ADEKUNLE AJASIN UNIVERSITY
P.M.B. 001 AKUNGBA AKOKO,
ONDO STATE, NIGERIA

HAEMATOLOGICAL AND TRANSCRIPTOMIC ALTERATIONS OCCURING IN SHORT-TERM EXPOSURE OF RATS TO SELECTED HERBS

BY

ABIMBOLA OMOWUMI FADIPE
MATRIC NO. 159416006

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ABIMBOLA OMOWUMI FADIPE

AMLSCN, B.Sc. (Ikeji, Arakeji)

(159416006)

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OCTOBER, 2018

CERTIFICATION

This is to certify that the investigations reported in this thesis were carried out by, Abimbola Omowumi Fadipe (MST/159416006) under my supervision in the Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko. Nigeria.

Dr. J. A. Saliu 05/4/18

Supervisor Signature Date

Dr. A. O. Olusola

Head of Department 23/1/19

Signature Date

DEDICATION

Dedicated to my God, the Alpha and the Omega

ACKNOWLEDGEMENTS

I owe God a great deal of gratitude, for His blessings, and protection over my life throughout the period of undertaking the course and this research work.

I would like to express my sincere appreciation to my Supervisor, Dr. J. A. Saliu for sparing time to advice, criticize and for making necessary corrections at each stage of the work and to the Head of Department, Dr A. O. Olusola, for his fatherly disposition and words of encouragement.

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ABSTRACT

Non communicable diseases like diabetes mellitus, hypertension, cancer and infertility are currently on the increase across the globe. This therefore necessitates the increase in the search for appropriate therapies which are more effective, less expensive and of minimal or no side effects. Herbal medicines are being regarded as suitable alternative to orthodox medicines considering their wide acceptability and availability. Many plants have shown hypoglycemic, hypolipidemic and insulinogenic properties but there is dearth of information on the transcriptomic alterations following exposure of normal rats to *Tapinanthus bangwensi, Momordica foetida, Peperomia pellucida* and *Mangifera indica.*

In this study, male albino rats of wistar strain were placed on four different herbs-supplemented diets containing 30% and 70% respectively on each plant for three days after which they were sacrificed by cervical dislocation. Blood samples were collected into anti-coagulated containers and their haematological parameters were measured using auto-haematological analyzer. Tissues were also collected and various gene expression assays were carried out.

The results showed that the genes expressed in the pancreas include Glut4, Insulin, CACNA1a and KCJN5. *Mangifera indica*, *Peperomia pellucida* and *Momordica foetida* groups expressed insulin and KCJN5 genes in significant measures, (upregulated). In the small intestine, GLP-1 and GLUT-2 were expressed in all groups at higher concentration, except for *P. pellucida* which was expressed at low concentration (30%). In the testis, FSH-R and LH-R genes were significantly expressed (upregulated) by *P. pellucida* (30%) and *M. indica* (70%). In the kidney, TNF-alpha gene was downregulated by all the extracts while IL1-alpha was upregulated. In the liver, PFK1, Pyr-Kinase and G6pD genes were expressed by all the extracts.

It was observed that there is modulation of gene expression and transcriptomic alterations after the exposure of normal rats to these herbs, with *M. indica*, *P. pellucida* and *M. foetida* showing to be less toxic and more promising in expression of genes that are important in diabetic management. Also, *M. indica* and *P. pellucida* with the upregulation of FSH-R and LH-R genes show pro-fertility ability. Haematological alteration of interest seen in the results include increase in Red blood cells (RBC), packed cell volume (PCV), and Haemoglobin (Hb) by the extracts of *M. indica*, *M. foetida* and *Tapinanthus bangwensi*. In addition, Platelets count was increased by the extracts of *M. foetida* and *T.bangwensi*. This study offers a key to unlock new therapeutic targets for the treatment of diabetes, inflammation, anaemia, haemorrhage and infertility.

CHAPTER ONE

1.0 INTRODUCTION

During the last century, the practice of herbalism has become main stream throughout the world. In spite of great advances observed in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medical systems and the identification of plants from indigenous pharmacopoeias, which have significant healing power. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (Calixto, 2000; Lewis, 2001). The search for natural products to cure diseases has received considerable attentions in which plants have been the most important source (Okwu, 2005; 2007). In Brazil alone, about 80,000 species of higher plants were described which offer enormous prospects for discovering new compounds with therapeutic property (Nakamura *et al.*, 1999).

Herbal preparations form the basis for many therapeutic drugs and are the first line treatment for many of the world's population, being readily available, traditional and relatively inexpensive (Okpara *et al.*, 2007). Herbal medicinal products are assuming greater roles in the lives of the people across the world in the face of global upsurge of drug resistance, toxicity, adverse effects, and increasing costs of synthetic products (Okpara, *et al.*, 2007). In Nigeria, several thousands of plant species have been claimed to possess medicinal properties and employed in the treatment of many ailments (Iweala and Oludare, 2011). Many of these indigenous medicinal plants are used as spices, food and for medicinal purposes. Medicinal plants are believed to be an essential source of new chemical substances with potential therapeutic effects. Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have access to conventional anti-diabetic therapies (Grover, *et al.*, 2002).

Metabolomics, pharmacogenomics, and toxicogenomics can be utilized to examine the chemical processes involving metabolites of medicinal herbs, to investigate the variations within the host genome and herbs, and to analyze the toxic effects of herbs (Youns *et al.*, 2010; Kuete and Efferth, 2011; Barlow *et al.*, 2012; Sertel *et al.*, 2012). Omics, such as functional genomics, transcriptomics and proteomics, can be applied to study the gene/protein functions of medicinal herbs and to evaluate the herb/host interactions.

Plants are known to be pharmacologically active, inactive or toxic by examining alterations caused by the plant extracts on the hematological and transcriptomics markers. One of the important methods of assessment of health in an animal is to assess the

hematological parameters. Several studies have reported the effect of plant extracts on hematological parameters of rats following administration of the plant spices or extracts (Ajeigbe *et al.*, 2013). Also, transcriptomics analysis is important in understanding how animals exposed to plant extract can undergo altered expression of genetic variants, which contributes to complex diseases such as diabetes (Ajeigbe *et al.*, 2013). Analysis of RNA expression provides insight into biological pathways and molecular mechanism that regulate cell fate, development, and disease progression. Although different studies have independently reported the effect of plants on the alteration of hematological parameters and transcriptomics library, this study aims to investigate the hematological and transcriptomic alterations occurring in experimental albino rats if exposed to the following herbs: Bitter gourd (*Momordica foetida),* Mango *(Mangifera indica),* Mistletoe(*Tapinanthus bangwensi* and Silver bush *(Pepperomia pellucida).*

Bitter gourd (*Momordica foetida*) is a popular vegetable in some Asian countries. Fresh bitter gourd is used as a nourishing food. It contains: 93.8% water, 0.9% protein, 0.1% lipid, 3.3% dietary fiber, 20 kJ energy per 100 g, and a small quantity, 0.05%, of vitamin C (Islam *et al.*, 2011). It is a good source of phenolic compounds (Islam *et al.*, 2011).The immature fruits of bitter gourd can be prepared in many ways such as frying or cooking as curries. In addition, fruits can be dehydrated, pickled or canned (Krawinkel and Keding 2006). They are usually blanched or soaked in salt water before cooking to reduce the bitter taste. Incorporating bitter foods in commonly consumed food dishes can mask the bitter taste of bitter gourd (Snee *et al.*, 2014). Bitter gourd treatments of cell cultures or feeding trials with laboratory animals such as mice or rats showed that it has blood glucose lowering properties. Most animal studies have shown a blood glucose lowering effect of the fruit of bitter gourd when fed orally as a single dose (Krawinkel and Keding 2006). The juice formulations of bitter gourd have proven to be more effective in lowering blood sugar level and HbA1c levels than its dried fruit products (Krawinkel and Keding 2006). Bitter gourd has been shown to be effective in treating Type I diabetes in rats or mice by increasing pancreatic insulin secretion (Fernandes *et al.*, 2007). Some preliminary evidence suggests that the consumption of bitter melon as whole fruit, extract, or dried powder may reduce blood sugar levels (Basch *et al.*, 2003). In human studies with diabetes patients, fresh bitter gourd juice was shown to significantly reduce plasma glucose concentrations and improve response to an oral glucose load (Islam *et al.*, 2011). Bitter gourd may have synergistic effects with oral hypoglycemics and it may aggravate hypoglycemia in type 2 diabetes patients (Basch *et al.*, 2003). One of the studies by Fuangchan *et al.,* (2011) effectively demonstrated the hypoglycemic effect of

bitter melon among type 2 diabetic individuals receiving 2,000 mg/day of dried bitter melon powder. However, the hypoglycemic effect of bitter melon was less than metformin 1,000 mg/day (Fuangchan *et al.*, 2011).

Leaves of *Mangifera indica* commonly known as mango (family *Anacardiaceae*), is a large evergreen tree of tropical and subtropical region that has been used in traditional medicine by a number of people for centuries. The leaves of *M. indica* plant are used as an antidiabetic agent in Nigerian folk medicine. Although aqueous extract given orally, it did not alter blood glucose level in either normoglycaemic or streptozocin induced diabetic rats; antidiabetic activity was seen when the extract and glucose were administered simultaneously and also when the extract was given to the rats 60 minutes before the glucose. The results indicate that aqueous extract of *M. indica* possess hypoglycaemia activity. This may be due to an intestinal reduction of the glucose absorption. (Aderibigbe, 2001).
Several studies in animal models with diabetes have shown both short and long term hypoglycemic effect of mango leaves. Its mechanism for lowering glucose level is unknown, and however, some studies suggest facilitation of glucose uptake peripherally (Campillo, 2001).

Peperomia pellucida is an annual shallow rooted herb that belongs to the family Piperaceae. It is found in various shaded damp habitats all over Asia and the America growing in clumps, thriving in loose, humid soils of tropical and subtropical climate. It usually grows to a height of about 15 to 45cm and is characterized by succulent stems, shiny, heart shaped, fleshly leaves and tiny dot like seeds attached to several fruiting spikes (Aziba *et al.*, 2001).
Extract of *P. pellucida* has anti-diabetic activities which might be due to systemic action in the sense that it stimulates the pancreatic B cells and improves the insulin secretor capacity. *P. pellucida* is safe and effective as an antiglycaemic with no undesirable effect (Hua *et al.*, 1999).

Plants have long played a significant role in maintaining human health and have also served as food for humans. WHO estimates that over 80% of people rely on traditional medical for their primary health care needs and most of this therapy involves the use of plant extracts or their active components (Campillo *et al.*, 2001).

Tapinanthus bangwensi which belongs to the family Loranthaceae is commonly known as mistletoe, common mistletoe or mistletoe. This plant is originally native to Nigeria, Europe, North Africa, Western and Southern Asia (Jurin *et al.,* 1993). As a semi-parasitic evergreen growing on host tree, it depends on the host for minerals and water, and synthesizes its carbohydrate using green leathery, oblong leaves (Osadebe and Uzochukwu, 2006). The chemical make-up of mistletoe may differ according to host tree species, the time of harvest and the process employed in processing. Such chemical constituents include caffeic acid, alkaloids, amines, phenols, flavonoids, terpenoids and viscotoxins, flavonoids, flavonols agylcones, lecitins, triterpenes, saponins, acetylcholine derivatives, vitamin C, histamine, resins, thionins, and cardenolides (Jolanta and Przemysław, 2015).

Tapinanthus bangwensi (mistletoe) has been in use medicinally for ages to cure ailments such as symptoms of menopause, infertility, cancer, nervous tension, asthma, hypertension, headache, diabetes and dermatitis (Obatomi *et al.,* 1994; Grossarth-Maticek and Ziegler, 2007). Immune system modulating ability of extracts of mistletoe is also well documented (Jurin *et al.,* 1993; Ladokun *et al.,* 2015).

Mistletoes grow on many trees and tree crops indigenous to West Africa and which are of economic importance; among them are shea butter, neem, citrus (Bright *et al.*,1998) and cocoa (Overfield *et al.*,1998).

1.11 STATEMENT OF RESEARCH PROBLEM

Herbal preparations form the basis for many therapeutic drugs and are the first line treatment for many of the world's population, being readily available, traditional and relatively inexpensive . In Nigeria, several thousands of plant species have been claimed to possess medicinal properties and employed in the treatment of many ailments.

Reports are available on the therapeutic use of *M. indica, P. pellucida, M. foetida and T. bangwensi* but there is little or no information on the transcriptomic alterations of these selected herbs hence the need to do this work. Also there is need to determine the haematological alterations they may cause when ingested orally. This study employed the quantitative real-time polymerase chain reaction (qPCR) technique to assess the transcriptomic alteration in rats exposed to these selected plants.

1.12 AIMS AND OBJECTIVES

The general aim of this research work was to determine the haematological and transcriptomic alterations occurring in acute culinary exposure of normal rats to *M. indica, P. pellucida, M. foetida and T. bangwensi.*

1.13 SPECIFIC OBJECTIVES

- To determine the effects of the extracts on the haematological parameters of rats fed with the *M. indica, P. pellucida, M. foetida and T. bangwensi* extracts.
- To determine the transcriptomic alterations occurring in the rats exposed to *M. indica, P. pellucida, M. foetida and T. bangwensi* extracts.
- To assess the toxic effects of *M. indica, P. pellucida, M. foetida and T. bangwensi* extracts on the rats
- To determine the mechanism of action of *M. indica, P. pellucida, M. foetida and T. bangwensi.*

CHAPTER TWO
LITERATURE REVIEW

In general, herbs are any plant used for food, flavoring, medicine, or fragrances for their savory or aromatic properties (Tapsell *et al.,* 2006). Culinary use typically distinguishes herbs from spices. Herbs refer to the leafy green or flowering parts of a plant (either fresh or dried), while spices are produced from other parts of the plant (usually dried), including seeds, berries, bark, roots and fruits(Tapsell *et al.*,2006). In botanical English, the word "herb" is also used as a synonym of "herbaceous plant". Herbs have a variety of uses including culinary, medicinal, and in some cases, spiritual. General usage of the term "herb" differs between culinary herbs and medicinal herbs. In medicinal or spiritual use any of the parts of the plant might be considered "herbs", including leaves, roots, flowers, seeds, root bark, inner bark (and cambium), resin and pericarp (Tapsell *et al.*,2006). As far back as 5000 BCE, Sumerians used herbs in medicine. Ancient Egyptians used fennel, coriander and thyme around 1555 BCE. In ancient Greece, in 162 CE, a physician by the name of Galen was known for concocting complicated herbal remedies that contained up to 100 ingredients (Tapsell *et al.,* 2006).

Culinary herbs are distinguished from vegetables in that, like spices, they are used in small amounts and provide flavor rather than substance to food. Herbs can be perennials such as thyme or lavender, biennials such as parsley, or annuals like basil. Perennial herbs can be shrubs such as rosemary, *Rosmarinus officinalis*, or trees such as bay laurel, *Laurus nobilis* – this contrasts with botanical herbs, which by definition cannot be woody plants (Dilhey *et al* .,2006). Some plants are used as both herbs and spices, such as dill weed and dill seed or coriander leaves and seeds. Also, there are some herbs such as those in the mint family that are used for both culinary and medicinal purposes (Dilhey *et al.,* 2010). Some plants contain phytochemicals that has effects on the body. There may be some effects when consumed in the small levels that typify culinary "spicing", and some herbs are toxic in larger quantities (Chinese Herbal Medicine, 2014). For instance, some types of herbal extract, such as the extract of St. John's-wort (*Hypericum perforatum)* or of kava (*Piper methysticum*) can be used for medical purposes to relieve depression and stress. Herbs have long been used as the basis of traditional Chinese medicine, with usage dating as far back as the first century CE (Chinese Herbal Medicine, 2014). In India, the Ayurveda medicinal system is based on herbs.

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Medicinal use of herbs in Western cultures has its roots in the Hippocratic (Greek) elemental healing system, based on a quaternary elemental healing metaphor.

2.1 CLASSIFICATION AND DESCRIPTION OF PLANTS

2.11 MISTLETOE (*Tapinanthus bangwensi*)

Mistletoe is a common name used generally for woody shoot parasites in various plant families, mostly in the *Loranthaceae* and *Viscaceae* families (Parker and Riches1993). Mistletoe was prominent in the folklore and religions of ancient Europe before Christianity started. It was used as a remedy for evil as it was reputedly endowed with magical powers; it is used for decorations during the Christmas and New Year celebrations, and it is still a common practice to kiss under a branch of mistletoe (Redmond and Redmond 2007). Mistletoe is commonly found growing on tree crops like cocoa, kolanut, coffee, bush mango etc. in the South western, Nigeria. Mistletoe can also grow on citrus trees like orange (*Citrus sp.*) and guava (*Psidium guajava* L.).

Mistletoes grow on many trees and tree crops indigenous to West Africa and which are of economic importance; among them are sheabutter, neem, citrus (Bright and Okunsanya 1998), and cocoa (Guyot and Ntawanga, 1997; Overfield, *et al.*, 1998).

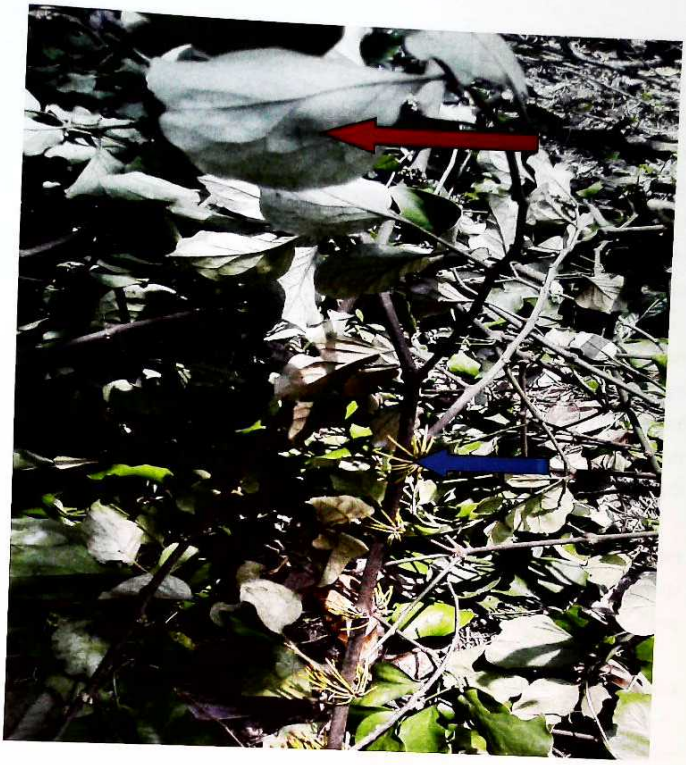

Plate 1: Photograph of *Tapinanthus bangwensi* harvested from cocoa tree.
(Blue arrow is pointing to the "Haustoria", while Red arrow is to the Leaf)

Mistletoe is of interest in botany especially because it is a partial parasite (a "hemiparasite") (Redmond and Redmond 2007). It grows on the branches of tree trunks and sends out roots tagged "haustoria" that penetrate into the tree and take up nutrients (Williams, 1990). Mistletoe can also grow on its own as it can manufacture its own food by photosynthesis like other plants. However, it is more common to find it growing as a parasite. The name 'mistletoe' given to the plant was derived from the belief of old that it sprang up from bird droppings. This belief was connected to the principle accepted in those days that life could arise suddenly from dung. In ancient times it was noticed that mistletoe often would appear on a branch or twig where birds' droppings were found (Hoagy, 2008).

In Europe, mistletoe is used mainly as an anti-cancer agent. In contrast to American mistletoe which is toxic, European mistletoe is believed to have medicinal properties (Hoagy, 2008). European mistletoe has been reported to treat a wide variety of physical and mental conditions (Williams, 1990). It is best known currently as a therapy in addition with other drugs and or radiation for cancer treatment. It was also reported by some HIV/AIDS Organizations (NGO's) to help restore immune systems (Hoagy, 2008).

In Germany, mistletoe extracts serve as the most unorthodox oncology therapy (Hoagy, 2005). Mistletoe was documented as a treatment for skin diseases and prostate cancer in Palestine as discovered through ethno botanical surveys carried out (Khammash, 2005).

In Nigeria, *Loranthus bengwensis* L. (Loranthaceae), African mistletoe, has found popularity in its being widely used to treat diabetes mellitus in folk medicine (Obatomi *et al.*, 1994). Another species of African mistletoe *Tapinanthus dodoneifolius* exhibited a wide range of antimicrobial activities against some bacterial and fungal isolates of farm animals that are resistant to multiple drugs. The ability to inhibit the growth of certain bacteria species, such as *Agrobacterium tumefaciens*, *Bacillus sp. Escherichia coli, Proteus sp., Pseudomonas sp.* and *Salmonella sp.* linked with crown gall or gastrointestinal tract and wound infections by this mistletoe extract serves as a clue to its being used ethno-medicinally (Deeni and Sadig 2002). Osadebe and Ukwueze (2004) had presented the wide range of data regarding the antimicrobial activities of *Loranthus micranthus*, an eastern Nigeria species of the African mistletoe.

2.12 *Peperomia pellucida*

Peperomia pellucida (also known by common names pepper elder, shining bush plant, and man to man) is an annual, shallow-rooted herb, usually growing to a height of about 15 to 45 cm. It is characterized by succulent stems, it flowers all year-round, and the plant is found in various shaded, damp habitats all over Asia and the Americaş. *P. pellucida* has been used as a food item as well as a medicinal herb. Although mostly grown for its ornamental foliage, the entire plant is edible, both cooked and raw. It is also said that it can be a good refrigerant (Aziba *et al.*, 2001).

The analgesic properties of the plant seem to be related to its effect on prostaglandin synthesis (Aziba *et al.*, 2001). It may have potential as a broad-spectrum antibiotic, as demonstrated in tests against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* (*Bojo et al.*, 1994). Chloroform extracts from dried leaves *of P. pellucida* have been shown to exhibit antifungal activity against *Trichophyton mentagrophytes in vitro* (Ragasa *et al.*, 1998).

Anti-inflammatory activity (in paw edema) and analgesic activity has been demonstrated in rats and mice. Although the plant can cause asthma-like symptoms in patients with known hypersensitivity reactions to the species, no clinical data have yet been reported on human toxicity (Arigoni-Blank *et al.*, 2002).

Plate 2: Photograph of *Peperomia pellucida* (Shiny bush leaf -arrowed) from site

against roundworm (Burkil, 1985). The leaf sap is used to treat severe headache and ear-ache. In Malawi headache is treated by binding the head with the plant stem. In the Ivory Coast a preparation of the leaves is used as an aphrodisiac and is taken by women as an emmenagogue and as child-birth helper. In Uganda tea from leaves or roots are used as an abortifacient and an embolic (Burkil, 1985). In the Ivory Coast a leaf-decoction is used to treat smallpox (Burkil, 1985).

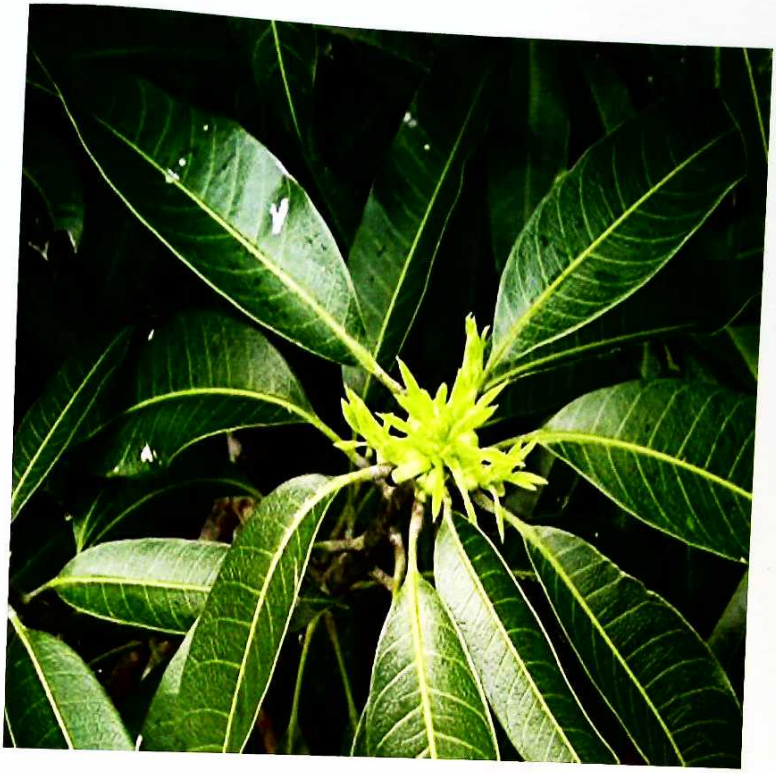

Plate 4: Photograph of *Mangifera indica* leaves (Mango leaves)

The root is used in Tanganyika to wash small children and mothers' breasts. In South Africa a root decoction with other plants is taken for boils (Burkil, 1985). The inflammation caused by the venom of the spitting cobra, (*Naja nigrocollis*) can be prevented by promptly rubbing the skin with crushed leaves and chewing them. The leaf sap is drunk to treat snakebite (Burkil, 1985). The leaf sap is used to stop nose bleeding. In Tanganyika, the young leaves are used to treat dropsy and malaria (Burkil, 1985).

2.14 *Mangifera indica* (Mango)

Mangifera indica, commonly known as mango, is a species of flowering plant in the sumac and poison ivy family, Anacardiaceae. It is found in the wild in Bangladesh, India and Pakistan where it is indigenous and cultivated varieties have been introduced to other warm regions of the world. It is a large fruit-tree, capable of a growing to a height and crown width of about 100 feet and trunk circumference of more than twelve feet. The species appears to have been domesticated in India at around 2000 B.C. (Sauer and Jonathan, 1993). The species was brought to East Asia around 400-500 BCE from India; next, in the 15th century to the Philippines; and then, in the 16th century to Africa and Brazil by the Portuguese. The species was described for Science by Linnaeus in 1753. Mangiferin (a pharmacologically active hydroxylated xanthone C-glycoside) is extracted from mango at high concentrations from the young leaves (172 g/kg), bark (107 g/kg), and from old leaves (94 g/kg) (Barneto *et al.*, 2008).

In Ayurveda, it is used in a Ramayana formula sometimes with other mild sours and shatavari (*Asparagus racemosus*) and guduchi (*Tinospora cordifolia*). In this oriental system of traditional medicine, varied properties are attributed to different parts of the mango tree, both as food and medicine (Shah *et al.*, 2010). Extracts of the bark, leaves, stems, and unripe fruits have demonstrated antibiotic properties *in vitro*. (Shah *et al.*, 2010). The different chemical constituents of the plant are the polyphenols, flavonoids and triterpenoids. Mangiferin, a xanthone glycoside, is the major bio-active constituent; other constituents are isomangiferin, tannins and garlic acid derivatives (Shah *et al.*, 2010). The bark is reported to contain protocatechic acid, catechin, mangiferin, alanine, glycine, γ-amino butyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoids cycloart-24-en-3β,26diol, 3-ketodammar-24 (*E*)-en-20S,26-diol, C-24 epimers of cycloart-25 en 3β,24,27-triol and cycloartan-3β,24,27-triol (Scartezzini and Speroni, 2000). Indicoside A and B, manghopanal, mangoleanone, friedelin, cycloartan-3β-30-diol and derivatives, mangsterol, manglupenone, mangocoumarin, n-tetacosane, n-heneicosane, n-triacontane and mangiferolic acid methyl

ester and others isolated from stem bark of the plant (Khan *et al.*, 1993). Mangostin, 29-hydroxy mangiferonic acid and mangiferin also have been isolated from the stem bark together with common flavonoids the flower yielded alkyl gallates such as gallic acid, ethyl gallate, methyl gallate, n-propyl gallate, n-pentyl gallate, n-octyl gallate, 4-phenyl gallate, 6-phenyl-n-hexyl gallate, and dihydrogallic acid (Khan *et al.*, 1989). Root of mango contains the chromones, 3-hydroxy-2-(4'-methylbenzoyl)-chromone, and 3-methoxy-2-(4'-methyl benzoyl)-chromone. The leaf and flower yield an essential oil containing humulene, elemene, ocimene, linalool, nerol and many others (Nunez *et al.*, 2002). The fruit pulp contains vitamins A and C, β-carotene and xanthophylls (Ross, 1999). An unusual fatty acid, cis-9, cis-15-octadecadienoic acid was isolated from the pulp lipids of mango (Shibahara *et al.*, 1993). Quantitative analysis of the compounds has been performed by HPLC, and mangiferin was found to be the predominant component in the plant (Nunez et *al.*, 2002).

Plate 3: Photograph of *Momordica foetida* with the fruits (Bitter gourd)
(Blue arrow is pointing to the fruit, while Red arrow is to the Leaf)

The bark of *M. indica* and fruits are known to contain vitamins, polyphenols, terpenoids, steroids, fatty acid and trace elements, it is also reported to possess various biological activities such as anti-tumour, anti-neoplastic, anti-oxidant, and anti-inflammatory (Garrido, *et al.*, 2001; Nunez, *et al.*, 2002), analgesic and immunomodulatory properties (Garcia, 2002). Methods such as haematology and transcriptomics analysis has been used in different studies to decipher the pharmacological and toxic effect of medicinal herbs (extract) on animals, and to evaluate the herb/host interactions both at the tissue and molecule level (Garcia, 2002).

2.15 Haematology

Haematology refers to the study of the numbers and morphology of the cellular components of the blood, which include the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes), and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Haematological parameters are good indicators of the physiological status of animals (Khan and Zafar, 2005) as the information from this test are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al.*, 2007).

The examination of blood provides the opportunity to clinically investigate the presence of several metabolites and other constituents in the body and also plays a vital role in the physiological, nutritional and pathological status of animals (Doyle, 2006). It also helps to distinguish normal state from state of stress which can be induced by external factors such as feed, drug or plant extract (Aderemi, 2004). They are also excellent medium for the measurement of potential biomarkers, because its collection is relatively non-invasive and it encompasses an enormous range of physiological process in the body at any given time (Aderemi, 2004). Haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed, drug or plant administration. According to Daramola *et al.* (2005), haematological values could serve as baseline information for comparisons of nutrient deficiency, physiology and health status of animals.

Blood which is a vital special circulatory tissue is composed of cells suspended in intercellular fluid substance (plasma) with the major function of maintaining homeostasis (Isaac *et al.*, 2013). Haematological components, which consist of red blood cells (RBC),white blood cells (WBC) or leucocytes, mean corpuscular volume (MCV), mean

corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are valuable in monitoring herb toxicity especially with plant constituents that affect the blood as well as the health status of animals (Oyawoye and Ogunkunle, 2004). Red blood cells (erythrocytes) serve as a carrier of haemoglobin, which reacts with oxygen carried in the blood to form oxy-haemoglobin during respiration (Chineke *et al.*, 2006). The red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Soetan *et al.*, 2013; Isaac *et al.*, 2013).

Red blood cell distribution width (RDW or RDW-CV or RCDW and RDW-SD) is a measure of the range of variation of red blood cell (RBC) volume that is reported as part of a standard complete blood count. Usually red blood cells are a standard size of about 6-8μm in diameter. Certain disorders, however, cause a significant variation in cell size. Higher RDW values indicate greater variation in size. Normal reference range of RDW-CV in human red blood cells is 11.5-14.5%. If anaemia is observed, RDW test results are often used together with mean corpuscular volume (MCV) results to determine the possible causes of the anaemia. It is mainly used to differentiate anaemia of mixed causes from an anemia of a single cause. An elevation in the RDW is not characteristic of all anaemia. Anaemia of chronic disease, hereditary spherocytosis, acute blood loss, aplastic (anaemia resulting from an inability of the bone marrow to produce red blood cells), and certain hereditary haemoglobinopathies (including some cases of thalassemia minor) may all present with a normal RDW (Evans and Jehle, 1991).

Platelet count and platelet indices such as mean platelet volume (MPV) and platelet distribution width (PDW) are parameters used for the routine check of complete blood count CBC. Being natural sources of growth factors like insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), or transforming growth factor β (TGF-β) caused the platelets to have important role in different processes such as inflammation, angiogenesis, repair and regeneration of the tissues . Previous studies suggest that in inflammation via some cytokines, the platelets size and volume alter differently: in low grade inflammatory disorders, by the involvement of the large platelets in thrombi, MPV values may increase. On the other hand, in high grade inflammatory conditions, the consumption of the large platelets at the inflammation site cause a decrease in MPV levels (Gasparyan *et al.*, 2011).

The major functions of the white blood cell and its differentials are to fight infections, defend the body by a process called phagocytosis, against invasion by foreign organisms and to produce, transport, and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan *et al.*, 2013), thereby enhancing adaptability to local environmental and disease prevalent conditions (Okunlola *et al.*, 2012).

Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot-formation (blood clotting) will be extended, resulting in excessive loss of blood in the case of even slight injury. Platelets play an essential role in cancer development, progression and metastasis through their direct interaction with tumor cell. Platelet actions trigger autocrine and paracrine activation processes that cause phenotypic changes in stromal cells which contribute to the development of cancer. Increased platelets were associated with poor prognosis in patients with a wide spectrum of malignancies, such as pancreatic cancer, gastric cancer, colorectal cancer, endometrial cancer, and ovarian cancer (Suzuki, 2004; Ekici, 2015; Long *et al.*, 2016; Pietrzyk, 2016). However, platelet count is determined by the balance between the rate of production and consumption of platelets. A normal platelet count could conceal the presence of highly hyper-coagulative and pro-inflammatory cancer phenotypes in the presence of efficient compensatory mechanisms (Sertel *et al.*, 2012).

Mean platelet volume (MPV), the most commonly used measure of platelet size, is an index of platelet activation and is available in clinical practice (Gasparyan, 2011). Platelet distribution width (PDW), another platelet index, indicates variation in platelet size (Kaito, 2005). Altered MPV levels were reported in gastric cancer, ovarian cancer, lung cancer, colon cancer, and breast cancer (Gasparyan, 2011).Packed cell volume (PCV) which is also known as haematocrit (HCT) or erythrocyte volume fraction (EVF)), is the percentage (%) of red blood cells in blood (Purves *et al.*, 2003). Packed cell volume is involved in the transport of oxygen and absorbed nutrients (Isaac *et al.*, 2013). Increased packed cell volume shows a better transportation and thus results in an increased primary and secondary polycythemia. (Purves *et al.*, 2003).

Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates with the exception of the fish family,*Channichthylidae* as well as tissues of invertebrates (Sidell and O' Brien, 2006) . Haemoglobin has the physiological

function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Soetan *et al.*, 2013 and Isaac *et al.*, 2013). Previous reports stated that packed cell volume, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Awodi *et al.*, 2005; Chineke *et al.*, 2006). Furthermore, it has been posited that high packed cell volume (PCV) indicate either an increase in number of red blood cells (RBCs) or reduction in circulating plasma volume (Chineke *et al.*, 2006). Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) indicate blood level conditions. A low level of this parameter is an indication of anaemia (Aster, 2004).

Table1.1: Haematological parameters of laboratory rats and the accepted normal range.

Haematological Parameters	Male Range	Female Range
HCT (%)	41.70-57.00	33.10-47.70
HGB (g/L)	147.0-208.0	108.0-175.0
WBC (×109/L)	5.90-18.30	5.80-20.10
MCHC (g/L)	317.0-368.0	315.0-368.0
Granulocyte (×109/L)	1.70-12.10	1.70-14.70
Granulocyte % (%)	27.00-73.00	24.00-79.00
L+M (×109/L)	2.90-8.90	1.40-8.20
L/M % (%)	27.00-73.00	21.00-76.00
PLT (×109/L)	135.0-1005.0	62.0-1188.0

Source: Özkan *et al.*, 2003

2.20 TRANSCRIPTOMICS

Transcriptomic is an unbiased, sensitive, and personalized approach with the potential to reveal new predictive biomarkers of disease and ultimately improve the decision-making process in the assessment of biological activities of medicinal herbs or drug (Sandvik *et al.*, 2006). When performed in a dose response format, the observed transcriptional changes can provide both quantitative and qualitative information on the dose of the administered agent at which cellular processes are affected. Transcriptomic approaches have transformed the way in which physicians approach diagnosis, prognosis, and treatment, and in which regulators approach risk assessment (Heidecker *et al.*, 2008).

The transcriptome includes the total complement of messenger RNA (mRNA) molecules (also called transcripts) produced in a specific cell or the population of cells comprising a tissue (Heidecker *et al.*, 2008). Transcriptomics is regarded as a high throughput technology concerned with determining how the transcriptome changes with respect to various factors at a certain time point and at a given biological state. Regulation of gene expression is highly complex and underlies many fundamental biological processes such as growth, differentiation, and disease pathogenesis with the ability to adapt rapidly with tremendous variability in different tissues and in response to stimuli (Sandvik *et al.*, 2006). Transcriptomics and global gene expression are powerful tools used in the field of toxicology and, as in most cases; toxicity is not expected to occur without alterations at the transcriptional level (Gibb *et al.*, 2011). A common application of transcriptomics in toxicology is to compare gene expression after a chemical exposure from a diseased and non-diseased tissue to provide a list of genes that show altered expression in the diseased group. These findings not only advance researchers' understanding of disease pathogenesis, but they also reveal transcripts that can be qualitatively or quantitatively assessed as new biomarkers (Gibbs *et al.*, 2011).

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathologic processes, or pharmacologic responses to a therapeutic intervention (Ilyin *et al.*, 2004). Although a single biomarker can be easily understood, transcriptomic studies have shown that aggregate measures composed of multiple genes are also informative as biomarkers of complex disease. Efforts in transcriptomic biomarker development are not restricted to medical diagnostics but include environmental

chemical risk assessment and determination of exposure to chemical residues in food or plants (Riedmaier *et al.*, 2009a; Pinel *et al.*, 2010).

2.21 TYPES OF TRANSCRIPTOMICS BIOMAKERS.

There are three commonly used types of transcriptomic biomarkers:

- messenger RNA (mRNA),
- micro RNA(miRNA), and
- Long noncoding RNA (lncRNAs).

mRNA biomarkers are already an established method in several scientific fields. Pathogenic tissue can be distinguished from nonpathogenic ones by analyzing the expression of specific genes. Using mRNA gene expression analysis is helpful in validating different types or stages of diseases. Noncoding RNAs can be subdivided into small noncoding RNAs shorter than 200nt and long noncoding RNAs with more than 200nt (Gibb *et al.*, 2011).

miRNAs are small noncoding RNA molecules with about 20-22nucleotides which are involved in post transcriptional processing of mRNA. In this way they are able to regulate physiological pathways and metabolic processes and therefore impact the entire cellular physiology, organ development, and tissue differentiation. The expression of miRNA can be measured in cell culture samples. These miRNAs are also present in body fluids, such as urine, blood, and breast milk (Kroh *et al.*, 2010).

Noncoding RNAs with a length of more than 200nt belong to the group of long noncoding RNAs (lncRNAs) and have been identified as biomarkers in several disease conditions. Interestingly, the identification of single biomarkers on the mRNA or the miRNA level is not possible in most pathological disorders. In such cases, a set of multiple biomarkers must be present to distinguish between specific disease types, disease states, or applied treatments (Gibbs *et al.*, 2011).

2.22 TRANSCRIPTOMIC METHODS

There are three commonly used methods for assessing transcriptomes:

- quantitative real-time polymerase chain reaction (qPCR),
- microarrays, and
- RNA sequencing (RNAseq).

Each of these target methods have advantages and disadvantages which can be further subdivided into targeted or untargeted approaches. The qPCR is a targeted method that uses short DNA sequences called primers to anneal and amplify known transcripts in a biological sample. Rapid quantitation of hundreds of transcripts using qPCR is relatively inexpensive, but knowledge of sequences of the candidate transcripts is required (Morin *et al.*, 2008). The two main technologies for untargeted global transcriptome screening that dominate the diagnostic field are gene expression microarrays and RNAseq. In microarray-based methods, RNA is isolated from a specific sample and converted to a chemically labeled form. Labeled RNA is then incubated with a small chip that measures transcript abundance by hybridization of labeled RNA to each of the probes on the microarray.

Microarrays offer genome-wide coverage of the transcriptome, have high throughput, and have become relatively inexpensive but complicated normalization methods and, similarly to qPCR, are limited by the requirement to know existing transcript sequences up front. Despite these limitations, microarrays have thus far been the most widely applied technology (Chu, 2012). RNAseq is the most recent transcriptomic approach where the total complement of RNAs from a given sample is isolated and sequenced using high-throughput technologies (often called Next-Generation Sequencing). The abundance of each transcript is quantitated by counting the number of copies. RNA sequencing has the advantage, as compared to microarrays and qPCR, in that no previous knowledge on sequence is necessary. However, RNAseq methods are costly and complex and require enormous computational capacity. In some situations, RNAseq has already begun to replace microarrays in basic research, but clinical studies will likely use both approaches depending on scientific goals, sample size, and cost (Morin *et al.*, 2008; Wang *et al.*, 2009; Krohn *et al.*, 20 10; Chu and Corey 2012).

2.23 STEROL REGULATORY ELEMENT BINDING PROTEIN (SREBP)

The transcription factor SREBP (sterol regulatory element binding protein), is a key regulator of cholesterol and lipid homeostasis (Yang *et al.*,2006) which is encoded by the genes SREBF1 and SREBF2, it belongs to the basic-helix-loop-helix leucine zipper class of transcriptional factors (Yang *et al.*, 2006). It is important to note that there are three related SREBP proteins: SREBP1a, which regulates fatty acid and cholesterol biosynthesis; SREBP1c, which regulates fatty acid synthesis; and SREBP2, which primarily regulates genes, involved in cholesterol metabolism; of which SREBP1c and SREBP are highly expressed in the Leydig cells of the testis (Yang *et al.*, 2006).

and spermatids, with the effects being more significant when administered through the intraperitoneal route (Naseem *et al.*, 1998).

2. 25 ANDROGENS

Androgens are critical steroid hormones that determine the expression of the male phenotype including the outward development of secondary sex characteristics as well as initiation and maintenance of spermatogenesis. Their actions are mediated by the androgen receptor (Wang *et al.*, 2009). Androgen receptor is a type of nuclear receptor that is activated by binding either of the androgenic hormones (e.g., testosterone) in the cytoplasm and then translocating into the nucleus; it functions as a DNA-binding transcription factor that regulates gene expression (You and Sar 1998; Suárez-Quian *et al.*, 1999).

2. 26 INSULIN AND GLUCOSE TRANSPORTER-4(GLUT-4)

Glucose uptake and storage in peripheral tissues such as skeletal muscles and adipose tissue is a major regulatory process in the homeostatic control of blood glucose levels (Khan and Flier, 2000). It is widely accepted that skeletal muscle, by virtue of its large contribution to body mass, represents the major site of insulin-mediated glucose disposal. However, both tissues contribute toward the lowering of blood glucose (Turban *et al.*, 2005). Glucose uptake is mediated through the translocation of the Glut4 receptor from the interior to the cell surface which is stimulated by the insulin signaling pathway initiated by activation of the insulin receptor. Any defect in this pathway triggers the development of hyperglycemia in type II diabetes. Thus, measurement of glucose uptake into peripheral tissues is an important mechanism to assess insulin sensitivity (Turban *et al.*, 2005).

Medicinal plants offer a rich, yet inadequately explored source of potentially useful anti-diabetic drug. *Phyllanthus emblica* (amla), *Tinospora cordifolia* (guduchi), and *Curcuma longa* (haldi) are extensively used by ayurvedic practitioners for the treatment of diabetes (Gogte, 2000). Their anti-diabetic activity has been explored in various animal models of diabetes and its complications (Babu *et al.*, 2004; Rao, 2005; Suryanarayan *et al.*, 2007; Nwozo *et al.*, 2009; Mehta *et al.*, 2009; Qureshi *et al.*, 2009). Most of these studies have focused on the glucose lowering effects of these plants in experimental models.

GLUT4, also known as solute carrier family 2, is a protein encoded, in humans, by the *SLC2A4* gene. As glucose levels increase, insulin is released from the pancreas and into the blood stream. Increased insulin levels cause the uptake of glucose into the cells (Gogte,

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2000). GLUT4 is stored in the cell in transport vesicles, and is quickly incorporated into the plasma membrane of the cell when insulin binds to membrane receptors. Insulin binds to the insulin receptor in its dimeric form and activates the receptor's tyrosine-kinase domain. The receptor then recruits Insulin Receptor Substrate, or IRS-1, which binds the enzyme PI-3 kinase. PI-3 kinase converts the membrane lipid PIP2 to PIP3. PIP3 is specifically recognized by PKB (protein kinase B) and by PDK1, which can phosphorylates and activate PKB. Once phosphorylated, PKB is in its active form and phosphorylates TBC1D4, which inhibits the GTPase-activating domain associated with TBC1D4, allowing for Rab protein to change from its GDP to GTP bound state. Inhibition of the GTPase-activating domain leaves proteins next in the cascade in their active form, and stimulates GLUT4 to be expressed on the plasma membrane (Nwozo et al., 2009). Insulin is a hormone made in the pancreas, an organ located behind the stomach. The beta cells within the islets of the pancreas make insulin and release it into the blood when glucose levels rise. Increased production of insulin seen in the pancreas results most times from the body's demand for it when there is a case of insulin resistance during which muscle, fat, and liver cells do not respond properly to insulin and thus cannot easily absorb glucose from the bloodstream. Over time, insulin resistance can lead to type 2 diabetes because the beta cells fail to keep up with the body's increased need for insulin (Turban *et al.*, 2005; Nwozo, *et al.*, 2009).

2.27 CALCIUM VOLTAGE-GATED CHANNEL SUBUNIT ALPHA1 A (CACNA1-α)

Calcium voltage-gated channel subunit alpha1 A (CACNA1-α) mediates the entry of calcium ions into excitable cells, and are also involved in a variety of calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, and gene expression(Mojiminiyi *et al.*, 2008). It was reported that, although not as high as the expression by the brain, stomach or testis, the pancreas produced a significant level of CACNA1-α. The contractile mechanism in smooth muscle is activated by a rise in the concentration of free intracellular Ca^{2+} concentration. The source of (Ca^{2+}) is the extracellular fluid or release from internal pools. Thus, vascular smooth muscle relaxant agents may produce their effects by inhibiting either or both sources of Ca^{2+} (Mojiminiyi *et al.*, 2008).

A growing number of diseases are recognized as being caused by mutations in genes encoding voltage gated ion channels of skeletal muscle (Hoffman *et al.*, 1995). Naturally occurring mutations in channel genes or antibodies directed against channel proteins have also been found for other excitable tissues such as the heart and the brain as well as unexcitable organs such as the kidney (Greenberg, 1997; Lehmann-Horn and Jurkat-Rott,

1999). Mutations in calcium channels or channel subunits cause malignant hyperthermia susceptibility types 1 and 5 (Monnier *et al.*, 1997), hypokalemic periodic paralysis, and various forms of hemiplegic migraine, episodic ataxias, and epilepsies (Jurkat-Rott *et al.*, 1994; Ptacek *et al.*, 1994; Terwindt *et al.*, 1998).

2.28 POTASSIUM CHANNEL (KCJN5).

Potassium channels are the most widely distributed type of ion channel and are found in virtually all living organisms (Littleton and Ganetzky, 2000). They form potassium-selective pores that span cell membranes. Furthermore potassium channels are found in most cell types and control a wide variety of cell functions. . The protein encoded by this gene is an integral membrane protein and inward-rectifier type potassium channel. The encoded protein, which has a greater tendency to allow potassium to flow into a cell rather than out of a cell, is controlled by G-proteins. It may associate with two other G-protein-activated potassium channels to form a hetero-multimeric pore-forming complex (Jessell *et al.*, 2000; Hille and Berti, 2001).

Potassium channels function to conduct potassium ions down their electrochemical gradient, doing so both rapidly (up to the diffusion rate of K^+ ions in bulk water) and selectively (excluding, most notably, sodium despite the sub-angstrom difference in ionic radius) (Lim *et al.*, 2016). Biologically, these channels act to set or reset the resting potential in many cells. In excitable cells, such as neurons, the delayed counter flow of potassium ions shapes the action potential. By contributing to the regulation of the action potential duration in cardiac muscle, malfunction of potassium channels may cause life-threatening arrhythmias. Potassium channels may also be involved in maintaining vascular tone. They also regulate cellular processes such as the secretion of hormones (*e.g.*, insulin release from beta-cells in the pancreas) since their malfunction can lead to diseases (such as diabetes) (Rang, 2003).

FOUR TYPES OF POTASSIUM CHANNEL

- Calcium-activated potassium channel - open in response to the presence of calcium ions or other signaling molecules.
- Inwardly rectifying potassium channel - passes current (positive charge) more easily in the inward direction (into the cell).

- Tandem pore domain potassium channel - are constitutively open or possess high basal activation, such as the "resting potassium channels" or "leak channels" that set the negative membrane potential of neurons.
- Voltage-gated potassium channel - are voltage-gated ion channels that open or close in response to changes in the trans-membrane voltage (Rang, 2003).

2.29 GLUCAGON LIKE PEPTIDE-1 (GLP-1)

Glucagon like peptide (GLP) is a 30 amino acid long peptide hormone derived from the tissue-specific posttranslational processing of the proglucagon gene expressed in several organs including the gut (intestinal enteroendocrine L-cells),. GLP-1 is the only known incretin describing its ability to decrease blood sugar levels in a glucose-dependent manner by enhancing the secretion of insulin. The most noteworthy effect of GLP-1 is its ability to promote insulin secretion in a glucose-dependent manner (Kristine and Catherine, 2003).

2.30 TUMOUR NECROSIS FACTOR –alpha (TNF-alpha)

TNF-alpha is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer (Vassali, 1992; Pfeffer, 2003). Reduced production of TNF-alpha may limit the release of acute phase mediators and thereby facilitate autoimmunity (Paul and Carroll, 1999). Mangiferin mediates the down-regulation of necrosis factor kappa B (NF-κB), suppresses of NF-κB activation induced by inflammatory agents, including tumor necrosis factor (TNF), increases the intracellular glutathione (GSH) levels and potentiates chemotherapeutic agent-mediated cell death; this suggests a possible role in combination therapy for cancer. It is likely that these effects are mediated through mangiferin ROS quenching and GSH rising; increased intracellular (GSH) levels are indeed known to inhibit the TNF-induced activation of NF-κB (Vassali, 1992; Paul and Caroll, 1999; Pfeffer, 2003).

2.31 INTERLEUKIN 1 alpha (IL- 1 alpha)

Interleukin 1 alpha is a cytokine of the interleukin 1 family. Interleukin 1 is responsible for the production of inflammation, as well as the promotion of fever and sepsis (Nicklin *et al.*, 1994). IL-1α inhibitors are being developed to interrupt those processes and treat diseases. A wide variety of other cells only upon stimulation can be induced to transcribe

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IL-1α genes and produce the precursor form of IL-1α, (Fieldmann and Saklatvala, 2001). Although there are many interaction of IL-alpha with other cytokines, the most consistent and most clinically relevant is its synergism with TNF (Dinarello, 2001).

2.32 PHOSPHOFRUCTOKINASE-1(PFK).

Phosphofructokinase-1 (PFK-1) is one of the most important regulatory enzymes of glycolysis, as it catalyzes the conversion of fructose 6-phosphate and ATP to fructose 1,6-bisphosphate and ADP. This enzyme is controlled by many activators and inhibitors. PFK1 is allosterically inhibited by high levels of ATP, and enzyme activity increases when the cellular ATP/AMP ratio is lowered. PFK1 is also inhibited by low pH levels, PEP, and citrate. Amongst others, the most potent activator of PFK-1 is fructose 2, 6-bisphosphate. It has been reported that the L isoform of PFK-1 is expressed in the liver (Usenik, 2010). The precise regulation of PFK1 prevents glycolysis, gluconeogenesis from occurring simultaneously (Usenik, 2010).

2.33 GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD).

The G6PD gene provides instructions for making an enzyme called glucose-6-phosphate dehydrogenase. This enzyme is involved in the normal processing of carbohydrates. It also protects red blood cells from the effects of potentially harmful molecules called reactive oxygen species. Reactive oxygen species are byproducts of normal cellular functions. Chemical reactions involving glucose-6-phosphate dehydrogenase produce compounds that prevent reactive oxygen species from building up to toxic levels within red blood cells (Agbafor, 2015)

2.34 PYRUVATE KINASE

Pyruvate kinase is the enzyme responsible for catalyzing the final step of glycolysis. One of the isozymes of this enzyme is found in the liver (Gupta and Bamezai, 2010). Pyruvate kinase serves as a regulatory enzyme for gluconeogenesis, a biochemical pathway in which the liver generates glucose from pyruvate and other substrate (Berg *et al.*, 2002).

Genetic defects of this enzyme cause the disease known as pyruvate kinase deficiency. In this condition, a lack of pyruvate kinase slows down the process of glycolysis. This effect is especially devastating in cells that lack mitochondria, because these cells must use anaerobic glycolysis as their sole source of energy because the TCA cycle is not available. For example, red blood cells, which in a state of pyruvate kinase deficiency, rapidly become

deficient in ATP and can undergo haemolysis. Therefore, pyruvate kinase deficiency can cause chronic non-spherocytic haemolytic anaemia (CNSHA) (Grace *et al.*, 2015).

Due to the allosteric inhibitory effects of ATP on pyruvate kinase, a decrease in ATP results in diminished inhibition and the subsequent stimulation of pyruvate kinase. Consequently, the increase in pyruvate kinase activity directs metabolic flux through glycolysis rather than gluconeogenesis (Arguad *et al.*, 1993).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Collection and Identification of plant materials:

Fresh leaves of mango plants (*Mangifera indica*) were harvested from School of Health Technology Akure, also fresh leaves of mistletoe (*Tapinanthus bangwensi*) of cocoa plant and giant bitter lemon cucumber (*Momordica foetida*) were harvested from cocoa plantation in Emiloro near Oda town in Akure, while fresh leaves of Sliver bush (*Peperomia pellucida*) were harvested from the female hostel of Adekunle Ajasin University Akungba-Akoko. These plant specimens were identified at the Forestry Herbarium, Ibadan (FHI) and later deposited as voucher materials at Adekunle Ajasin University (AAU) Herbarium with voucher numbers, *Mangifera indica* no 131, *Momordica foetida* no138, *Peperomia pellucida* no 165 and *Tapinanthus bangwensi* no 201.

3.2 Sample Preparation

The leaves of *M. indica*, *T. bangwensi* and *M. foetida* were thoroughly washed and air dried for two months while that of *Peperomia pellucida* were freeze dried (Lyophilized) using a lyophilizer at the Biochemistry Laboratory of Adekunle Ajasin University Akungba. The dried leaves were blended into fine powder using an electric blending machine (Marlex CM/L7371373).

The ground plant materials were weighed into 30%weight/weight (30g leaf extract and 70g growers mash) and 70%weight/weight (70g leaf extract and 30g growers mash). The control group was 100% growers mash. 100ml of honey was measured using a graduated cylinder into the weighed powdered extract and into 100% (100g) growers mash. The honey was thoroughly mixed with the powdered leaf extracts. This is to facilitate the eating of the extracts by the rats especially those extract that are bitter like the Bitter gourd *(M. foetida).*

3.3 Ethical approval:

The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH Publication No. 85-23, Revised) (NIH, 1985) and Animal Welfare Act on the care and use of laboratory animals. All procedures were examined and approved by the Faculty's ethics committee (I R B).

3.4 Animal Treatment:

A total number of twenty albino rats were used; five groups with four animals in a group. The fifth group is the control group. Prior to the experiment, the animals were weighed and stabilized for a period of seven days by giving them water and growers mash prepared by Guinea feed Nig. Ltd. This was done to ascertain the animals were in good state of health. Different weights of the prepared extract were used to feed the rats for three consecutive days after which the rats were fasted over night for 12 hours. Clean water and 100% growers mash were used to feed the control group. After the administration of extract the rats were weighed. On the fourth day the rats were sacrificed and vital organs like liver, kidney, small intestine and pancreas were removed and processed for genetic expression. Blood samples were collected for hematological analysis (packed cell volume PCV, white blood count WBC, platelets, haemoglobin estimation (HB) etc.

3.5 Haematology analysis:

The haematological analysis was carried out in Inland Medical Centre, Ikare Akoko using an auto Haematology analyzer (URTI) 3300. The haematology parameters include Packed Cell Volume (PCV), White Blood Count (WBC), Platelets and others.

3.6 Gene expression:

The maps of the organs were made and were arranged into the PCR machine (Multigene Optimax) made by Labnet international Inc. prior to polymerization reaction.

3.6.1 RNA isolation:

Tissues excised from each animal were homogenized in Eppendorf tubes containing 50ul Trizol reagent. 100µl gradient separation medium (chloroform) was added to homogenates, vortex and centrifuged for 30 minutes at 1500rpm and the supernatant was aspirated, which contains the RNA, into new labeled tubes. 100µl of precipitating medium (isoamyl alcohol) was added to the supernatant and vortexes for 30mins at 1500rpm, RNA is recovered in pellet form and the supernatant decanted. 50µl of 70 percent ethanol was added and centrifuged for 5mins at 15000rpm, the supernatant was decanted and the cap left open to allow the ethanol to escape. 50µl of nuclease-free water was added to the total RNA isolated from tissues. The concentration of total RNA was determined by UV absorbance spectrophotometry (JENWAY 6305) for RNA (mRNA) quantification and all samples were diluted to the same concentration.

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3.6.2 Reverse transcription:

Reverse transcription was performed by adding 2µL reverse transcriptase from the cocktail which contain (1) the random oligo primer (2) the dNTPs (3) the reverse transcriptase (4) the reverse transcriptase buffer and (5) nuclease-free water.

2µl reverse transcriptase was aliquoted into 20µl of total RNA across the samples for the conversion into complimentary DNA (cDNA). The sample is then incubated in the thermocycler running for 4 hours at 65-degree Celsius in a total reaction volume of 22µl for the conversion.

3.6.3 Polymerase Chain Reaction (PCR):

Synthesized cDNA was diluted in nuclease-free water and 5µL was aliquoted into primer-specific PCR cocktail. In total, a 5.0µL PCR reaction mix containing 5µL cDNA template, 2µL forward and reverse primers (Inqaba Biotech, South Africa) and 2µL PCR Master Mix. As a rule, β-actin specific primer was used to track basal gene expression in each representative animal. The PCR was carried out using multigen optimax PCR machine. PCR amplification was done under 30 cycles with each cycle consisting of denaturation, annealing and extension.

Amplification conditions were: 94°C pre-denaturation for 5mins, 94°C for 30sec, Annealing 55°C (Tm) for 30 secs and Extension 72°C for 30 secs and then 5 min at 72°C by 30 cycles.

3.6.4 Agarose Gel Electrophoresis:

PCR amplicons were submitted for densitometric run in agarose gel electrophoresis. Briefly, 0.5X agarose was prepared in 50ml TBE-buffer. Under buffered conditions, gel was allowed to solidify at room temperature. 5µL PCR amplicon together with 5µL DNA loading buffer was loaded into pre-formed wells. Snapshots revealing the relative density of DNA bands were taken under UV-gel documentation. The metrics of average band density after 15 minutes of buffered run were analyzed with ImageJ (NIH).

3.7 Statistical Analysis: The data obtained for the haematological analyses were subjected to graphical analyses, while the band densities of the gene expression were quantified and subjected to analysis of variance, using ordinary one way ANOVA at 95% level of significance using graphpad prism 7.

CHAPTER FOUR

RESULTS

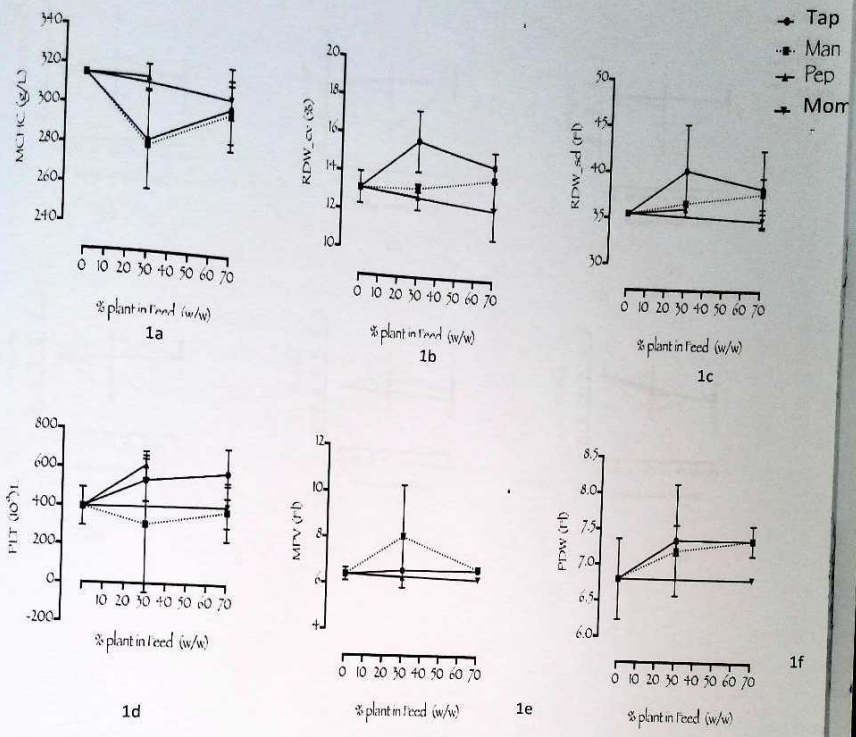
4.1 Haematological alterations that occurred on culinary exposure of normal albino rats to leaf extract of mistletoe (*Tapinanthus bangwensi*), mango (*Mangifera* indica), silver bush (*Peperomia pellucida*) and large bitter melon (*Momordica foetida*).

M. indica and *T.bangwensi* caused a decrease in the production of MCHC at 30% concentrate but increased the production at 70% compared to the control, while *M. indica* also increase the production of RDW and MPV at both 30% and 70% concentrate.

P. pellucida and *T.bangwensi* increased the platelets counts and PDW at both 30% and 70% concentrate respectively.

P. pellucida reduced the production of red blood cells (RBC), haemoglobin (HB), HCT (PCV), MCH and MCV at 30% concentrate while *M. indica* at 70% increased the production of RBC, HCT, MCV and HB but decreased them at 30% concentrate.

T.bangwensi, M. indica, P. pellucida and *M. foetida* caused a reduction in WBC and LYM except granulocytes which was slightly increased by *M. indica* and *T. bangwensi* at 30% and 70% concentrate compared to the control (Figures 1, 2 and 3).



Key: Tap= *Tapinanthus bangwensi,* **Man**= *Mangifera indica*

Pep = *Peperomia pellucida*, **Mom** = *Momordica foetida* (**NB:** Applies to Fig. 1 -. 3).

Figure 1: The effects of Plant extracts on platelets, mean platelet volume (MPV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and platelet distribution (PWD).

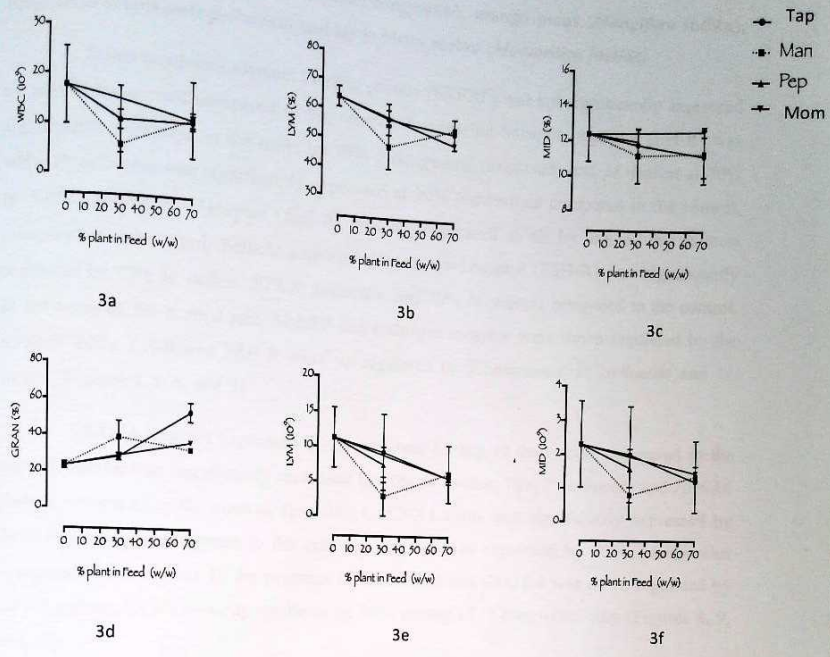


Figure 3: The effects of Plant extracts on white blood cells (WBC), lymphocytes (LYM) and granulocytes of normal rats.

4.2 Transcriptomic alterations that occurred on culinary exposure of normal albino rats to leaf extract of mistletoe (*Tapinanthus bangwensi*), mango plant (*Mangifera* indica), silver bush (*Peperomia pellucida*) and large bitter melon (*Momordica foetida*).

Sterol regulatory element binding protein (SREBP) was not significantly expressed by any of the extract compared to the control. Luteinizing hormone receptor (LH-R) was significantly expressed in the testis by 70% *T.bangwensi* (mistletoe) and *M. indica* at 70% while *P. pellucida* was significantly expressed at 30% concentrate compared to the control, ($p<0.05$). Androgen –Receptor (And-R) was not expressed at all by any of the extracts compared to the control. Follicle stimulating hormone-Receptor (FSH-R) was significantly expressed by 70% *M. indica*, 30% *P. pellucida*, and 70% *M. foetida* compared to the control. In the testis of the normal rats, SREBP and androgen receptor were down regulated by the extracts while LH-R and FSH-R were up regulated by *T.bangwensi, P. pellucida* and *M. indica* (Figures 4, 5, 6, and 7).

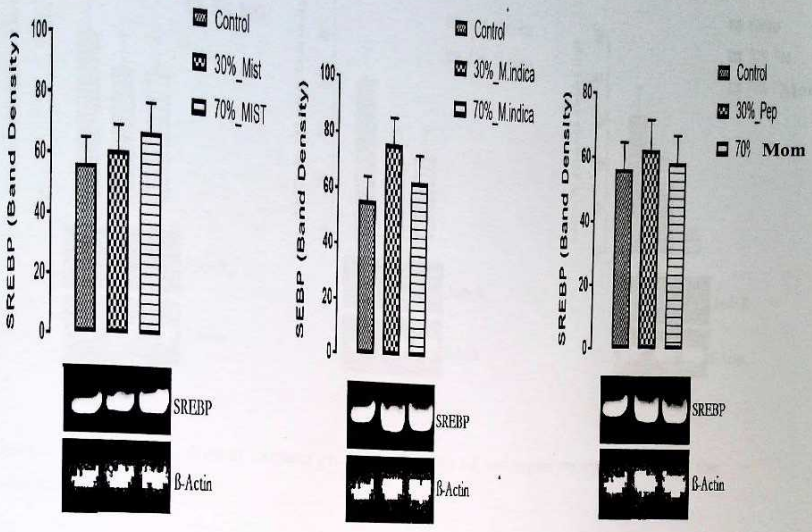
GLUT4 was not expressed in the pancreas by any of the extract compared to the control. Insulin was significantly expressed by 70% *M. indica*, 70% *P. pellucida* and *70% M. foetida* compared to the control, ($p<0.05$). CACNA1-alpha was significantly expressed by 30% *P. pellucida* compared to the control. KCJN5 was expressed by 70% *T.bangwensi* compared to the control. In the pancreas of the normal rats GLUT-4 was down regulated by all the extract, KCJN5 was up regulated by 70% extract of *T.bangwensi* only (Figures 8, 9, and 10).

Insulin gene was up regulated by a higher dose of *M. indica, M. foetida,* and a lower dose of *P. pellucida* (Figure 11).

GLP-1 was expressed in the small intestine by 70% *T.bangwensi*, 70% *M. indica*, 30% *P. pellucida,* and 70% *M. foetida* compared to control, ($p<0.05$). GLUT-2 was expressed significantly by 70% *T.bangwensi*, 30% and 70% *M. indica,* 30% *P. pellucida* and *70% M. foetida* compared to the control. In the small intestine GLP-1 and GLUT-2 genes were up regulated by *V. album* , *M. indica, M. foetida,* and *P. pellucida* (Figure 12 and 13).

TNF-α was down regulated in the kidney by all the extracts. IL-1-alpha was significantly expressed by all the extract compared to the control, ($p<0.05$). PFK-1 was expressed significantly by 70% *T.bangwensi,* 30% and 70% *M. indica,* 30% *P. pellucida* and 70% *M. foetida* compared to the control (Figures 14, 15, and 16).

G6PD was significantly expressed in the liver by 30% and 70% *T.bangwensi*, 30% and 70% *M. indica*, 30% *P. pellucida* and 70% *M. foetida* compared to the control, ($p<0.05$). GLUT-4 was significantly expressed in the liver by 30% and 70% mistletoe, 70% *M. indica* and 30% *P. pellucida* compared to the control. Pyr-kinase was significantly expressed by 30% and 70% *T.bangwensi*, *M. indica*, *P. pellucida* and *M. foetida* respectively compared to control ($p<0.05$). In the liver, glucose-6-phosphate dehydrogenase (G6PD), glucose transporter- 4(GLUT-4) and pyruvate kinase were up regulated by *T.bangwensi*, M. *indica, P. pellucida* and *M. foetida*.



Key: Mist= Mistletoe (*Tapinanthus bangwensi*), **M. indica**= Mango (*Mangifera indica*), **Pep**= *Peperomia pellucida,* **Mom**= *Momordica foetida.* (**NB:** Applies to Figures.4 -19)

Figure 4: The effects of plant extracts on the expression of sterol regulatory element binding protein (SREBP) on the testis of rats.

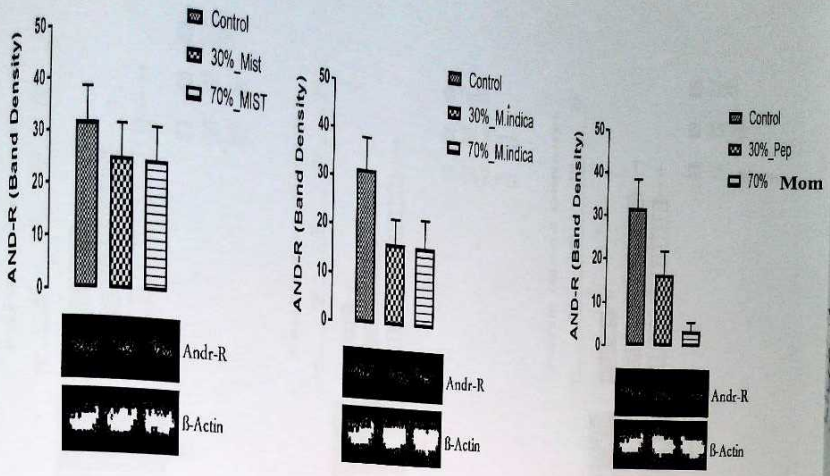


Figure 5: The effects of plant extracts on the expression of androgen receptor gene on the testis of rats.

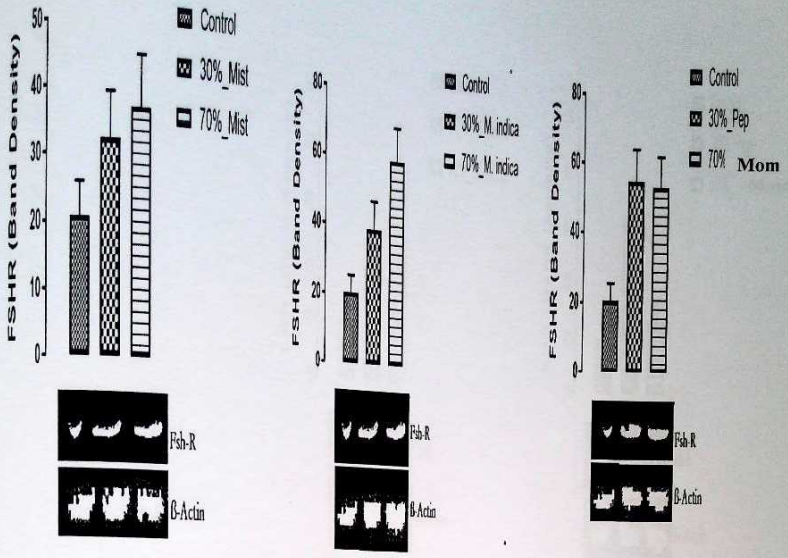


Figure 6: The effects of plant extracts on the expression of follicle stimulating hormone (FSH) receptor on the testis of albino rats.

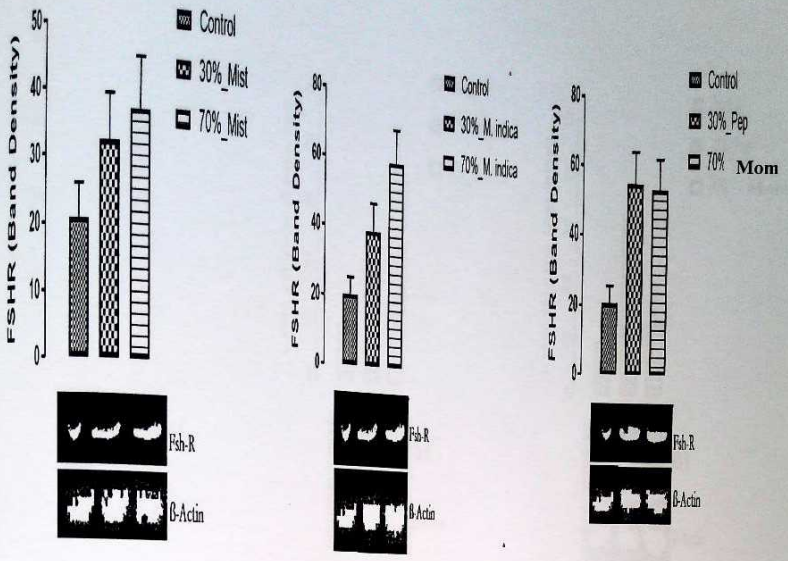


Figure 6: The effects of plant extracts on the expression of follicle stimulating hormone (FSH) receptor on the testis of albino rats.

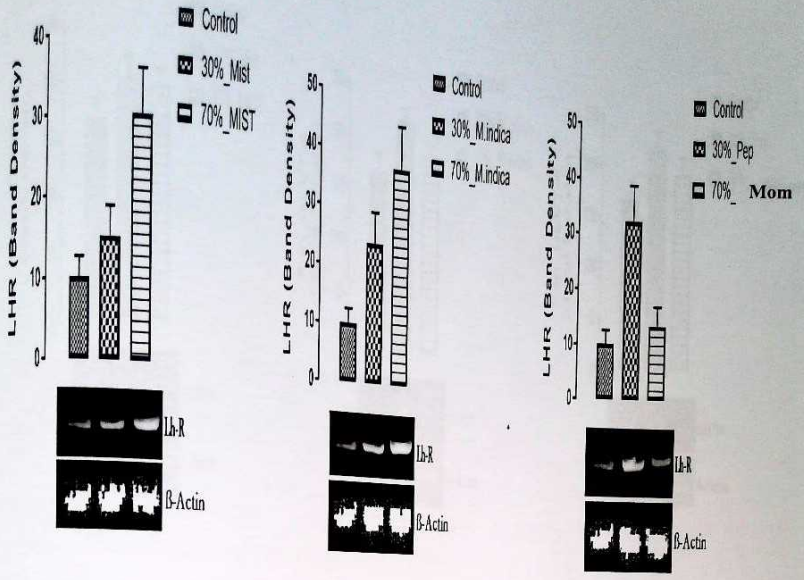


Figure 7: The effects of plant extracts on the expression of luteinizing hormone (LH) receptor on the testis of albino rats.

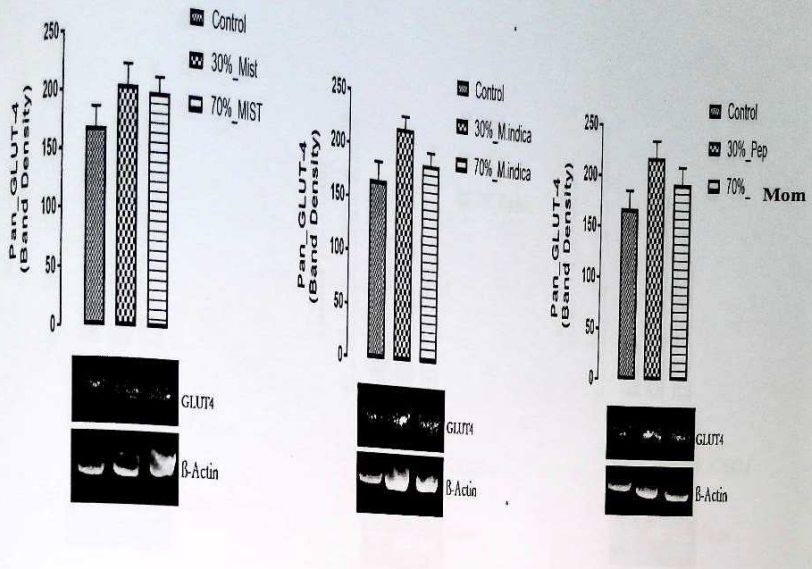


Figure 8: The effects of plant extracts on the expression of Glucose transporter-4 (GLUT-4) gene on the pancreas of rats.

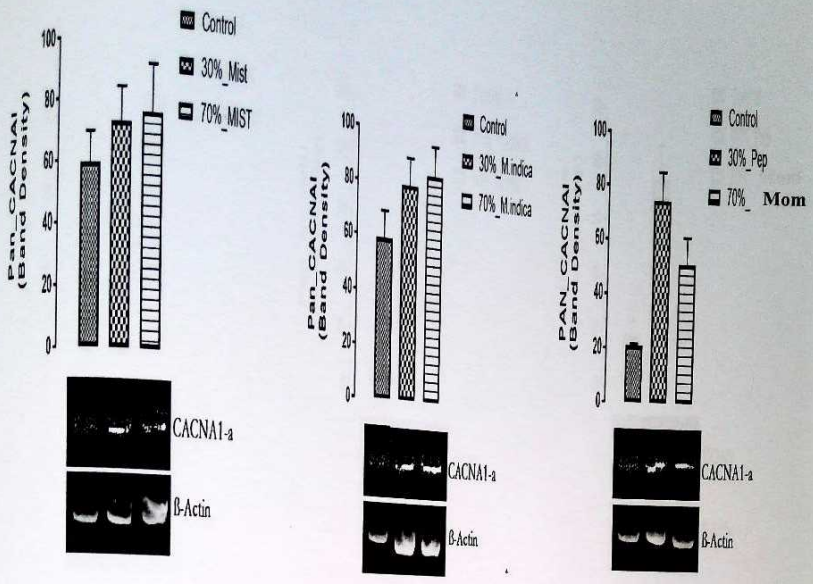


Figure 9: The effects of plant extracts on the expression of calcium channel voltage-gated lalpha (CACNA1-a) gene on the pancreas of rats.

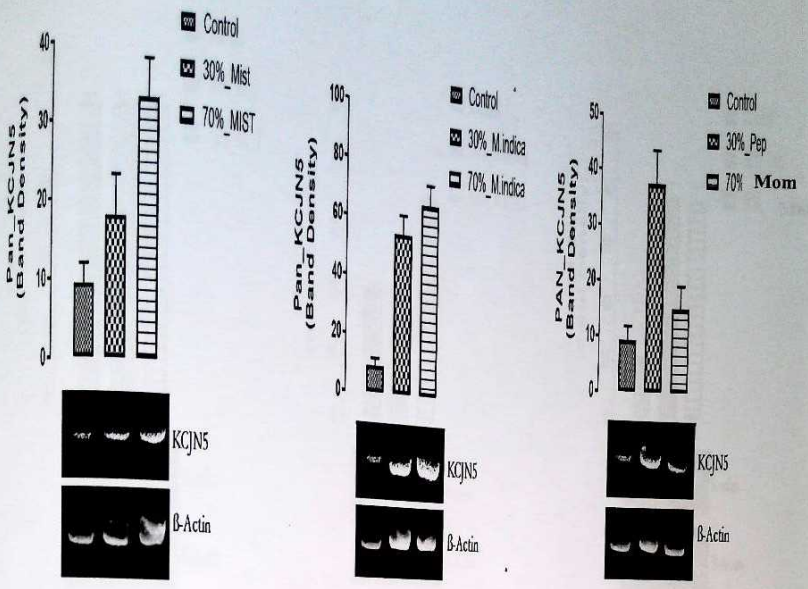


Figure 10: The effects of plant extracts on the expression of potassium channel on the pancreas of albino rats.

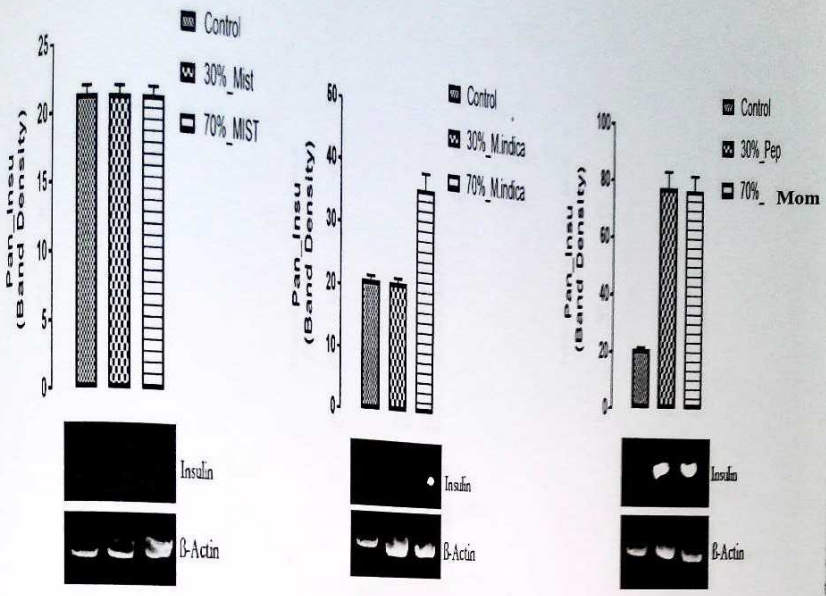


Figure 11: The effects of plant extracts on the expression of Insulin gene on the pancreas of albino rats.

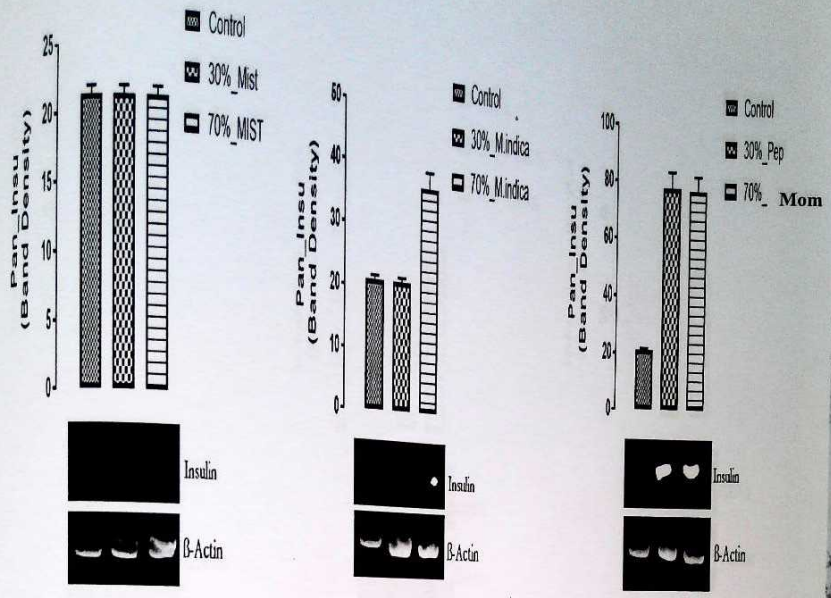


Figure 11: The effects of plant extracts on the expression of Insulin gene on the pancreas of albino rats.

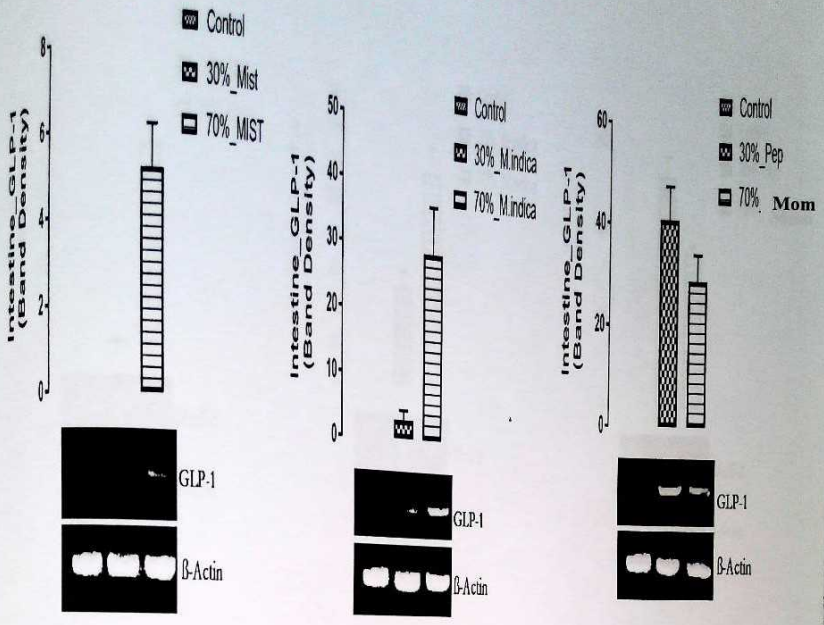


Figure 12: The effects of plant extracts on glucagon- like peptide (GLP) gene on the small intestine of albino rats.

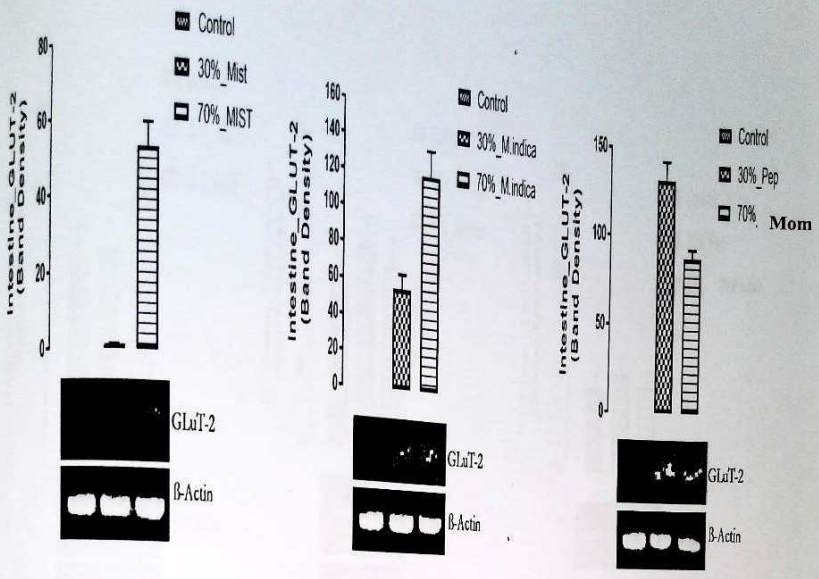


Figure 13: The effects of plant extracts on the expression of glucose transporter 2 (GLUT-2) gene in the small intestine of albino rats.

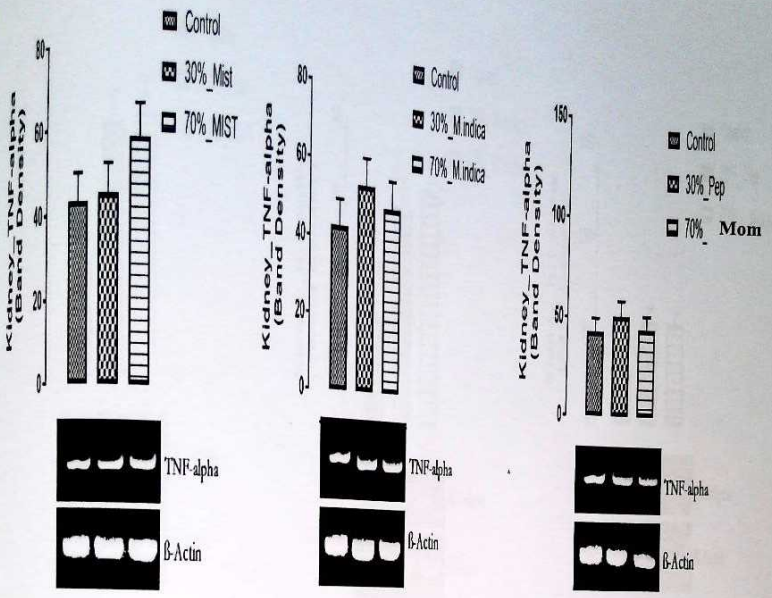


Figure 14: The effects of plant extracts on the expression of tumour necrosis factor-α (TNF-α) gene in the kidney of albino rats.

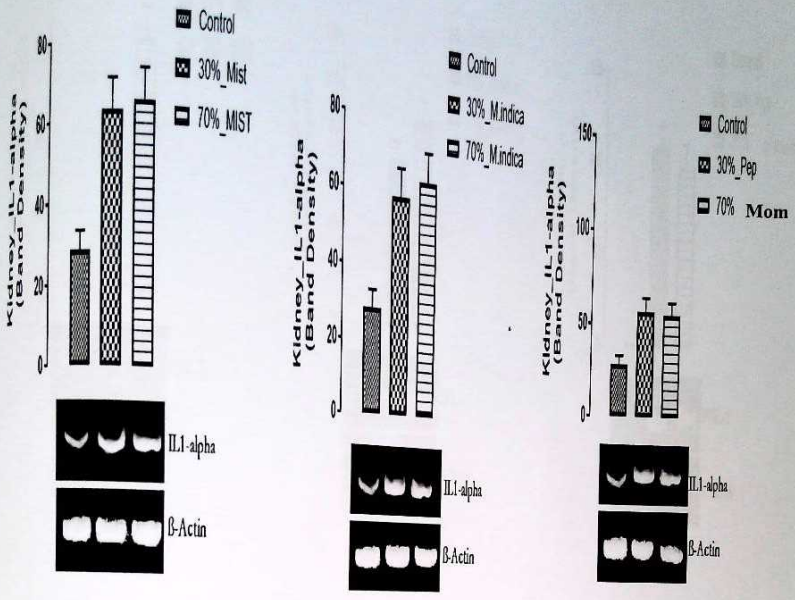


Figure 15: The effects of plant extracts on the expression of interleukin-1α (IL-1α) gene on the kidney of albino rats.

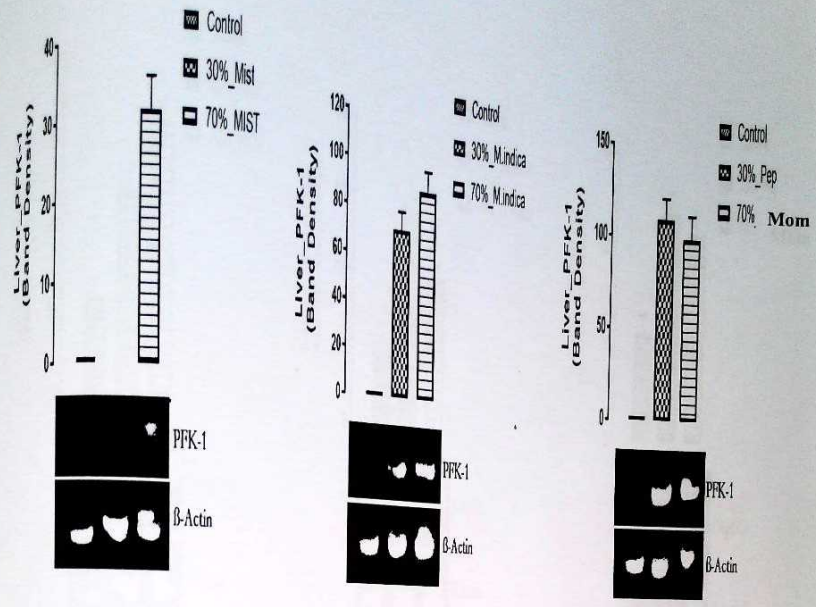


Figure 16: The effects of plant extracts on the expression of phosphofructokinase gene on the liver of albino rats.

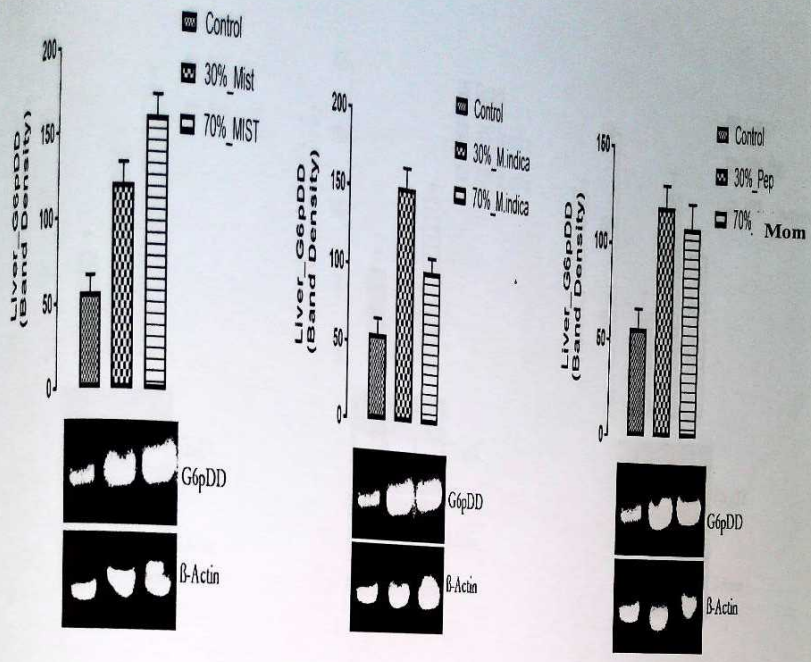


Figure 17: The effects of plant extracts on the expression of Glucose-6-phosphate dehydrogenase gene on the liver of albino rats.

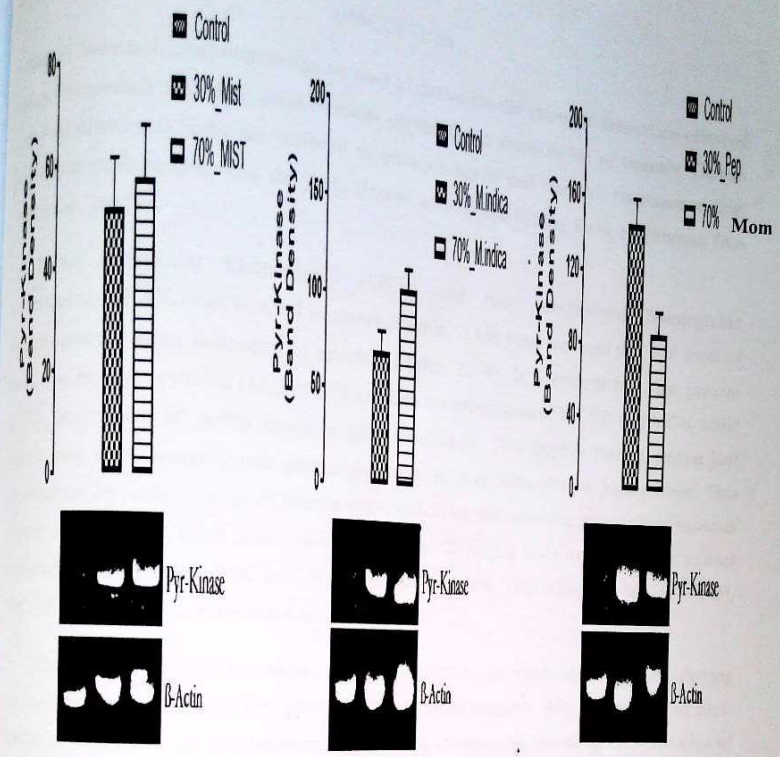


Figure 19: The effects plant extracts on the expression of pyruvate kinase gene in the liver of albino rats.

CHAPTER FIVE

DISCUSSION

Testing of haematological indices can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood composition of animals. Certain medicinal plants and herbs are believed to enhance health and improve resistance against infection through conditioning the body tissues and re-establishing body equilibrium (Al-kindi, *et al.*, 2011).

Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) can be used to assess anemia. It has been reported that low level of these parameters is an indication of anaemia (Aster, 2004). It is evident from the present study, that *Mangifera indica* (*M.I*) at 30% reduces the production of MCHC and MCH while at 70% concentrate *M. indica* increases their production. This implies that *M. indica* leaf extract may have hematopoietic properties when it is consumed at a higher dose. This corroborates the early findings of Izunya *et al.*, (2010) on anti-anaemia potentials of aqueous extract of stem bark of *M. indica* that 25, 50 and 75 mg/kg body weight of the extract increased PCV, HGB and RBC in a dose dependent manner. This is because MCHC, MCH, RBC, HCT and HGB indicate blood level condition.

Experimental model has shown blood platelets to be implicated in blood clotting processing. In view of this, low platelet concentration suggests that the process of clot-formation (blood clotting) will be extended, resulting in excessive loss of blood in the case of even slight injury. Interestingly, leaf extract of *Tapinanthus bangwensi* (mistletoe) and *Peperomia pellucida* increased the production of platelets and platelet distribution width in the experimental rats compared to the control. *M. indica* caused a reduction in platelet count and only to increase the mean platelet volume (MPV) at a low dose of 30% and lower it at 70% concentrates. From this result it is logical to surmise that leaf extract of *T.bangwensi* (mistletoe) and *P. pellucida* may help in the stimulation of thrombopoetin production and can thus be used to manage hemostatic activity of blood since platelet is involved in blood clotting while *M. indica* has the tendency to cause thrombopenia. (Adedapo *et al.*, (2007) had pointed out that reduced blood platelets affect the viscosity of blood which is co-related positively to blood pressure. This work corroborates the work done by Mishra and Tandom, (2011) on *Hibiscus Rosa Sinensis* which adversely affect blood platelet count after treatment.

serve as a carrier of haemoglobin, which reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Chineke *et al.*, 2006). The red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of animals' blood volume (Ugwuene, 2011, 2012; Soetan *et al.*, 2013; Isaac *et al.*, 2013). In this study there is a notable increase in red blood cells (RBC) and RDW of rats fed with leaf extract of *M. indica* and *T.bangwensi* (30%) compared to the control (Fig 2), While *P.pellucida* at 30% concentrate caused a decrease in the levels of RBC.

Previous reports stated that packed cell volume, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow's capacity to produce red blood cells in mammals (Awodi *et al.*, 2005; Chineke *et al.*, 2006). Furthermore, high packed cell volume (PCV) indicates either an increase in number of red blood cells (RBCs) or reduction in circulating plasma volume (Chineke *et al.*, 2006).

At higher concentration *M. indica* (70%) caused an increase in the production of packed cell volume (HCT), RBC and HB while *P. pellucida* at higher concentration (70%) caused a reduction in PCV, HB and RBC compared to the control. This may be as a result of the level of iron present in the plant. Asif and Wahid (2003) reported that saponin in *Fagonia cretica* L. reduced red blood cell indices, Harikrishnan *et al.*, (2012) reported that saponin increased red blood cell indices. Iron, an important factor in the synthesis of haemoglobin, a component of RBC, was reported by Babatunde, (2012) to be the highest amount of trace element in the *M. indica* stem bark. The iron content of the extract may therefore contribute to its effect in boosting the red blood cell indices of the rats. Based on these earlier reports and the results of this study, it can be hypothesized that the combined effects of both iron and phytochemical content of *M. indica* extract are responsible for its ability to boost RBC indices. *M. foetida* (African large bitter gourd) and *T. bangwensi* (mistletoe) also increase the production of red blood cells and hemoglobin (HB) even at a low dose of 30%. This is an indication that *M. indica, T.bangwensi* and *M. foetida* at concentrated amount can enhance erythropoiesis while *P. pellucida* may cause anaemia even at lower dosage of 30% concentrate.

The major functions of the white blood cell and its differentials are to fight infections, defend the body by a process called phagocytosis, against invasion by foreign organisms and

...dies in immune response. Thus, animals with low ...hite blood cells are exposed to high risk of disease infection, while those with high counts ...e capable of generating antibodies in the process of phagocytosis and have high degree of ...esistance to diseases (Soetan *et al.*, 2013), thereby enhancing adaptability to local ...nvironmental and disease prevalent conditions (Okunlola *et al.*, 2012). Immune system ...odulating ability of extracts of *T .bangwensi* is also well documented (Jurin *et al.*, 1993; Ladokun *et al.*, 2015). According to Fig 3, the leaf extract of *M. indica and T.bangwensi* decreased the production of white blood cells and lymphocytes compared to the control. This is in agreement with work done by Mishra and Tadon, (2015). Reports about WBC counts have pointed out that whereas increased count of WBC is supposed to be helpful in boosting immune system (Adedapo *et al.*, 2007; Mohajeri, 2007), a decreased count of WBC shows the suppression of leucocytes and their production from bone marrow (Osuigwe *et al.*, 2007). Therefore an increased count of WBC *in B. spectabilis* treated animals, as observed by Mishra and Tadon (2015), suggests that *B. spectabilis* might have a good potential to boost immune system.

Luteinizing hormone receptor (LH-R) has been reported to be found predominantly in the ovary and leydig of the testis where it plays pivotal role in testosterone production and support spermatogenesis. This trans-membrane receptor specifically acts to up-regulate the enzyme cholesterol side chain cleaving enzyme, which leads to the greater conversion of cholesterol into androgen precursors required to make many steroid hormones, including testosterone and estrogens (Ofusori *et al.*, 2007). In this study the increase in LH receptor levels in *M. indica* and *P. pellucida* compared to the control may be due to low testosterone levels which then facilitated increased response of LH receptor as explained by the statement above. Many beneficial effects of medicinal plants on male reproductive function are associated with antioxidant effects (Ofusori *et al.*, 2007). This suggests that the potential of phytomedicines to improve male fertility is due to presence of antioxidants. Furthermore, antioxidants have been shown to improve various processes (spermatogenesis, steroidogenesis) of male reproductive function (Sheweita *et al.*, 2005; Elumalai *et al.*, 2009). This result is not in agreement to the work done by Baghsamaneh *et al.*, (2015) that concluded that the aqueous extract of mango leaves reduces male reproductive activity and can be used for birth control.

Androgen receptor is a type of nuclear receptor that is activated by binding either of the androgenic hormones (e.g. testosterone) in the cytoplasm and then translocating into the

...that presence of KCNJ5 gene may ...duced production of the hormone insulin thereby preventing ...glycaemia and hypoglycaemia.

There was a significant expression of GLP-1 in the small intestine of the animals fed ...*T.bangwensi*, *M. indica*, *P. pellucida*, and *M. foetida* compared to the control. It has ...reported that GLP-1 is a 30 amino acid long peptide hormone derived from the tissue-...ecific posttranslational processing of the pro-glucagon gene expressed in several organs ...luding the gut (intestinal entero-endocrine L-cells) (Kristine and Catherine, 2003). GLP-1 ...the only known incretin describing its ability to decrease blood sugar levels in a glucose-...pendent manner by enhancing the secretion of insulin, alongside GIP (Kristine and ...therine, 2003).

...*pellucida*, which up- regulated insulin levels in the pancreas as seen in this study, also ...regulated GLP-1 levels. It can be inferred that the plant may be a potent incretin-mimetic ...the enhancement of insulin secretion.

This study also revealed the expression of glucose transporter-2 (GLUT-2), there was ...significant expression of GLUT-2 in the small intestine of the animals fed with higher ...ncentration of the extracts compared to the control group, disagreeing therefore with past ...orks done by Thorens, (2015).

TNF-alpha was down regulated in all the groups compared to the control. TNF-alpha is ...cell signaling protein (cytokine) involved in systemic inflammation and is one of the ...ytokines that make up the acute phase reaction. This cytokine has been implicated in a ...riety of diseases, including autoimmune diseases, insulin resistance, and cancer (Pfeffer, ...003). Mangiferin, a phytochemical present in *M. indica,* mediates the down-regulation of ...F-ĸB, suppresses NF-ĸB activation induced by inflammatory agents, including tumor ...uclear factor (TNF), increases the intracellular glutathione (GSH) levels and potentiates ...hemotherapeutic agent-mediated cell death; this suggests a possible role in combination ...herapy for cancer. It is likely that these effects are mediated through mangiferin ROS ...quenching and GSH rising; increased intracellular (GSH) levels are indeed known to inhibit ...e TNF-induced activation of NF-κB. This result suggests that these selected herbs may have ...nti-inflammatory and anti-toxicity properties (Vassali, 1992; Paul and Carroll, 1999; Pfeffer, ...003).

Interleukin1-α (IL-1α), was expressed significantly in this present work. Interleukin 1 ...alpha is a cytokine of the interleukin 1 family. In general, interleukin 1 is responsible for the

developed to interrupt those processes and treat diseases. Thus IL,-1α appears to be
pressed at low levels in some organs under steady state conditions, while some may
press IL-1α during stress (Moshe *et al.*, 2002).

PFK in the liver was up-regulated by *T.bangwensi*, *M. indica*, *P. pellucida* and *M.*
alis. Since PFK-1 is one of the most important regulatory enzymes of glycolysis, it's up-
gulation is not far-fetched from the facts that *T. bangwensi*, *M. indica*, *P. pellucida* and *M.*
alis expressed insulin gene and therefore may be needed for the conversion of glucose to
ruvate to prevent hypoglycaemia (Usenik *et al.*, 2010).

This study showed that G6pD was significantly expressed in all the experimental
oups compared to the control. The result of this study may be because of the presence of
me phytochemicals in these extracts that have anti-oxidant properties. This is in contrast to
e work done by Agbafor, (2015) on leaf extract of *Senna hirsuta* that lowered the
oduction of this enzyme in the rats used.

Glucose transporter type 4 (GLUT-4) is the insulin-regulated glucose transporter found
imarily in adipose tissues and striated muscle (skeletal and cardiac), although it has been
ported that Glut-4 is not expressed in liver cells, there was a significant expression of Glut-
in the liver cells of the rats fed with *T.bangwensi*, *M. indica* and *P. pellucida* this is in
greement with result from a study on expression of GLUT-4 mRNA, (Aschenbach *et al.*,
009).

CHAPTER SIX

CONCLUSION

an be concluded from this research work that these selected herbs (*M. indica, P. pellucida, angwensi and M. foetida*), were observed to cause some haematological and scriptomics alterations when administered orally to laboratory rats.

- Extracts of *M. indica, T.bangwensi* and *M. foetida* can be said to boost red blood cells production, when administered at a slightly high dose of 70% concentrate i.e. erythropoetic ability.
- Extracts of *P. pellucida* and *T. bangwensi* has the ability to boost platelets production i.e. thrombopoetic ability.
- Extracts of M. *indica, P. pellucida and M .foetida* up regulated the expression of insulin gene, glucose transporter-4- and glucagon-like peptides gene which are involved in glucose metabolism. It can therefore be said that these extracts have anti diabetic agents.
- Extracts of *M. indica and P. pellucida* can be said to prevent infertility due to the up regulation of luteinizing hormone and follicle stimulating hormone i.e. pro-fertility ability.
- All the extracts *M .indica, P. pellucida, T.bangwensi* and *M. foetida* have the ability to alleviate inflammation due to the down regulation of tumour necrosis factor which is one of the cytokines responsible for acute phase reaction i.e. anti-inflammatory ability.
- All the extracts are observed to be non-toxic by the down-regulation of TNF-α gene, except for *T.bangwensi* that is known to be cytotoxic on cancerous cells. Some of these observations have justified the traditional claims or use of these herbs for medicinal purposes.

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