oblanceolate blades, green on top, paler and dull on under side with fine lateral nerves; a strong, pungent odour; petioles short, 3-10 mm long.

Flowers are terminal or lateral, large; stalks stout, green, 1.3-1.9 cm long; 3sepals, minute, inconspicuous, broad, green, 3 mm long, triangular; petals yellowish-green, 6 in 2 whorls of 3, outer petals larger, ovate-acute, valvate, cordate with pointed apex (heart shaped), 4-5 x 3-4 cm, 3 mm thick and fleshy, fitting together at edges in bud and rough on the outside;3 inner petals, narrow, smaller, nearly 3.8 cm long, thinner, rounded,concave with fingernail-shaped base and overlapping edges; stamens are numerous, shield shaped, united below; anthers are parallel and opening longitudinally; carpels are numerous, overtopping the stamens, each with 1ovule; pistils white, narrow, 5 mm long, with sticky stigmas.

Fruits, 14-40 x 10-18 cm, weighing up to 7 kg, ovoid, heart shaped, anoblong syncarp composed of numerous united pistils, pistils end in a fleshy spine or short base of spine 1.5 mm or more in length, which grows from the style; often asymmetric due to incomplete fertilization of the ovules; epidermis often shining, dark green, with short, fleshy spines covering each carpel; pulp white, fibrous and juicy; seeds shiny, dark brown or black, oblong, up to 2 cm long, 0.7 cm wide (Orwa *et al.,* 2009)

Figure 1: *Annona muricata* L (A) Whole plant (B) leaves; (C) flowers (D) fruits (E) Seeds. (Frontiers in Pharmacology, 2018)

1.4.4 DISTRIBUTION

Annona muricata is tolerant of poor soil (PIER, 2008) and prefers lowland areas between the altitudes of 0 to 1,200 metres (3900ft). It cannot stand frost. It is native to the tropical regions of the Americas and is widely propagated (CABI, 2018). It is an introduced species on all temperate continents, especially in subtropical regions.

1.4.4 ECOLOGY

A. muricata survives in the humid tropical and subtropical lowlands at an altitude of below 1200m above sea level, with temperature between 25 and 28 C, relative humidity between 60 and 80%, and annual rainfall above 1500mm. It is common on the coast and is found on slopes. Planted for its fruit, it has become wild or naturalized in thickets, pastures and along roads. The species is commonly cultivated in home gardens and is found in rural garden areas on volcanic and raised limestone islands, where it is occasionally naturalized (CABI, 2019).

Climatic requirements for cultivation

Mean annual temperature: 25-30 deg.C

Mean annual rainfall: Over 1000mm

Soil type: Prefers loose, fairly rich, deep loam with pH range of 5.5-6.5

1.4.5 GEOGRAPHICAL SPECIES DISTRIBUTION

Native of Argentina, Bahamas, Barbados, Bolivia, Brazil, Chile, Colombia, Cuba, Dominica, Honduras, Jamaica, Martinique, Mexico, Montserrat, Netherlands Antilles, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, Sri Lanka, St Kitts and Nevis, St Lucia, St Vincent and the Grenadines, Surinam, Trinidad and Tobago, Uruguay, Venezuela, Virgin Islands (US), Zanzibar, Benin, Cambodia, China, Cote d'Ivoire, Eritrea, Ethiopia, Ghana, Guinea, India, Indonesia, Laos,Exotic plant in Liberia, Mauritania, Nigeria, Papua New Guinea, Philippines, Reunion, Senegal, Sierra Leone, Tanzania, Thailand, Togo, Uganda, US, Vietnam.Dominican Republic, Ecuador, French Guiana, Grenada, Guatemala,Guyana, Haiti.

1.4.6 PRODUCTS OF *Annona muricata*

1.4.6.1 FOOD

A. muricata can be consumed fresh for dessert when fully ripe or mixed with ice cream or milk to make a delicious drink, as is done in Java and in Cuba and other parts of America. The pulp can be made into fruit jelly, juice (with sugar added), nectar or syrup. In Indonesia sweetcake (‘dodol sisrak’) is made by boiling *A. muricata* pulp in water and adding sugar until the mixture hardens. In the Philippines, young fruits with seeds that are still soft are used as a vegetable. Mature but firm fruits may be made into candy of delicate flavour and aroma.

1.4.6.2 TIMBER

Sapwood is whitish and heartwood brown. The wood is soft, light (specific gravity of 0.4), not durable; it is rarely used as timber but has been used for ox yokes.

1.4.6.3 POISON

Powder of dried leaves and sap from fresh ones are useful in destroying vermin. Powder or oil from the seeds has been used to kill lice and bedbugs. All tree parts have insecticidal properties and can be used, with fruit as bait to kill fish.

1.4.6.4 MEDICINE

The crushed leaves are applied to mature boils and abscesses or are used as a remedy for distention and dyspepsia, scabies and skin diseases, rheumatism, coughs and colds. The leaves may also be used to make a decoction, which is taken orally with salt for digestive tract ailments and to relieve fatigue. A crushed leaf and seed decoction is taken orally for intestinal malaise. A massage of the leaves is good for nervous shock, while a leaf or bark decoction is used for anxiety attacks. Flower or flower bud tea is mixed with honey for colds, chest pain and nerve disorders, and the bark and young fruits, which contain tannin, are used to treat diarrhoea and dysentery. The green bark is rubbed on wounds to stop bleeding.

Sour sop leaves are sold and consumed in Indonesia as herbal medicine. The leaves are usually boiled to make tea. It is believed to have medicinal benefits such as for preventing cancer and anti-inflammation.

1.4.7 NUTRITION

Raw sour sop is 81% water, 17% carbohydrates, 1% protein and has negligible fat (table 1). In a 100gram reference amount, the raw fruit supplies 66 kilocalories, and contains only vitamin C as a significant amount (25%) of the daily value, with no other micronutrients in appreciable amounts (table 1)

Table 1.4.7 Nutritional value per 100 g (3.5 oz) of Sour sop

Nutritional value per 100 g (3.5 oz)	
Energy	276 kJ (66 kcal)
Carbohydrates	16.84 g
Sugars	13.54 g
Dietary fiber	3.3 g
Fat	0.3 g
Protein	1 g
Vitamins	**Quantity %DV**
Thiamine (B1)	6% 0.07 mg
Riboflavin (B2)	4% 0.05 mg
Niacin (B3)	6% 0.9 mg
Pantothenic acid (B5)	5% 0.253 mg
Vitamin B6	5% 0.059 mg
Folate (B9)	4% 14 µg
Choline	2% 7.6 mg

Vitamin C	25% 20.6 mg

Minerals	**Quantity %DV**
Calcium	1% 14 mg
Iron	5% 0.6 mg
Magnesium	6% 21 mg
Phosphorus	4% 27 mg
Potassium	6% 278 mg
Sodium	1% 14 mg
Zinc	1% 0.1 mg

Other constituents	**Quantity**
Water	81 g

Units
μg = micrograms • mg = milligrams
IU = International units

Source: USDA Food Data Central

1.4.8 PHYTOCHEMICAL AND PHARMACOLOGICAL/BIOLOGICAL ACTIVITY

A number of bioactive compounds and phytochemicals present in *Annona muricata* has been reported and isolated. This is linked to the ethnomedicinal properties of the plant and its antioxidant properties.(Agu, Okolie, Eze,Anionye, & Falodun,2017; Agu, Okolie, Falodun, et al.,2017; Ahalya, Shankar,& Kiranmayi,2014; Okolie, Agu, & Eze,2013; Gupta, et al., 2011; Baskar, et al., 2007). Many bioactive compounds and phytochemicals mainly annonaceous acetogenins and essential oils have been isolated from *A.muricata.* The phytochemicals present in *Annona muricata* are alkaloids, flavonoids, cardiac glycosides, saponins, tannins, phytosterols, terpenoids (Edeoga, *et al.,* 2005). Agu,Okolie, Eze,*et al.*(2017) reported the presence of alkaloids, flavonoids and phenols in high quantities especially in fruit pulp and leaf.

Table 1.4.8 Phytochemical screening of the various parts of Annona muricata.

Phytochemical	Fruit	Leaf	Root- bark	Stem- bark
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Phlobatannins	–	–	–	–
Terpenoids	+	+	+	+
Carbohydrates	+	+	+	+
Cardiac glycosides	–	+	+	–
Reducing sugars	+	+	+	+
Monosaccharides	+	+	+	+
Pentoses	+	+	+	+
Ketoses	+	+	+	+
Starch	+	+	+	+
Protein	+	+	+	+
Arginine	+	+	+	+

Cysteine	+	+	+	+
Aromatic amino acids	+	+	+	+
Phenolic amino acids	+	+	+	+
Anthraquinones	–	–	–	–
Alkaloids	+	+	+	+
Steroids	+	+	+	+
Phenolics	+	+	+	+
Nitrogen and halides (Cl^-)	+	+	+	+
Sulfur and sulfate ion	+	+	+	+
Nitrate ion	+	+	+	+

Source: Agu and Okolie, 2017.

The isolated compounds from this plant have also displayed some interesting In Vivo and In Vitro biological and pharmacological actives such as anti-tumoral, cytotoxicity, anti-parasitic, pesticidal properties (Gleye et al; 1999), anti- hyperglycemic activity, antinoceptive and anti-inflammatory activity,anti-depression, antihypertensive, antimicrobial, anti-nociceptive activities anti-hyperlipidemia Insecticidal, larvicidal and repellent activity etc.

1.4.8.1 ANTI-HYPERLIPIDEMIA

Study of methanolic extracts of *A.muricata* on serum lipid profiles in experimentally-induced diabetic wistar rats showed antihyperlipidemic activities with significant reductions in total cholesterol, LDL and VLDL and a significant increase in HDL and anti-atherogenic index (Adeyemiet al., 2008).

1.4.8.2 HEPATOPROTECTIVE ACTIVITY

The alcoholic extract of *A.muricata* renders an overall protection against CCl4 induced toxicity by scavenging the free radicals produced by CCl4 metabolism. Thus it provides protection against increase in serum glutamic oxaloacetic transminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), liver and brain lipid peroxidation (LOP)levels and decrease in liver and brain protein levels. All these data suggest that the plant drugs possess possible antihepatotoxic activity. Traditional medical practitioners in India administer the decoction against liver disorders (Bedd et al., 1999).

The leaf aqueous extract of *A. muricata* reduced the harmful effect and preserved the hepatic physiological mechanism of the liver damaged by a hepatotoxin such as paracetamol (Acetaminophen), a drug widely used as antipyretic and analgesic, which can cause liver damage if taken in excessive (Arthur et al., 2012). Soursop extract reduces bilirubin levels due the glucosides present in the extract, which might be converted into glucuronic acid, conjugating with bilirubin for excretion, or because the extract active regulators increase the activity of enzymes, synthesis of transporter, and steps related to bilirubin clearance pathway (Arthur et al., 2012)

1.4.8.3 ANTIHYPERTENSIVE ACTIVITY

Leaf extract of *A. muricata* caused a dose-dependent reduction in mean arterial pressure (MAP) in normotensive rats (Nwokocha et al., 2012). The suggested hypotensive mechanism of action of aqueous extract of *A. muricata* did not involve the endothelial or nitric oxide-dependent pathways. Studies suggested that plant extracts lower blood pressure through the blockage of calcium ion channel, and this Ca^{+} antagonism is further demonstrated by its ability to relax high K^{+} induced contractions. The hypotensive effect has been attributed to the action of alkaloids (Nwokocha et al., 2012).

1.4.8.4 GASTROPROTECTIVE ACTIVITY

Gastroprotective activity of *A. muricata* leaves was examined against ethanol-induced gastric injury. The results of the oral administration of the ethyl acetate extract (200 and 400 mg/kg) showed significant antiulcer potential, which was mediated through protective effects against gastric wall mucosal damages (Hamid *et al*., 2012)

1.4.8.5 ANTIPLASMODIAL ACTIVITY

Malaria, one of the most debilitating diseases, afflicts a substantial population in tropical and subtropical zones (Snow *et al.,* 1999). The available antimalarial drugs demonstrate varying degrees of failure due to rapid spread of parasite resistance (Winstanley, 2000). Therefore, research into new antiplasmodial agents against the pathogenic parasites is definitely warranted. The pentane leaf extract of *A. muricata* was assayed against two strains of Plasmodium falciparum: The Nigerian chloroquine-sensitive strain and FcM29-Cameroon (chloroquineresistant strain); a promising antiplasmodial effect was obtained with an IC50 value of 16 and 8 μg/mL after 72 h, respectively. The leaf extract, also at 20 μg/mL, showed a 67% inhibition against an asynchronous F32 strain of P. falciparum (Bidla *et al.,* 2004)

1.4.8.6 ANTICONVULSANT ACTIVITY

In African countries, the decoction of the *A. muricata* leaves is traditionally used to control fever and convulsive seizures (N'gouemo*et al.,* 1997). Gouemo and colleagues (N'gouemo*et al.,* 1997) investigated the effect of the ethanolic extract of the leaves against pentylenetetrazol-induced tonic-clonic seizures in mice. The result showed that the plant extract at 100 and 300 mg/kg doses significantly decreased the incidence and the mortality rate of tonic seizures. Administration of the extract to mice also lengthened the onset of clonic seizures. This study showed that a subsequent bioassay-guided investigation may lead to the isolation of a bioactive compound that can be used as an anticonvulsant drug.

1.4.8.7 ANTIOXIDANT ACTIVITY

Immoderate generation of intracellular reactive oxygen species (ROS) is a precursor of oxidative stress which subsequently catalyzes metabolic deficiency and cellular death through biochemical and physiological lesions (Chance *et al.,* 1979). Agu *et al.,* (2017) investigated the influence of the methanolic extracts of the fruit, leaf, stem-bark and root-bark of *Annona muricata* on in vivo antioxidant enzymes activities (superoxide dismutase, SOD; Catalase, CAT and (glutathione peroxidase, GPx) and lipid peroxidation status (malondialdehyde, MDA) of Wistar Rats(WR). Rats weighing 72-120g were divided into four groups of plant parts; fruit, leaf, stem-bark and root-bark) and each group were further sub-dived into six sub-groups that were administered (1ml distilled water), 100, 200, 400, 600 and 800mg/kg body weights extracts per rat. The extracts were administered to the rats for a period of 28days, after which the rats were fasted over-night before they were sacrificed using a decapitator. The results obtained for MDA level showed significant decrease for the groups administered the fruit and stem-bark extracts but an increase for the groups administered leaf and root-bark extract. From these results it was concluded that *Annona muricata* indicates good potentials to avert lipid peroxidation and ameliorate oxidative stress that could arise due to increased production of pro-oxidants.

1.4.8.8 ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE ACTIVITIES

The antinociceptive effect of ethanolic and hydroalcoholic extracts of *A. muricata* has been reported using various chemical and thermal nociceptive models. *A. muricata* produced antinociception action of activity in both neurogenic and inflammatory phases (Roslida et al., 2012). Metabolites of arachidonic acid (called eicosanoids) are involved in inflammation process. These metabolites are produced via cyclooxygenase and lipoxygenase when a cell is activated by mechanical trauma, cytokines, growth factors or other stimuli. It has been proposed that the mechanism of antinociception may be by inhibition of cyclooxygenase (COX) and lipoxygenases (LOX) and other inflammatory mediators by flavonoids present in the plant extract.

1.4.8.9 ANXIOLYTIC AND ANTI-STRESS ACTIVITIES

It is possible to attribute this bioactivity to the alkaloid compounds; especially because two of the isolated alkaloids (anonaine and asimilobine) have relaxing activity. These compounds can influence the central nervous system via the $5HT_{1A}$ receptor. The $5HT_{1A}$ receptor binds with the endogenous neurotransmitter serotonin and is involved in the modulation of emotion (Hasrat et al., 1997a, 1997b). This bioactivity can validate the reason for the traditional use of *A. muricata* as sedative.

1.4.8.10 WOUND HEALING

Bark and leaf extracts showed elevation in wound contraction compared with wound without treatment (Moghadamtousi et al., 2015b; Padmaa et al., 2009). Wound healing consists of four complex phases: coagulation, inflammation, proliferation and maturation. *A. muricata* accelerates some of these phases. In inflammatory phase the protein expression of heat shock proteins (Hsp70) is important for healing due to their role in cell proliferation. *A. muricata* induced upregulation of Hsp70 in wound tissues. In this phase the inflammatory cells produce cytokines and free radicals that in great quantity can produce lipid peroxidation in wound. Tissues treated with *A. muricata* extracts showed elevated activity of CAT, GPx and SOD (Catalase, CAT, glutathione peroxidase, GPx and superoxide dismutase, SOD) that protect tissue against oxidative damage to accelerate the wound healing process. Additionally, *A. muricata* extracts reduce MDA, the biomarker of lipid peroxidation that can cause defect in endothelial cells, fibroblast and collagen metabolism necessary for wound healing. During the maturation phase, the collagen accumulation and fibroblast proliferation occurred. *A. muricata* extracts elevated the deposition of collagen fibers in the wound as observed in histological analysis (Moghadamtousi et al., 2015c).

1.4.8.11 NEUROTOXICOLOGY

Annonacin is a chemical compound with toxic effects, especially in the nervous system, found in sour sop and other plants from the family Annonacaea. It is a member of the class of compounds known as Acetogenins. Annonacin-contaning fruit products are regularly consumed throughout the West Indies for their traditional medicine uses. Annonacin is a disabling and potentially lethal neurotoxin (Levine *et al.,* 2015). Like other acetogenins, it is a mitochondrial complex I (NADH-dehydrogenase) inhibitor (Potts *et al.,* 2012). As NADH-dehydrogenase is responsible for the conversion of NADH to NAD+ as well as the establishment of a proton gradient in the mitochondria, annonacin disables the ability of a cell to generate ATP through oxidative phosphorylation, leading to cell apoptosis or necrosis (Potts *et al.,* 2012).

1.4.9 TOXICOLOGY

Toxicology is a discipline of science that deals with toxins and poisons and their effects and treatment. Toxicological screening is very vital for the development of new drugs and extension of the therapeutic potential of existing molecules. The US Food and Drug Administration (FDA) stated that it is expedient for new molecules to be screened for their pharmacological activity and toxicity potential in animals. The toxic effects of chemicals, food substances, pharmaceuticals have attained great significance in the 21st century. Toxicity testing helps to calculate the no observed adverse effect level (NOAEL) dose and it is also helpful in clinical studies (Setzer and Kimmel, 2003).

1.4.9.1 TOXICITY STUDIES

Toxicity is the extent to which a substance can harm a living organism. Toxicity studies are performed to determine the safety or hazard elicited by substances such as laboratory chemicals and consumer products. Toxicity studies can be acute, sub-chronic or chronic.

1.4.9.2 ACUTE TOXICITY STUDIES

Acute toxicity testing is carried out to determine the effect of a single dose on a particular animal species within 24 hours. In general, it is recommended that acute toxicity testing be carried out with two different animal species (one rodent and one non-rodent). Studies of acute toxicity however tends to establish the dose-dependent effect (s), which may take place and this includes all information that is important in the assessment of acute toxicity including mortality, morphological, biochemical, pathological, and histological changes in the dead animals. The assessment of the lethal dose (LD_{50}) (the dose that kills 50% of test animal's population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity. Acute toxicity study solely gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent (Akhila *et al.,* 2007). Acute oral toxicity is also performed in animals to evaluate the safety of plant-based products and other formulations for humans. The safe dose in these animals is extrapolated to human doses. It has been reported that pathophysiological observations for gastrointestinal and hematological disorder in animals have close relativeness with those observed in humans (Ogbonnia *et al*., 2010). Although doses can be extrapolated for safe uses, it is difficult to ascertain the replication of some pharmacokinetic behavior of plant-based product in humans compared to animals. Prior to any specific kind of pharmacological model, acute toxicity studies are recommended to be performed. In the psychopharmacological studies, acute oral toxicity studies can provide the researcher an estimate of various toxic effects of a single dose of any plant-based product and the effect of each dose and each extract can vary depending on its source. The toxicity assessment of pharmacological agents is a very important procedure that is usually carried-out before they are allowed to enter the market for sale. Conversely, different methods have been developed and adopted for acute toxicity testing. These include the Lorke's method, Karber's method and the Up and down method.

1.4.9.3 SUB-ACUTE TOXICITY STUDIES

Sub-acute toxicity tests are intended to evaluate the toxicity of the chemical after repeated administration and also to help in establishing doses for the longer-term sub-chronic studies. These studies are conducted to evaluate a new drug's potential adverse effects following a treatment period of 2-4 weeks' duration. Most sub-acute studies utilize three to four different dosages of the chemicals, administered by mixing it in the feed. The test substance is administered daily for a certain period through the oral route. If this route is not convenient, the test substance may be administered peritoneally. The test substance is administered regularly at a specific time. Usually, a rodent of any gender and age 5–6 weeks is used for repeated dose toxicity testing. There should be little individual variation between the animals: the allowable variation in the weight is ± 20%. Baseline parameters such as the behavioral and biochemical parameters of the animals should be recorded. These will be helpful in calculating percentage changes (Parasuraman, 2011). At the end of the study, tissues from most of the organs are removed, and histological changes are recorded. Biochemical analysis is carried out on the blood and organ harvested and results of test groups are compared to that of the control group to ascertain toxicity. Consequently, a significant difference between results of test groups and control may indicate toxicity. Sub-acute toxicity studies are conducted to evaluate a new drug's potential adverse effect or effect of a chemical or plant based product. In addition, sub-acute toxicity studies are relevant in choosing dosage levels to be used in subsequent sub-chronic or chronic toxicity studies (Parasuraman, 2011). Sub-acute and sub-chronic toxicity are similar; the difference lies in the duration of the studies.

1.4.9.4 SUB-CHRONIC TOXICITY STUDIES

Rodents and non -rodents are used to study the sub-chronic toxicity of a substance. The test substance is administered orally for 30-90 days, and weekly body weight variations, monthly biochemical and cardiovascular parameters and behavioral changes are observed (Parasuraman, 2011). During this type of study, data about observed effects, mortality, body weight, food/water consumption, physical examinations, hematology, bone marrow, coagulation, blood chemistry, urinalysis, organ weights, gross pathology, and histopathology are collected and analyzed. At the end of the study, the experimental animals are sacrificed. Gross pathological changes are observed, and all the tissues are subjected to histo-pathological analysis. There should be little individual variation between the animals, and the allowed weight variation range is ± 20%. A satellite group may be included in the study protocol, and this group has both a control group and a high-dose group (Muralidhara *et al.,* 2001).

1.4.9.5 CHRONIC ORAL TOXICITY STUDIES

Chronic toxicity studies are conducted with a minimum of one rodent and one non-rodent species. The test compound is administered over more than 90 days, and the animals are observed periodically (Parasuraman, 2011). A chronic toxicology study provides inferences about the long-term effect of a test substance in animals, and it may be extrapolated to the human safety of the test substance. The report on chronic oral toxicity is essential for new drug entities. There should be little individual variation between the animals, and the allowable weight variation range is ± 20%. A satellite group may be included in the study protocol. During the study period, the animals are observed for normal physiological functions, behavioral variations and alterations in biochemical parameters. At the end of the study, tissues are collected from all parts of the animal and subjected to histological analyses (Jaijoy *et al*., 2010). Biochemical analysis is carried out on the blood and organ harvested and results of test groups are compared to that of the control group to ascertain toxicity. Consequently, a significant difference between results of test groups and control may indicate toxicity. These studies are designed to determine: The potential target organs of toxicity; the reversibility of toxicities observed; the NOAEL; and the potential clinical risk in relation to the anticipated clinical dose following long-term treatment.

1.4.10 HEMATOLOGICAL INVESTIGATION

The full blood count (FBC) is one of the most recurrently demanded pathological tests by doctors and typically consists of 13–19 parameters. The clinical use of this particular test is elaborate; an increased or decreased total white blood cell count (WBC) may be as a result of irregular bone marrow pathology; Leukocytosis with an associated lymphocytosis or neutrophilia could deduce the existence of a viral or microbial infection. Effect of chemotoxin during chemotherapy may also reduce the amount of WBC. Anemia may occur due to decreased hemoglobin, or red blood cell or both and relying on the red blood cell indices values, the etiology of the anemia can be inferred. The degree of increase in hemoglobin could be applied in monitoring the management of anemia and determine the quantity of blood needed for transfusion. The platelet count and its size can also be used to determine the thrombopoietic activity of the bone marrow, and a decrease or increase in platelet count can also point to liver infection or haemostatic disorder (Osei-Bimpong *et al.,* 2012).

1.4.11 WHITE BLOOD CELLS

White blood cells (WBCs) or leukocytes play a vital role in immune defense, and it includes various sub-populations: basophil, granulocytes, monocytes, lymphocytes, neutrophil, and eosinophil. Leukocytes are formed in the bone marrow and lymphocytes are produced in the lymphoid tissues. The quantity of leukocytes in the blood is considered a small percentage of the total population and undergoes wide fluctuation. The marginal neutrophils are linked to the endothelial cells, but detach and join the circulatory pool in cases when the blood pressure and blood flow velocity increases. Thus, alteration in blood pressure can also alter the amount of leukocytes existing in the blood (Summers *et al.,* 2010).

1.4.12 RED BLOOD CELLS

Red blood cell indices, traditionally have been the derived parameters of mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC); more recently, the red cell distribution width (RDW) has also been included and for some instruments, hemoglobin distribution width (HDW). These indices can provide basis for classifying anemia's and in various combinations, they have been useful in the distinction between iron deficiency and thalassemias. Wintrobe introduced in 1929; MCH, MCHC and MCV of red blood cells. MCV describes the size of red blood cells and is expressed in cubic microns (μm^3) or femtoliters (10−15; fl). Thus, anemia is categorized as macrocytic or microcytic if the value of MCV is higher or lower than the expected normal range and normocytic if MCV value falls within the accepted range. RDW signifies the coefficient of variation of the red blood cell volume distribution (size) and is expressed in percentage (Walker *et al.,* 1990). MCH measures the quantity of hemoglobin per red blood cell. MCHC specifies the quantity of hemoglobin per unit volume. It is expressed as grams per deciliters (g/dl) of red blood cells or as a percentage value. Hemoglobin is considered the most direct pointer to detect clinical severity in hemolytic infection or diseases. In the mild stage, Its levels may not be far from the normal values (Hb > 10 g/dL) , moderate stage is characterized by significant reduction (Hb 8–10 g/dL), severe stage may have values (Hb 6–8 g/dL), and the very severe stage, values (Hb 6g/dL). Hemoglobin levels should be monitored closely in patients with hemolysis, so as to evaluate their response to medications or treatment. (Barcellini and Fattizzo, 2015).

1.4.13 PLATELET COUNTS

Platelets which are also called thrombocytes are anucleated (having no nucleus) blood cells that play critical roles in the maintenance of homeostasis (Assinger, 2014). They arise from the fragmentation of the cytoplasm of megakaryocytes in the bone marrow (Assinger, 2014). In spite of the dynamic nature of the platelets, they actually remain inactive and activation occurs when there is a severe damage to the blood vessels. It is expedient to note that blood coagulation or haemostasis is not the lone purpose of the platelets. It also possesses multifunctional attributes that are essential in monitoring body homeostasis as well as disease pathology (Ghoshal and Bhattacharyya, 2014). Platelet influences inflammation and innate immune response by expressing the toll-like receptors (TLRs), which initiate the innate immune response. They also store and release upon activation many inflammatory mediators such as interleukin-1 that can exacerbate the immune response (Ware *et al.,* 2013).

Platelet indices (PI) — Plateletcrit (PCT), platelet distribution width (PDW) and mean platelet volume (MPV) are platelet parameters that form a part of the automatic complete blood count.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS AND EQUIPMENTS

Hand gloves, nose mask, universal container, EDTA bottle, plain bottle, ceramic plates, syringe (2ml, 5ml and 10ml), drug sachets, micropipette, beakers, measuring cylinder, conical flask, test tubes, volumetric flask, Pasteur pipette, dissecting kit, spatula, wooden cage, foil paper, cotton wool, filter paper, masking tape, padlock, drum, funnel, slide, muslin cloth and cuvette.

2.1.1 EQUIPMENTS

Centrifuge, homogenizer, refrigerator, weighing balance, cotton wool, gavage, weigh balance and hematological analyzer.

2.1.2 REAGENTS

Chloroform, normal saline, sulfolyser, diluents and distilled water

2.2 METHODS

2.2.1 PLANT MATERIAL COLLECTION

The *Annona muricata* bark were obtained from Edo state Trade fare Garden Benin City, Edo State, Nigeria and were identified in the Department of Plant Biology and Biotechnology (PBB) herbarium, University Of Benin, Benin City, Edo State.

2.2.2 PREPARATION OF PLANT SAMPLE.

The *Annona muricata* stem bark collected were washed and shade dried. After drying, the stem bark was pulverized and 500g of the pulverized stem bark was macerated in 2.5l of water and methanol respectively. The extraction process took 3 days (72 hours) and the solution was filtered using a muslin cloth. The residue obtained was discarded and the filtrate solution was concentrated by subjecting it to a rotary evaporator and then freeze dried at the National Centre for energy and environment, University of Benin to obtain a crude extract of the plant in powdery form, from which the stock solution is prepared.

2.2.3 EXPERIMENTAL ANIMALS

Adult male rats used in this experiment were procured from a local breeder in Benin City, Edo State. They were acclimatized for two weeks at the Animal Unit of the Department of Pharmacology, Faculty of Pharmacy, University of Benin. They were given grower pellets and water throughout the period of acclimatization.

2.2.4 ACUTE TOXICITY

Acute toxicity describes the adverse effects or unwanted effect of a substance that result from a single exposure or from multiple exposure in a short period of time (24 hours). The Lorke's method of acute toxicity was employed in this experiment to determine the toxic effects and behavioral changes of the albino rats from the normal (Lorke, 1983). This method involves two steps which are phase 1 and phase 2 and finally the LD_{50} value is determined.

A total of 21 animals (wistar rats) were used for the phase 1. The rats were divided into two groups (A and B). Group A served as the control with 3 albino rats. Group B served as the treated group and has doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight of extract for both aqueous and methanol, with each group having 3 animals (wistar rats) making a total of 9 rats for aqueous and 9 rats for methanol. After oral administration of the extracts at doses of 10mg/kg, 100mg/kg and 1000mg/kg to animals in both aqueous and methanol extract groups, the treated animals were then observed for 24 hours for mortality and general behavior.

Phase 2 was carried out after 24 hours observation of the animals with no death recorded from the various doses of the extracts administered in phase 1. For both the aqueous and methanol extract, a total of 6 rats were used for the phase 2 at doses of 1600mg/kg, 2900mg/kg and

5000mg/kg body weight administered orally, with 1 albino rat per dose. They were also observed for 24 hours for any death or behavioral changes. At the end of the 24 hours duration, no death was recorded in the phase 2. This suggests that the plant extracts were not toxic at the end of the acute toxicity study.

CALCULATION FOR LD50

The value of LD50 was calculated using the formula:

$LD50 = \sqrt{D0 \times D100}$

D0 = Highest dose that gave no mortality

D100 = Lowest dose that resulted in mortality

2.2.5 EXPERIMENTAL DESIGN FOR SUBCHRONIC TOXICITY

A total number of 60 male wistar rats were acquired and kept in the pharmacology animal house for acclimatization. on the first day the rats were randomly divided into twelve (12) groups with six groups for aqueous extract, and another six groups for methanol extract. They were weighed, and marked with different colors (using markers), identifying them according to their groups and numbers in their groups and were given access to rat feed and water. The cages were named; control, 200mg/kg, 500mg/kg, 1000mg/kg 3000mg/kg, and 5000mg/kg, of their body weight, from group 1 to 6. Group 2 group 3, group 4, group 5 and group 6 served as the test groups given different and regulated doses of the extract according to their body weight.

The rats were remarked whenever the marks faded. Fasting blood sugar taken and recorded whenever the rats were weighed once a week. The weight determinations were carried out weekly to aid feed and sample administration, and the extract doses calculated. The shavings in the cages were changed every two days; the rat feed and water were given afresh daily after the administration of the extract doses every morning. The progress of the rats was monitored for 28days and changes made when necessary. Groups 2, 3, 4 5and 6 were given calculated doses of the extract.

Extract was administered to the rats orally with the aid of an oral gavage at stated doses of (200, 500, 1000, 3000 and 5000) mg/kg body weight, one dose per 24 hours was given for 28 days.

Table 2.2.5.1 Aqueous Extract sample doses

GROUPS	**SAMPLE DOSES**
CONTROL	Received rat growers pellet and distilled water
2	Received 200mg/kg body weight
3	Received 500mg/kg body weight
4	Received 1000mg/kg body weight
5	Received 300mg/kg body weight
6	Received 5000mg/kg body weight

Table 2.2.5.2 Methanol Extract sample doses

GROUPS	SAMPLE DOSES
CONTROL	Received rat growers pellet and distilled water
2	Received 200mg/kg body weight
3	Received 500mg/kg body weight
4	Received 1000mg/kg body weight
5	Received 3000mg/kg body weight
6	Received 5000mg/kg body weight

2.2.6 DETERMINATION OF EXTRACT INTAKE

The volume doses for the sample were determined according their weight

$$\text{Volume of the extract (ml)} = \frac{\text{Dosage (kg/ml)} \times \text{Average weight (g)}}{\text{Concentration of the extract (kg/ml)}}$$

2.2.7 ADMINISTRATION OF PLANT EXTRACTS

The rats in the control group for both aqueous and methanol were orally administered water and groups 2, 3, 4 and 5 were orally administered 200mg/kg, 500mg/kg 3000mg/kg and 5000mg/kg body weights doses respectively of the plant extract for 28 days.

2.2.8 SACRIFICING OF THE WISTAR RATS

The rats were sacrificed on the 29th day based on their groups in each cage and their organs extracted and weighed. The rats were subjected to overnight fast, were selected and placed in a container containing cotton wool soaked in chloroform; this was to make the animal unconscious. Chloroform is an anesthetic intoxicant which is used to saturate the container in which the animal is to be inserted to inactivate the rat before sacrifice.

The sacrificing process was done through incision with the aid of a dissecting set. The abdomen was cut opened and blood was collected from the abdominal aorta and from the heart using a 5ml syringe (1 syringe for each rat). The blood sample was put in an EDTA sample container (anticoagulant tube) to prevent coagulation and to ensure that enough plasma was obtained from the blood sample of each rat after spinning in a centrifuge.

2.3 HEMATOLOGICAL INDICES

The hematological indices of the blood were determined with the aid of an automated hematology analyzer Urit 3010.

Manufacturer: Urit Medical Electronics, Guangxi, China.

Series: URIT

Sample Types: Whole Blood

Figure 2. Hematological Analyzer (URIT 3010)

2.3.1 DATA ANALYSIS

All data were represented as mean ± standard deviation (SD). Values obtained were examined using analyses of variance (ANOVA), and the least significance difference (LSD) analysis, to evaluate the differences among groups and for multiple comparisons among different groups. $p < 0.05$ was the level of significance used. SPSS/PC software was used in all calculations.

CHAPTER THREE

3.0 RESULTS

3.1. ACUTE TOXICITY

The acute toxicity studies revealed no death or signs of toxicity after a single oral administration of aqueous and methanolic extract of *A.muricata* stem bark up to 5000mg/kg b.wt. However, the rats showed hypo activity.

3.2. SUBCHRONIC TOXICITY

3.2.1 THE RESULT OBTAINED IN THIS STUDY USING METHANOLIC EXTRACTS ARE PRESENTED IN TABLE 3.2.1.1 TO TABLE 3.2.1.3

Table 3.2.1.1: Effect on WBCs, lymphocytes and granulocytes count in the plasma of rats administered methanol extract of the stem bark of *Annona muricata*

Groups	WBCs ($\times 10^9$/l)	Lymphocytes (%)	Granulocytes (%)
Control	12.96±3.56	73.88±10.19	17.60±7.23
200mg/kg b.wt	10.26±3.53	70.20±7.97	20.02±6.57
500mg/kg b.wt	12.50±6.15	71.76±4.48	18.80±2.99
1000mg/kg b.wt	15.00±6.26	55.57±13.15^a	33.87±10.72^a
3000mg/kg b.wt	10.20±1.34	64.37±4.76	24.16±5.32
5000mg/kg b.wt	7.93±2.62	57.37±11.84^a	31.76±11.43^a

WBCs, white blood cells. Values are represented as mean±SD for six determinations.
Values with superscript [a] are significantly different (p<0.05) from the control group. n=5

The lymphocyte count of rats administered 1000mg/kg b.wt and 5000mg/kg b.wt were significant lower (p<0.05) when compared to the control group. Whereas the granulocyte count of rats administered 1000mg/kg b.wt and 5000mg/kg b.wt increased significantly (p<0.05) with respect to the control group. WBCs were altered in a non-dose dependent manner compared with control.

Table 3.2.1.2: Effect on RBCs, HCT, HGB, MCV, MCH, and MCHC of rats administered methanol extract of the stem bark of Annona muricata

Groups	**RBCs($\times10^{12}$/l)**	**HCT (%)**	**HGB (g/dl)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/dL)**
Control	6.50±0.83	42.74±4.66	14.24±1.55	65.84±3.23	21.94±1.07	33.30±0.00
200mg/kg b.wt	5.62±1.70	39.48±9.12	13.16±3.04	72.20±9.19	24.04±3.08	33.30±0.00
500mg/kg b.wt	6.47±0.66	41.18±2.67	13.72±0.89	63.92±3.88	21.29±1.29	33.30±0.00
1000mg/kg b.wt	6.68±0.49	47.50±9.47	15.83±3.16	70.73±9.49	23.57±3.17	33.30±0.00
3000mg/kg b.wt	6.24±0.34	41.56±3.09	13.86±1.04	66.50±1.32	22.16±0.49	33.33±0.05[a]
5000mg/kg b.wt	6.97±0.83	43.06±6.83	14.35±2.28	61.56±2.66	20.50±0.87	33.30±0.00

Values are represented as mean±SD for six determinations.
Values with superscript [a] are significantly different (p<0.05) from the control group. n=5

There was no significant difference (p<0.05) in the red blood cells count compared to the control (Table 3.2.1.2).

The effect of methanol extract of the stem bark of *Annona muricata* on the concentration of HGB, HCT, MCV, MCH and MCHC of all test groups were not significantly different (p>0.05) when compared to the control group except for a singular significant difference (p<0.05) in the MCHC level of rats administered 3000mg/kg b.wt extract in comparism with the control.

Table 3.2.1.3: Effect on platelets count in the plasma of rats administered methanol extract of the stem bark of Annona muricata

Groups	Platelets($\times 10^9$/l)	MPV(fL)	PCT (%)
Control	535±102.84	8.44±0.22	0.45±0.09
200mg/kg b.wt	498±137.68	8.40±0.41	0.41±0.10
500mg/kg b.wt	466±86.15	8.20±0.24	0.38±0.06
1000mg/kg b.wt	518±91.5	8.52±0.61	0.44±0.11
3000mg/kg b.wt	580±74.87	8.56±0.38	0.49±0.046
5000mg/kg b.wt	490±104.51	8.00±0.26	0.39±0.08

Values are represented as mean±SD for six determinations.
Values with superscript [a] are significantly different ($p<0.05$) from the control group. n=5

The Platelets count, MPV and PCT of all test groups were not significantly different ($p>0.05$) when compared to the control group.

3.2.2 THE RESULT OBTAINED IN THIS STUDY USING AQUEOUS EXTRACTS ARE PRESENTED IN TABLE 3.2.2.1 TO TABLE 3.2.2.3

Table 3.2.2.1: Effect on WBCs, lymphocytes and granulocytes count in the plasma of rats administered aqueous extract of the stem bark of Annona muricata

Groups	WBCs ($\times 10^9$/l)	Lymphocytes (%)	Granulocytes (%)
Control	14.16±3.08	62.66±8.11	24.98±6.60
200mg/kg b.wt	12.28±3.07	62.96±9.34	25.48±7.39
500mg/kg b.wt	13.08±3.35	62.90±1.70	24.38±1.30

1000mg/kg b.wt	9.30±3.53	66.23±6.80	23.37±5.88
3000mg/kg b.wt	9.85±4.65	72.42±5.56	18.15±4.60
5000mg/kg b.wt	13.62±6.29	66.48±15.48	23.78±12.53

WBCs, white blood cells. Values are represented as mean±SD for six determinations.
Values with superscript [a] are significantly different ($p<0.05$) from the control group. n=5

The WBCs, lymphocytes and granulocytes of all test groups were not significantly different ($p>0.05$) when compared to the control group with their values altered in a non-dose dependent manner compared with control.

Table 3.2.2.2: Effect on RBCs, HCT, HGB, MCV, MCH, and MCHC of rats administered aqueous extract of the stem bark of Annona muricata

Groups	RBCs($\times 10^{12}$/l)	HCT (%)	HGB (g/dl)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control/kg bw	5.35±1.33	33.22±7.13	11.07±2.38	62.46±2.71	22.03±3.27	33.32±0.01
200mg/kg bw	6.84±0.54[a]	42.36±2.98[a]	14.11±1.00[a]	62.06±3.76	20.67±1.27	33.31±0.04
500mg/kg bw	6.65±0.58[a]	40.28±3.30[a]	13.41±1.07[a]	60.62±1.21	20.18±0.38	33.28±0.07
1000mg/kg bw	5.26±1.05	34.07±6.26	11.35±2.09	65.04±4.17	21.67±1.39	33.32±0.01
3000mg/kg bw	6.56±0.53[a]	46.53±3.03[a]	15.49±1.02[a]	70.93±1.41[a]	23.57±0.44	33.27±0.07
5000mg/kg bw	5.88±0.28	40.00±2.18[a]	13.32±0.74[a]	68.05±2.26[a]	22.87±0.97	33.30±0.04

Values are represented as mean±SD for six determinations.
Values with superscript [a] are significantly different ($p<0.05$) from the control group. n=5

The effect of the aqueous extract of the stem bark of *Annona muricata* on RBCs count in wistar rats (Table 3.2.2.2) shows significant differences ($p<0.05$) with higher RBCs count at 200mg/kg .wt, 500mg/kg b.wt and 3000mg/kg b.wt in comparism with the control group.

The haematocrit and haemoglobin levels of rats administered aqueous extract of *A. muricata* both show significant differences ($p<0.05$) from control at 200mg, 500mg, 3000mg and 5000mg/kg b.wt respectively.

MCV values at higher doses of 3000mg and 5000mg/kg b.wt are significantly different ($p<0.05$) from the control.

MCH and MCHC of all test groups were not significantly different ($p>0.05$) when compared to the control.

Table 3.2.2.3: Effect on platelets count in the plasma of rats administered aqueous extract of the stem bark of Annona muricata

Groups	Platelets($\times 10^9$/l)	MPV(fL)	PCT (%)
Control	545±237.2	7.10±0.42	0.38±0.15
200mg/kg b.wt	490±59.5	7.28±0.13	0.35±0.04
500mg/kg b.wt	505±51.7	7.56±0.15^a	0.38±0.03
1000mg/kg b.wt	595±59.3	8.06±0.64^a	0.48±0.09
3000mg/kg b.wt	470±54.2	8.0±0.26^a	0.37±0.04
5000mg/kg b.wt	380±101.4^a	8.04±0.27^a	0.30±0.08

Values are represented as mean±SD for six determinations.
Values with superscript ^{a} are significantly different ($p<0.05$) from the control group. n=5

Platelets count of wistar rats administered with aqueous extract show a gradual decrease at the 200mg/kg b.wt before an increase at 1000mg/kg b.wt and is significantly different ($p<0.05$)from the control group at 5000mg/kg b.wt.

MPV values at 500mg to 5000mg/kg b.wt differ significantly ($p<0.05$) when compared to the control group.

PCT of all test groups were not significantly different ($p>0.05$) when compared to the control group.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

Usage of herbal preparations without control dosage coupled with non-availability of adequate scientific studies on their safety has raised concerns on their toxicity (Saad *et al.,* 2006). Toxicity studies in animal model are commonly used to evaluate potential health risk in humans caused by intrinsic opposing effects of plant extracts. Such toxicity testing is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity, when data are translated from animal studies.

Acute oral toxicity studies are usually conducted in animals to estimate the toxic or adverse effects produced by a single large dose of any plant-based product within 24 hours. The acute toxicity results from this study revealed no mortality at a single oral administration of methanol and aqueous extract of stem bark of *A. muricata* up to a concentration of 5000 mg/kg body weight of the extract. This implies that at a single oral administration, the methanol and aqueous extract of stem bark of *A. muricata* may be safe and non-toxic. Hence the LD50 was estimated to be <5000mg/kg (orally). The LD50 result obtained from this study is similar to that reported by Arthur *et al*. (2011), who reported no death or signs of toxicity in rats fed hexane leaves extract of *A.muricata as* well as Ag*u et al.* (2017) who reported no death in rats fed methanolic stem bark extract of *A. muricata* up to 5000mg/kg (orally) although the rats were observed to have curled inward, to be less active, and to have developed increased heart rates at higher doses.

Hematological parameters can be used to determine the blood related functions of plant extracts. The hemopoietic system is one of the most delicate targets of toxins and an important index of physiological and pathological status in both humans and animals.

To study the influence of these extracts on immunity and hematological profiles FBC; WBCs, RBCs, PLT, HCT, HGB etc. statuses were assayed.

WBC's are the first line of cellular defence that responds to infectious agents, tissue injury, or any inflammation. WBCs and lymphocytes in the groups administered methanolic stem bark extract decreased in a dose dependent manner with a significant decline in WBC count for groups administered 5000mg/kg b.wt. Although the rats administered 1000mg/kg b.wt show an increase in WBC and granulocyte count. This insignificant difference and decrease in WBC and lymphocytes count in comparism with the control group is in agreement with the experiment of Agu *et al.* (2017), therefore indicating that the methanolic stem bark extracts of *A.muricata* may not possess toxins that challenge the immune system or that the methanolic stem bark extract of *AMC* possess no positive effect on immunity.

Rats administered aqueous extract of the stem bark of *Annona muricata* also show insignificant differences in the lymphocyte count and a decline in WBC concentration at higher doses. This result contradicts Arthur *et al.* (2011) who revealed that the lymphocytes of wistar rats were significantly increased at higher doses by aqueous extract of *A.muricta* leaves. Nonetheless, this contradiction maybe due to differences in the plant part used in the experiment. Generally, this result suggests that stem bark extract of *A.muricta* may not contain phytochemicals in substantial amounts that are able to boost immunity.

On observation of the influence of these extracts of *AMC* on the RBCs, HGB, HCT, and red blood cells indices (MCV, MCH, and MCHC). The groups administered methanolic stem-bark *AMC* extracts increase in their RBCs, HCT, and HGB level at higher doses compared with the control. This increase in RBCs, HGB, and HCT is most distinguished in groups administered 1000mg/kg b.wt of methanol extract. This result also shows an increase in MCV levels significantly at 1000mg/kg b.wt which may indicate an increase in the size of red blood cells. This result is in concordance with the outcome of Agu *et al.* (2017) as well as Ola and Ebenezer's (2016) result using the ethanol extract of unripe *AMC* fruits in swiss albino rats infected with Salmonella typhi. Although it contradicts that of Syahida *et al.* (2012) reporting no significant difference in RBC concentration. This disagreement maybe due to differences in extraction solvent or plant part used in the experiment.

Rats administered aqueous extracts of the stem bark of *A. muricata* also show significant differences in RBC, HCT, HGB and MCV in a dose dependent manner compared to the control. This increase in RBC and its related indices is most distinguished in groups administered 3000mg/kg b.wt of aqueous extract. This result contradicts the report of Arthur *et al.* (2012) *AMC* aqueous leaf extract analysis that suggests no significant difference.

This result suggests that stem bark of_*AMC* extract has effect on erythropoiesis, morphology, or osmotic fragility of red blood cells.

Platelet is implicated in blood clotting and plays a crucial role in reducing blood loss and repair of vascular injury. The platelet levels of groups administered methanol stem-bark *AMC* extract decreased steadily with lowest level at 5000mg/kg b wt of extract (Agu *et al.,* 2017), except for groups administered 3000 mg/kg body weight of the extract which showed a significant increase in blood platelet count and MPV levels compared to control. This result is in agreement with that reported by Agu *et al.* (2017).

The platelet levels of groups administered aqueous stem-bark *AMC* extract decreased steadily with lowest level at 5000mg/kg b wt of extract except for groups administered 1000 mg/kg body weight of the extract which showed a significant increase in blood platelet count and MPV levels compared to control.

This suggests that at 3000 mg/kg b.wt and 1000mg/kg body weight, methanol and aqueous stem bark extract of *AMC* respectively may have effect on thrombopoiesis and morphology of blood platelets. The wound-healing potential of *A. muricata* has been identified, and some of the possible mechanisms behind this potential have been reported. Moghadamtousi et al. (2015) reported the relationship between the antioxidant capacities and wound healing. Platelets can adhere to the walls of the blood vessels, release bioactive compounds, and aggregate to each other, stimulating the biosynthesis of blood clotting factors.

Conclusion

The investigation into the sub-chronic toxicity of methanol and aqueous extract of the stem bark of *Annona muricata* revealed no significant effect on the immunity of Wistar albino rats. The acclaimed wound-healing properties of *A. muricata* were established and linked to the influence on blood platelets and also possible immunity against microorganisms that prevent wound healing. RBCs and its related indices were also significantly increased suggesting that the plant has hemomodulatory properties. The dose effect of *Annona muricta* stem bark extracts on hematological parameters may be further studied for possible therapeutic applications in the management of hematological-related disorders because its use by tradomedical practitioners is justified.

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APPENDIX

AQUEOUS EXTRACTS

Multiple Comparisons
Dependent Variable: White blood cell
LSD

(I) wbc	(J) wbc	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
control	200mgextract	1.88000	2.64951	.486	-3.6300	7.3900
	500mgextract	1.08000	2.64951	.688	-4.4300	6.5900
	1000mgextract	4.86000	3.05940	.127	-1.5024	11.2224
	3000mgextract	4.31000	2.81023	.140	-1.5342	10.1542
	5000mgextract	.54000	2.64951	.840	-4.9700	6.0500

ANOVA
White blood cell

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	80.034	5	16.007	.912	.492
Within Groups	368.546	21	17.550		
Total	448.580	26			

Multiple Comparisons
Lymphocytes
LSD

(I) LYM	(J) LYM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
control	200mgextract	-.30000	5.80349	.959	-12.3690	11.7690
	500mgextract	-.24000	5.80349	.967	-12.3090	11.8290
	1000mgextract	-3.57333	6.70129	.599	-17.5094	10.3628
	3000mgextract	-9.76500	6.15553	.128	-22.5661	3.0361
	5000mgextract	-3.82000	5.80349	.518	-15.8890	8.2490

ANOVA

Lymphocytes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	303.660	5	60.732	.721	.615
Within Groups	1768.226	21	84.201		
Total	2071.887	26			

ANOVA

Granulocytes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	149.299	5	29.860	.540	.744
Within Groups	1160.409	21	55.258		
Total	1309.707	26			

Multiple Comparisons

Granulocytes

LSD

(I) GRAN	(J) GRAN	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	200mgextract	-.50000	4.70139	.916	-10.2771	9.2771
	500mgextract	.60000	4.70139	.900	-9.1771	10.3771
	1000mgextract	1.61333	5.42869	.769	-9.6762	12.9029
	3000mgextract	6.83000	4.98657	.185	-3.5401	17.2001
	5000mgextract	1.20000	4.70139	.801	-8.5771	10.9771

ANOVA

Red blood cell

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.190	5	2.038	3.296	.024
Within Groups	12.986	21	.618		
Total	23.176	26			

Multiple Comparisons

Dependent Variable: Red blood cell

LSD

(I) RBC	(J) RBC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
control	200mgextract	-1.48400*	.49734	.007	-2.5183	-.4497
	500mgextract	-1.29200*	.49734	.017	-2.3263	-.2577
	1000mgextract	.09400	.57428	.872	-1.1003	1.2883
	3000mgextract	-1.20850*	.52751	.032	-2.3055	-.1115
	5000mgextract	-.52200	.49734	.306	-1.5563	.5123

ANOVA

Haemoglobin

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58.226	5	11.645	5.403	.002
Within Groups	45.265	21	2.155		
Total	103.491	26			

Multiple Comparisons

Dependent Variable: Haemoglobin

LSD

(I) HGB	(J) HGB	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	2	-3.04000*	.92854	.004	-4.9710	-1.1090
	3	-2.33400*	.92854	.020	-4.2650	-.4030
	4	-.28133	1.07219	.796	-2.5111	1.9484
	5	-4.41550*	.98486	.000	-6.4636	-2.3674
	6	-2.25200*	.92854	.024	-4.1830	-.3210

ANOVA

Haemotocrit

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	528.303	5	105.661	5.453	.002
Within Groups	406.902	21	19.376		
Total	935.205	26			

Multiple Comparisons

Dependent Variable: Haematocrit

LSD

(I) HCT	(J) HCT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	2	-9.14000*	2.78398	.004	-14.9296	-3.3504
	3	-7.06000*	2.78398	.019	-12.8496	-1.2704
	4	-.84667	3.21466	.795	-7.5319	5.8386
	5	-13.30500*	2.95285	.000	-19.4458	-7.1642
	6	-6.78000*	2.78398	.024	-12.5696	-.9904

ANOVA

Mean corpuscular volume

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	354.444	5	70.889	9.777	.000
Within Groups	152.263	21	7.251		
Total	506.707	26			

Multiple Comparisons

Dependent Variable: Mean corpuscular volume

LSD

(I) MCV	(J) MCV	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	2	.40800	1.70301	.813	-3.1336	3.9496
	3	1.84000	1.70301	.292	-1.7016	5.3816
	4	-2.57600	1.96647	.204	-6.6655	1.5135
	5	-8.46350*	1.80632	.000	-12.2199	-4.7071
	6	-5.58600*	1.70301	.004	-9.1276	-2.0444

ANOVA

Mean corpuscular haemoglobin

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	38.000	5	7.600	2.751	.046
Within Groups	58.011	21	2.762		
Total	96.011	26			

Multiple Comparisons

Dependent Variable: Mean corpuscular haemoglobin

LSD

(I) MCH	(J) MCH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	2	1.35800	1.05117	.210	-.8280	3.5440
	3	1.85000	1.05117	.093	-.3360	4.0360
	4	.35800	1.21379	.771	-2.1662	2.8822
	5	-1.53700	1.11494	.183	-3.8556	.7816
	6	-.84000	1.05117	.433	-3.0260	1.3460

ANOVA

Mean corpuscular haemoglobin concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.011	5	.002	1.111	.384
Within Groups	.043	21	.002		
Total	.055	26			

Multiple Comparisons

Dependent Variable: Mean corpuscular haemoglobin concentration

LSD

(I) MCHC	(J) MCHC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	2	.01600	.02867	.583	-.0436	.0756
	3	.04200	.02867	.158	-.0176	.1016
	4	.00067	.03311	.984	-.0682	.0695
	5	.05900	.03041	.066	-.0042	.1222
	6	.02000	.02867	.493	-.0396	.0796

ANOVA

Platelet

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	111133.117	5	22226.623	1.520	.226
Within Groups	307067.550	21	14622.264		
Total	418200.667	26			

Multiple Comparisons

Platelet

LSD

(I) PLT	(J) PLT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	2	54.80000	76.47814	.482	-104.2450	213.8450
	3	40.20000	76.47814	.605	-118.8450	199.2450
	4	-49.80000	88.30935	.579	-233.4493	133.8493
	5	74.45000	81.11732	.369	-94.2427	243.1427
	6	165.00000*	76.47814	.043	5.9550	324.0450

ANOVA

Plateletcrit

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.062	5	.012	1.772	.162
Within Groups	.147	21	.007		
Total	.209	26			

Multiple Comparisons

Dependent Variable: Plateletcrit

LSD

(I) PCT	(J) PCT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	2	.02800	.05294	.602	-.0821	.1381
	3	.00000	.05294	1.000	-.1101	.1101
	4	-.10200	.06112	.110	-.2291	.0251
	5	.00550	.05615	.923	-.1113	.1223
	6	.07600	.05294	.166	-.0341	.1861

ANOVA

Mean platelet volume

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.001	5	.800	7.714	.000
Within Groups	2.179	21	.104		
Total	6.180	26			

Multiple Comparisons

Dependent Variable: Mean platelet volume

LSD

(I) MPV	(J) MPV	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	2	-.18000	.20371	.387	-.6036	.2436
	3	-.46000*	.20371	.035	-.8836	-.0364
	4	-.96667*	.23523	.001	-1.4558	-.4775
	5	-.90000*	.21607	.000	-1.3493	-.4507
	6	-.94000*	.20371	.000	-1.3636	-.5164

METHANOLIC EXTRACTS

ANOVA

White blood cell

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	114.369	5	22.874	1.122	.382
Within Groups	387.411	19	20.390		
Total	501.780	24			

Multiple Comparisons

Dependent Variable: White blood cell

	(I) wbc	(J) wbc	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LSD	control	200mgextract	2.70000	2.85587	.356	-3.2774	8.6774
		500mgextract	.46000	2.85587	.874	-5.5174	6.4374
		1000mgextract	-2.04000	3.02911	.509	-8.3800	4.3000
		3000mgextract	2.76000	3.29768	.413	-4.1421	9.6621
		5000mgextract	5.02667	3.29768	.144	-1.8755	11.9288

ANOVA

Lymphocytes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1219.734	5	243.947	2.904	.041
Within Groups	1596.301	19	84.016		

Total	2816.034	24			

Multiple Comparisons

Dependent Variable: Lymphocytes

	(I) LYM	(J) LYM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LSD	control	200mgextract	3.68000	5.79710	.533	-8.4535	15.8135
		500mgextract	2.12000	5.79710	.719	-10.0135	14.2535
		1000mgextract	18.30500*	6.14875	.008	5.4355	31.1745
		3000mgextract	9.51333	6.69391	.171	-4.4972	23.5239
		5000mgextract	16.51333*	6.69391	.023	2.5028	30.5239

ANOVA

Granulocytes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	981.785	5	196.357	3.451	.022
Within Groups	1080.929	19	56.891		
Total	2062.714	24			

Multiple Comparisons

Dependent Variable: Granulocytes

	(I) GRAN	(J) GRAN	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LSD	control	200mgextract	-2.42000	4.77037	.618	-12.4045	7.5645
		500mgextract	-1.20000	4.77037	.804	-11.1845	8.7845
		1000mgextract	-16.27500*	5.05974	.005	-26.8652	-5.6848
		3000mgextract	-6.56667	5.50834	.248	-18.0958	4.9624
		5000mgextract	-14.16667*	5.50834	.019	-25.6958	-2.6376

ANOVA Red blood cell					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.477	5	.895	.929	.484
Within Groups	18.311	19	.964		
Total	22.788	24			

Multiple Comparisons

Dependent Variable: Red blood cell

	(I) RBC	(J) RBC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LSD	control	200mgextract	.88800	.62088	.169	-.4115	2.1875
		500mgextract	.04000	.62088	.949	-1.2595	1.3395
		1000mgextract	-.17200	.65854	.797	-1.5503	1.2063
		3000mgextract	.26133	.71693	.719	-1.2392	1.7619
		5000mgextract	-.46533	.71693	.524	-1.9659	1.0352

ANOVA Haemoglobin					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.571	5	3.514	.723	.615
Within Groups	92.390	19	4.863		
Total	109.961	24			

Multiple Comparisons

Dependent Variable: Haemoglobin

	(I) HGB	(J) HGB	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	Control	2	1.08600	1.39465	.446	-1.8330	4.0050
		3	.52000	1.39465	.713	-2.3990	3.4390
		4	-1.58900	1.47925	.296	-4.6851	1.5071
		5	.37933	1.61040	.816	-2.9913	3.7499
		6	-.11067	1.61040	.946	-3.4813	3.2599

ANOVA

Haematocrit

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	158.076	5	31.615	.723	.615
Within Groups	830.961	19	43.735		
Total	989.038	24			

Multiple Comparisons

Dependent Variable: Haematocrit

	(I) HCT	(J) HCT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	Control	2	3.26000	4.18257	.445	-5.4942	12.0142
		3	1.56000	4.18257	.713	-7.1942	10.3142
		4	-4.76000	4.43629	.297	-14.0453	4.5253
		5	1.17333	4.82962	.811	-8.9352	11.2818
		6	-.32667	4.82962	.947	-10.4352	9.7818

ANOVA

Mean corpuscular volume

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	333.968	5	66.794	1.743	.173
Within Groups	728.055	19	38.319		
Total	1062.023	24			

Multiple Comparisons

Dependent Variable: Mean corpuscular volume

	(I) MCV	(J) MCV	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	Control	2	-6.36000	3.91503	.121	-14.5543	1.8343
		3	1.91800	3.91503	.630	-6.2763	10.1123
		4	-4.88500	4.15252	.254	-13.5763	3.8063
		5	-.66000	4.52069	.885	-10.1219	8.8019
		6	4.27333	4.52069	.356	-5.1886	13.7352

ANOVA

Mean corpuscular haemoglobin

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37.067	5	7.413	1.732	.176
Within Groups	81.345	19	4.281		
Total	118.412	24			

Multiple Comparisons

Dependent Variable: Mean corpuscular haemoglobin

	(I) MCH	(J) MCH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	Control	2	-2.10000	1.30863	.125	-4.8390	.6390
		3	.64600	1.30863	.627	-2.0930	3.3850
		4	-1.63500	1.38802	.253	-4.5402	1.2702
		5	-.22667	1.51108	.882	-3.3894	2.9361

	6	1.44000	1.51108	.353	-1.7227	4.6027

ANOVA

Mean corpuscular haemoglobin concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	5	.001	1.672	.190
Within Groups	.007	19	.000		
Total	.010	24			

Multiple Comparisons

Dependent Variable: Mean corpuscular haemoglobin concentration

	(I) MCHC	(J) MCHC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	Control	2	.00000	.01185	1.000	-.0248	.0248
		3	.00000	.01185	1.000	-.0248	.0248
		4	.00000	.01257	1.000	-.0263	.0263
		5	-.03333*	.01368	.025	-.0620	-.0047
		6	.00000	.01368	1.000	-.0286	.0286

ANOVA

Platelet

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29614.477	5	5922.895	.546	.739
Within Groups	206021.283	19	10843.225		
Total	235635.760	24			

Multiple Comparisons

Dependent Variable: Platelet

	(I) PLT	(J) PLT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	Control	2	37.60000	65.85811	.575	-100.2426	175.4426
		3	68.80000	65.85811	.309	-69.0426	206.6426
		4	17.15000	69.85307	.809	-129.0542	163.3542
		5	-44.93333	76.04639	.562	-204.1003	114.2336
		6	45.06667	76.04639	.560	-114.1003	204.2336

ANOVA

Plateletcrit

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.032	5	.006	.880	.513
Within Groups	.140	19	.007		
Total	.173	24			

Multiple Comparisons

Dependent Variable: Plateletcrit

	(I) PCT	(J) PCT	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	Control	2	.03800	.05432	.493	-.0757	.1517
		3	.06800	.05432	.226	-.0457	.1817
		4	.00600	.05761	.918	-.1146	.1266
		5	-.04400	.06272	.491	-.1753	.0873
		6	.05933	.06272	.356	-.0719	.1906

ANOVA					
Mean Platelet Volume					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.794	5	.159	1.131	.378
Within Groups	2.666	19	.140		
Total	3.460	24			

Multiple Comparisons							
Dependent Variable: Mean Platelet volume							
	(I) MPV	(J) MPV	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
LSD	1	2	.04000	.23692	.868	-.4559	.5359
		3	.24000	.23692	.324	-.2559	.7359
		4	-.08500	.25129	.739	-.6110	.4410
		5	-.12667	.27357	.649	-.6993	.4459
		6	.44000	.27357	.124	-.1326	1.0126