EFFECTS OF AQUEOUS EXTRACT OF *Momordicabalsamina*L. ON MILK YIELD, LACTOGENIC HORMONES AND BIOMARKERS OF OXIDATIVE STRESS IN LACTATING WISTAR RATS

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MARCH, 2018

DECLARATION

I, AbubakarSani BUBA declare that the work in this dissertation entitled: "**Effects of Aqueous Extract of *Momordicabalsamina*L. on Milk Yield, Lactogenic Hormones and Biomarkers of Oxidative Stress in Lactating Wistar Rats**" has been carried out by me in the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, under the supervision of Dr. I.G. Bako and Dr. M. U. Kawu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree at this or any other institution.

AbubakarSani BUBA _____ _____
Signature Date

`

CERTIFICATION

This dissertation entitled EFFECTS OF AQUEOUS EXTRACT OF *Momordicabalsamina*L. ON MILK YIELD, LACTOGENIC HORMONES AND BIOMARKERS OF OXIDATIVE STRESS IN LACTATING WISTAR RATS by AbubakarSani BUBA,meets the regulations governing the award of the degree of Master of science degree (M.Sc) in Human Physiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my wonderful and beloved parents AlhajiSaniAbubakar and HajiaSalamatuSaniAbubakar for their love, care and support at all times.

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In full submission to the will of Almighty Allah for his guidance, wisdom, knowledge and health for making this work a reality. May the peace and blessings of Allah be on our noble prophet Muhammad (S.A.W) who is sent as mercy to humanity;*Ameen.*

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ABSTRACT

Momordicabalsamina L. (MBL) istaken by mothers to boost the production of milk.There is dearth of scientific literature on its lactogenic effects.This study was designed to evaluate theeffect of aqueous extract of MBL on milk yield, lactogenic hormonesand biomarkers of oxidative stress in lactating female Wistarrats. Twenty five (n = 25) adult lactating female Wistarrats were divided into five (5) groups of five (5) rats each and treated as follows: Group I: control,1ml/kg normal saline; group II:5 mg/kgmetoclopramide; group III-V: 200 mg/kg, 400mg/kg and 800 mg/kg of MBL respectively. Administration was done orally once daily at 18:00 h for a period of ten (10) days, (beginning from day 4 of lactation), during which the milk yield was calculated daily (starting from day 5 of lactation) at 18 h after gavage. On day 14 of lactation, the lactating female Wistar rats were sacrificed; bloodsamples and selected tissues were collected for biochemical assay and histological study respectively. Phytochemical screening of the aqueous extract of MBL revealed the presence of alkaloids, flavonoids, carbohydrates, saponins, cardiac glycosides and tannins.The result showed a significantly higher*($p<0.05$)* serum level of growth hormone and oxytocin in the MBL treated groupswhen compared to the control. There was a significantly lower*($p<0.05$)* serum prolactin level in the MBL 800 mg/kg group when compared to control, while there was no significant difference in serum prolactin level in the MBL 200 mg/kg and 400 mg/kg groups when compared to control. *Momordicabalsamina*L.stimulated a significant increase*($p<0.05$)*in mean milk yieldof MBL 400 mg/kg group when compared to the control group. Higher daily milk yield was observed in day 9 of theMBL 400 mg/kg group.Administration ofMBLcaused a significantly lower*($p<0.05$)*serum activity of superoxide dismutase at MBL 400 and 800 mg/kg groups; catalaseand glutathione peroxidase at MBL 800 mg/kg group, when

compared to control group.Significantly higher*(p<0.05)*serum malondialdehyde (MDA) concentrationwere observed inMBL treated groups when compared to the control group.*Momordicabalsamina*L.treated groupshad significantly higher *(p<0.05)* levels of alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) when compared to the controlgroup.There was minorstructural changes in the histology of mammary gland and livertissues at 800 mg/kg of MBL.In conclusion, aqueous extract of *Momordicabalsamina*L.caused:significant increased in milk yield, growth hormone and oxytocin, but it decreased prolactin level. It enhanced growth and proliferation of mammary gland tissue, resulting in increased milk synthesis and secretion. It also decreased serum antioxidant enzymes levels, with an increased in MDA concentration as well as serum liver enzymes; and mild fatty infiltration of binucleated hepatocytes at 800 mg/kg. It is therefore, suggested that the *Momordicabalsamina*L. can be used as a galactogogue at moderately low doses (≤400) and for a short periods to avoid its possible hepatotoxic effects, due to its bitter content “momordicin”.

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ABBREVIATIONS

$5HT_{2A}$	5-hydroxytryptamine
9-OH-RSP	9-hydroxy risperidone metabolite
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AST	Aspartate amino transferase
AVP	Arginine vasopressin
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CIS	Cytokine-inducible SH2- containing protein
COX	Cyclooxygenase
CYP	Cytochrome P enzyme
D2	Dopamine receptor 2
D2R	Dopamine receptor
DA	Dopamine
DNA	Deoxyribonucleic acid
ECM	Extra cellular matrix
eEF2	Eukaryotic translation elongation factor 2
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinases
FDA	Food and drug administration
GABA	Gamma aminobutyric acid
GH	Growth hormone
GHIH	Growth hormone inhibiting hormone

GHRH	Growth hormone releasing hormone
GPCR	G-protein coupled receptors
GPx	Glutathione peroxidase
Grb2	Growth factor receptor bound protein
GSH	Reduced glutathione
GTP	Guanosine triphosphate
IgA	Immunoglobulin A
IGF-II	Insulin-like growth factors
IMS	Insufficient milk syndrome
IP4	Inositol 1,3,4,5-tetrakisphosphate
IP6	Inositol hexakisphosphate
JAK/STAT	Janus kinase/signal transducers and activators of transcription
JNK	N-terminal protein kinases
LHD	Lactate dehydrogenase
M1	Muscarinic receptor 1
M2	Muscarinic receptor 2
MAPK	Mitogen activated protein kinase
MBL	*Momordicabalsamina*L.
MDA	Malondialdehyde
MEC	Mammary epithelial cells
MFGM	Milk fat globule membrane
MMI	Mothers milk insufficiency
MTP	Mitochondrial permeability transition
NAD	Nicotinamide adenine dinucleotide
OT	Oxytocin

OTRs	Oxytocin receptors
PI3K	Phosphatidylinositol 3-kinase
PIF	Prolactin inhibitory factors
PIM	Perceived insufficient milk
PKA	Protein kinase A
PKC	Protein Kinase C
PLC	Phospholipase C
PMNs	Polymorphonuclear neutrophils
PRFs	Prolactin releasing factors
PRL	Prolactin
PRLR	Prolactin receptors
PVN	Paraventricular nucleus
RNA	Ribonucleic acid
SC	Somatic cells
SD	Standard deviation
SEM	Standard error of mean
SIB	Suboptimal infant breastfeeding
SOCS	Suppressors of cytokine signalling
SOD	Superoxide dismutase
SON	supra optic nucleus
SOS	Sons of sevenless
Src	Sarcoma
SST	Somatostatin
STAT	Signal transducers and activators of transcription

CHAPTER ONE

1.0 Introduction

1.1 Background

Milk is a food that is of great biological significance for people from the moment they are born; it is accredited by the quantity and quality of nutrients it contains (high biological value proteins, and significant quantities of inorganic salts like phosphorus and calcium) which are structural materials for the skeleton (Amini*et al.,* 2013). Milk is produced by the breast through endocrine and autocrine systems (Walker, 2010).

A galactagogue is a substance that stimulates/improves lactation in humans and other animals (Tin*et al.,* 2015). It may be synthetic, plant-derived or endogenous in origin. Synthetic galactagogues such as domperidone and metoclopramide interact with the dopamine system in such a way to increase the production of prolactin (Forinash*et al.,* 2012).Prolactin (PRL) is a polypeptide hormone that is synthesized and secreted by the lactotropic cells of the anterior pituitary gland (Freeman *et al.,* 2000). It aids the development and maturation of the breast during pregnancy as well as assisting in the subsequent production of milk during lactation. Prolactin receptors are present in various organs of the body such as mammary gland, placenta and uterus(Freeman *et al.,* 2000; Mancini *et al.,* 2008). Prolactin has effects on mammogenesis (growth and development of breast), lactogenesis (synthesis of milk), galactopoiesis (maintenance of milk secretion) and acts as a luteotropic hormone that enhances integrity of the corpus luteum after mating (Hosseinzadeh *et al.,* 2014).It induces lactation by direct stimulation of the synthesis of milk proteins in the epithelial cells and indirect stimulation of the proliferation of the

secretory cells (Julie and Marta, 2010). Hypophysectomy during pregnancy inhibits subsequent lactation which shows that lactogenesis requires pituitary prolactin. Prolactin gene knockout results in abnormal mammogenesis characterized by complete absence of lobulo-alveolar units in homozygous females (Goyvaerts*et al.,* 2015). Prolactin levels are also elevated by certain drugs such as the dopamine receptor antagonists, like the neuroleptics (Khazali, 2010);and a good example of these drugs is metoclopramide.

Growth hormone (GH)is needed to support milk production in case of reduced prolactin level (Simelane*et al.,* 2012). It plays a vital role in proliferation and maintenance of mammary gland and lactation (Leung *et al.,* 2002). Oxytocin plays a significantrole in contraction of myoepithelial cells for milk ejection(Tenga *et al.,* 2013).

Oxidative stress is a disturbance in the prooxidant-antioxidant balance of the body by reactive free radicals which results in the development of many chronic diseases (Alessandra da Silva *et al.,* 2015). Reactive Oxygen Species (ROS) are believed to be a major contributor to the onset of oxidative stress in biological systems (Huang *et al.,* 2015).

Antioxidants are reducing agents capable of donating one or more electron(s) to a free radical, thereby terminating the chain reaction. Antioxidants can prevent or delay the oxidation of biological molecules (Ming-Chih*et al.,* 2011).

Momordica balsamina L.(Cucurbitaceae) is popularly known as Balsam Apple in the tropical regions of Africa. Itis locally known as Balsam apple (English), "*Garahuni"*(Hausa), "*Akbon-ndewe"* (Igbo) and "*Ejirin"*(Yoruba) (Burkill, 1985). In Hausa land of Nigeria and Niger Republic, the leaves are cooked as part of green vegetable

soup for lactating mothers, because it is believed to help the mother to regenerate blood lost during labour and to purify her breast milk (Hassan and Umar 2006).

1.2 Statement of Research Problem

Concern about insufficient milk letdown is a frequently cited problem among the sub Saharan African women (Heinig *et al*., 2006; Okasha *et al*., 2008). Global incidence of lactational insufficiency has been estimated to range from 23% to 63% during the first 4 months after delivery (Sultana *et al*., 2013). There is some evidence to suggest that mothers who are unable to meet their babies breastfeeding needs may benefit from galactogogues (McInnes and Chambers, 2008; Osadchy*et al.,* 2012).There are reports on short comings of the existing lactogenic drugs. For example, domperidone may be an option for mothers of preterm babies who at over 14 days of delivery and after full lactation support, still have difficulty expressing breast milk in sufficient quantity for their child's needs (Donovan and Buchanan, 2012).Ingram *et al*., (2012) reported an adverse side effects of metoclopramide in mothers such as anxiety, several gastrointestinal disorders, insomnia, severe depression, and seizures and in infants that consume milk from metcopramide treated mothers.Herbal galactagogues can be a better alternative considering the short comings and adverse side effects of the synthetic galactogogues (drugs)(Amir *et al.,*2011; Forinash*et al.,* 2012).

In lactation is the most energetically demanding period of female's life consequently, daily energy expenditure and basal metabolic rate increases in lactating females resulting in the generation of reactive oxygen species (Castilo *et al.,* 2006). Enzymatic antioxidant activities in the form of catalase, superoxide dismutase, andglutathione peroxidase levelsremain suppressed during pregnancy and lactation (Upreti and Misro, 2002).

The short comings as well as the side effectsof the currently available lactogenic drugs has stimulated the search for alternative medicine.

1.3 Justification

Breast milk is essential for normal growth and development during childhood and is central to establishing the foundation for healthy living (Sadoh *et al.*, 2011). It is therefore,paramount to help prevent any form of limitation to the natural source of sustenance in the infant. The World Health Organization recommends breast milk as the ideal food source for growth and development of infants (WHO, 2014).Breast milk functions not only as food for the infant, but also protects against infection, promotes intestinal, immune, and cognitive development of the breastfed infant (Zivkovic *et al.*, 2011, Neville *et al.*, 2012, Lassek and Gaulin, 2013).

When it comes to the situation where there is no milk at all or insufficient production, someplants are used which are believed to induce galactopoiesis(Nudrat *et al.*, 2007).Because of better cultural acceptability, better compatibility with the human body, lesser side effects and higher effectiveness, the use of traditional medicines is gradually being accepted by traditional medical practitioners(Mortel and Mehta,2013). More than 35,000 plant species are used in various human cultures around the world for medicinal purposes. Nearly 80% of the world population rely on traditional medicines for primary health care, most of which involve the use of plant extracts (Kaur *et al.*, 2011).

Momordica balsamina L. has been used traditionally to enhance and purify breast milk(Hassan and Umar 2006), hence the need to scientifically verify its lactogenic effects

as well as the safety of it usage on the body.*Momordica balsamina* L also known to possess an antioxidant activity (Akula and Odhav, 2008).

1.4 Aim of the Study

The aim of this study was to evaluate the lactogenic effects of the aqueousextract of *Momordica balsamina*L. on milk yield, lactogenic hormones and biomarkers of oxidative stress in lactating Wistar rats.

1.5 Objectives of the Study

The specific objectives of the study were:

(i) To determine the quantity of milk yield in lactating Wistar rat treated with aqueous extract of*Momordica balsamina* L.

(ii) To evaluate the effect of aqueous extract of*Momordica balsamina* L. on some lactogenic hormones(prolactin, growth hormone and oxytocin)in lactating Wistar rats.

(iii)To assess the effect of aqueous extract of*Momordica balsamina* L. on biomarkers of oxidative stress (MDA, SOD, GPx and CAT) in lactating Wistar rats.

(iv)To evaluate the effect of aqueous extract of *Momordica balsamina* L. on liver enzymes(ALT, AST, ALP) and histology ofmammary gland and liverof lactating Wistar rats.

(v) To determine the lethal dose (LD_{50}) and the preliminary phytochemical constituents of the aqueous extract of *Momordica balsamina* L.

1.6 Research Hypothesis

Mormodica balsamina L. extract has effecton milk yield,serum levels of prolactin, growth hormone, oxytocin andbiomarkers of oxidative stressin lactating Wistar rats.

CHAPTER TWO

2.0 Literature Review

Milk is an important natural food for infants from the minute they are born; it is the food for anew born for the period of first six months of his or her life (Fu *et al.,* 2015). The importance of milk is due tothe different nutrients it contains (high biological value proteins, essential amino acids andsignificant quantities of inorganic salts which are essential for the building up the skeleton)(Alsaweed *et al.,* 2015). Milk is the primary diet of infants, because it containsimmunoglobulins, antimicrobial peptides e.g. lactoferrin andlactoperoxidase and growth factors (Bode *et al.,* 2014; Bagci and Allmer*et al.,*2016).Breast feeding also prevents lactating mothers from developing ovarian cancer (Melnik and Schmitz, 2017).

2.1 Milk Production and Secretion

Milk secretion is a very complicated process that undergoes so many changes; most of thefactors influencing milk secretion are unacknowledged (Neville *et al,* 2002). Fully grownbreasts are comparable to a flowering tree in spring time**,** with lobular alveolar complexes,which are referred to as terminal duct lobular units (TDLU) by pathologists, developingfrequently from the main ducts. At times the breast reaches a stage of dormancy

which isrevealed by some waxing and waning of the TDLU controlled by the hormonal changes ofthe menstrual cycle (Hoddinott *et al.*, 2008). Thus, induction of the terminaldifferentiation of mammary alveolar cells before birth and after birth requires the presence ofhormones like prolactin (Binart, 2016).

Milk lactose biosynthesis is extremely dependent on the action of α-lactalbumin (LA). The LAconfers the binding of glucose to the complex and consequently facilitates the biosynthesisof lactose. Glucose is required to sustain milk production during lactationand its production can be obtained by increasing the process of gluconeogenesis. Glucagonis a key hormone that stimulates gluconeogenesis and increase glucose production fromliver (Williams *et al*, 2006; Hosseinzadeh *et al.*, 2014).

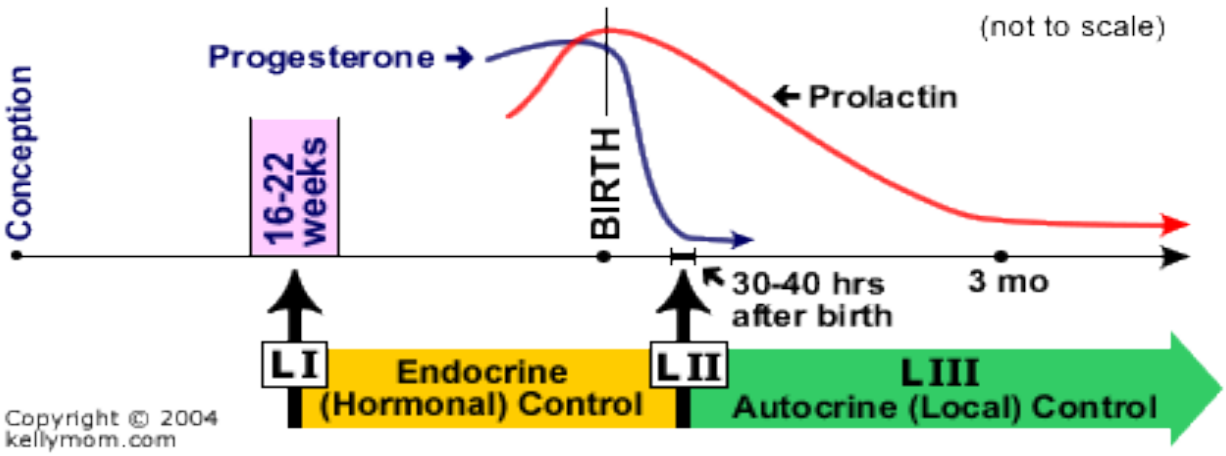


Figure 2.1: Hormonal changes during conception and lactation (L) at different time intervals(www.kellymom.com/bf/supply/milkproduction.html).

Milk secretion is suppressed by high circulating levels of progesterone (Figure 2.1). This phase of lactation isreferred to as lactogenesis I. Lactogenesis II occurs after birth, normally 30-40 hours afterthe delivery of a full-term infant. Once the placenta is detached,

progesterone levelsdecrease sharply and prolactin levels increase to initiate milk secretion (Fu *et al.,* 2015).

2.1.1 Some major lactatogenic hormones

One of the major roles of the endocrine system is to coordinate mammary function with the reproductive state of the animal. This physiological synchronization is a very complex process that involves the action and interaction of multiple hormones, as well as the interplay between hormones in circulation and local regulation of the mammary response to these hormones (Wall and Mcfadden, 2012). During lactation, several key hormones are involved in the regulation of mammary cell numbers, secretory activity, and consequent milk production potential (Boulanger *et al.,* 2015).

2.1.1.1 Prolactin

Prolactin (PRL) is mainly synthesized and secreted by the lactotrophic cells of the pituitary gland, but also by extrapituitary sites such as mammary gland, placenta, uterus and T lymphocytes (Riskin and mond, 2015). As the name indicates, PRL is known as the hormone of lactation. The varied effects of PRL on the mammary gland include growth and development of the mammary gland (mammogenesis), synthesis of milk (lactogenesis), and maintenance of milk secretion (galactopoiesis) (Hosseinzadeh *et al.,* 2014). Prolactin has been shown to maintain both the structural integrity and the functional activity of the mammary epithelium during lactation. Prolactin, as well as PL and primate GH, binds the same PRL receptor (PRLR). Multiple isoforms of membrane-bound PRLR resulting from alternative splicing of the primary transcript have been identified in several species (Nguyen*et al.,* 2016).

Prolactin receptor activation

The action of PRL in the mammary gland is mediated through its receptor, which activates the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (Voorhees*et al*., 2011). The understanding of how PRL transmits diverse signals to target cells is facilitated by the elucidation of the JAK/Stat pathway as the prototype signaling pathway used by all cytokine receptors. Prolactin is believed to activate sequentially the receptor by dimerizing two identical receptor subunits, leading to activation of Jak2 kinase associated with the cytoplasmic domain. Although the Jak2–Stat cascade is the major signaling pathway used by the PRL receptor, other transducing pathways are also involved in signal transduction by this receptor through MAP kinases (MAPK). Functional development of mammary epithelium during pregnancy depends mostly on PRL signaling. Proliferation and differentiation of secretory mammary epithelium is dependent on the presence of PRL receptor as well as its downstream Jak2–Stat5 pathway. Activation of prolactin-R involves ligand-induced sequential receptor dimerization driven by the prolactin molecule containing two binding sites (Chávez-Güitrón*et al*., 2016).

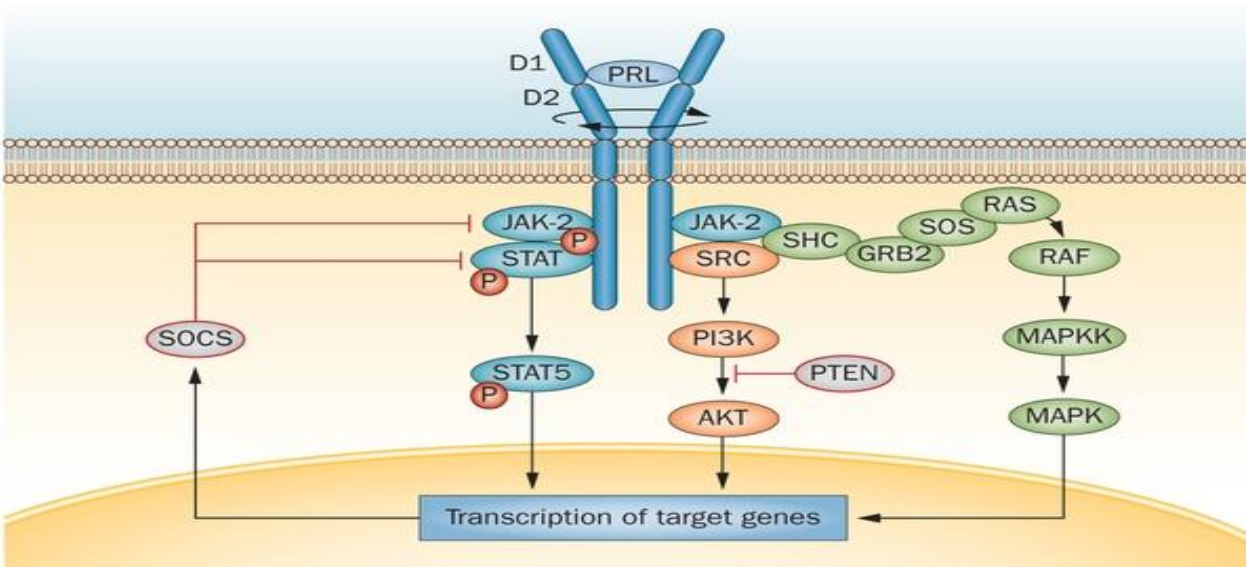


Figure 2.2: Mechanism of prolactin receptor activation (www. Nature reviews/Endocrionology).

Regulation of prolactin secretion

Prolactin secretion is affected by a large variety of stimuli provided by the environment and the internal milieu. The most important physiological stimuli that elevate pituitary prolactin secretion are suckling and stress and increased levels of ovarian steroids, primarily estrogen. Such stimuli are transduced by the hypothalamus which elaborates a host of prolactin releasing factors (PRF) and prolactin inhibiting factors (PIF)(Maria, 2015). In mammals, the control exerted by the hypothalamus over pituitary prolactin secretion is largely inhibitory(Binart, 2016). On the other hand, the hypothalamus is also involved in the acute stimulatory control of prolactin secretion by removal of the inhibition (disinhibition) and/or superimposition of brief stimulatory input. In addition, prolactin secretion is also influenced by numerous factors released by the lactotrophs themselves (autocrine regulation) or by other cells within the pituitary gland (paracrine regulation) (Kobayashi*et al*., 2016). Also circadian system plays a significant role in lactation and maternal behavior based on the fact that circadian disruptions play a role in the metabolic-hormonal link to delayed lactogenesis II (Fu *et al.*,2015).

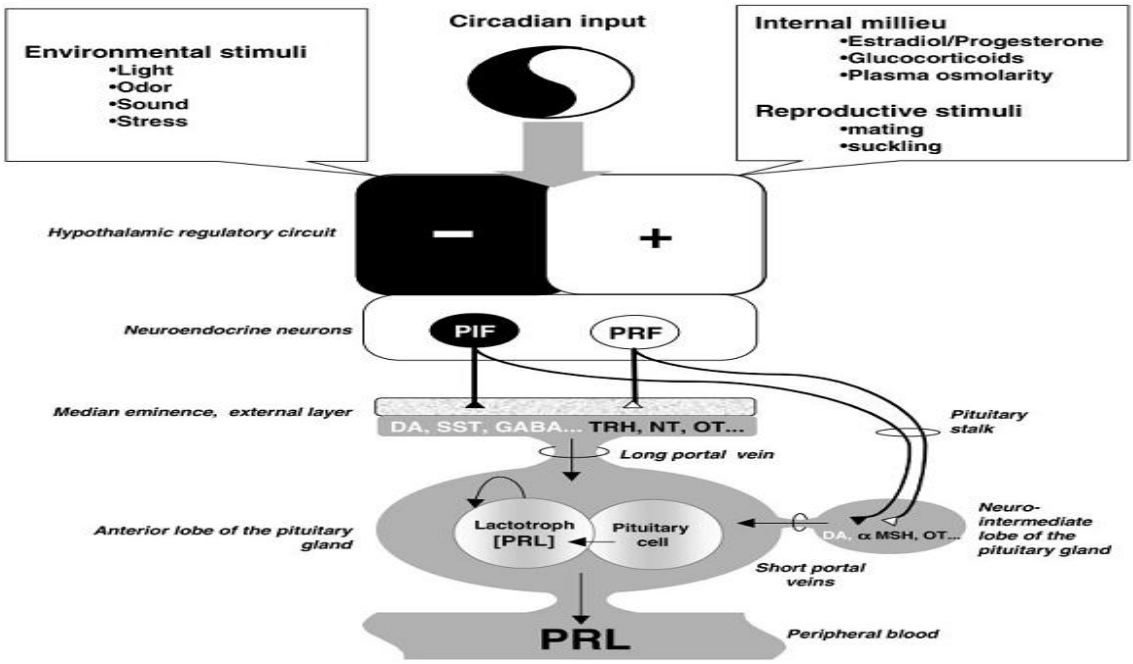


Figure 2.3: Regulation of prolactin secretion (Maria, 2015).

2.1.1.2 Growth hormone

Growth hormone (GH) is a peptide hormone consisting of 190 or 191 amino acids. Growth hormone releasing hormone, secreted from the hypothalamus, stimulates the secretion of GH from the pituitary gland. Some other tissues synthesize GH, including brain, placenta, mammary and pineal glands(Simelane*et al.,* 2012).Growth Hormone also promotes mammary epithelial proliferation.It has been suggested that prolactin stimulates paracrine release of insulin-like growth factor II (IGF-II) within the mammary gland,

which stimulates development of alveoli (Brisken *et al*., 2002; Hull and Harvey, 2014).

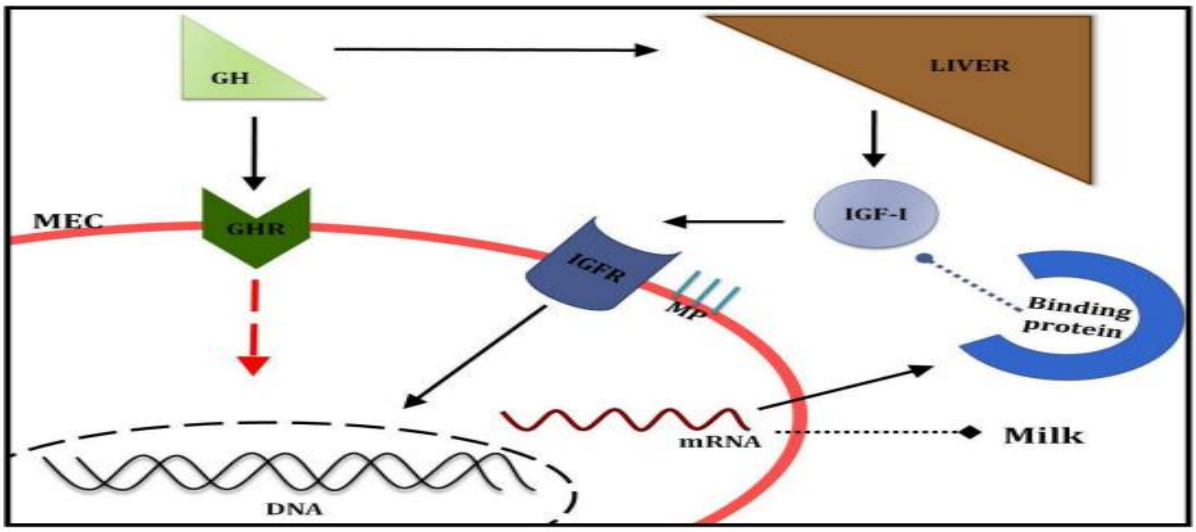


Figure 2.4: Activity of growth Hormone in lactation (Yamada and Lee, 2009).

Growth hormone receptor activation

The downstream signaling pathways mediated by the GHR are initiated upon the binding of GH to the extracellular domain of the GHR. Formation of this GH-GHR2 trimer complex was thought to be necessary and sufficient for GH responses (Schirra *et al.*,2008). However, other studies indicated that dimerization of the GHR was insufficient for activation of GH mediated signaling and that preformed GHR dimers exist prior to GH binding (Gent *et al*., 2002; Chávez-Güitrón*et al.*, 2016).

Signal transduction

Growth hormone binding to Growth hormone receptor (GHR) activates the tyrosine kinase, Janus kinase 2 (JAK2). Thus initiating a multitude of signaling cascades that result in a variety of biological responses including cellular proliferation, differentiation and migration, prevention of apoptosis, cytoskeletal reorganization and regulation of metabolic pathways. A number of signaling proteins and pathways activated by GH have been

identified, including JAKs, signal transducers and activators of transcription (Stats), the mitogen activated protein kinase (MAPK) pathway, and the phosphatidylinositol 3'-kinase (PI3K) pathway (Gent *et al.*, 2002; Koopman, *et al.*, 2016).

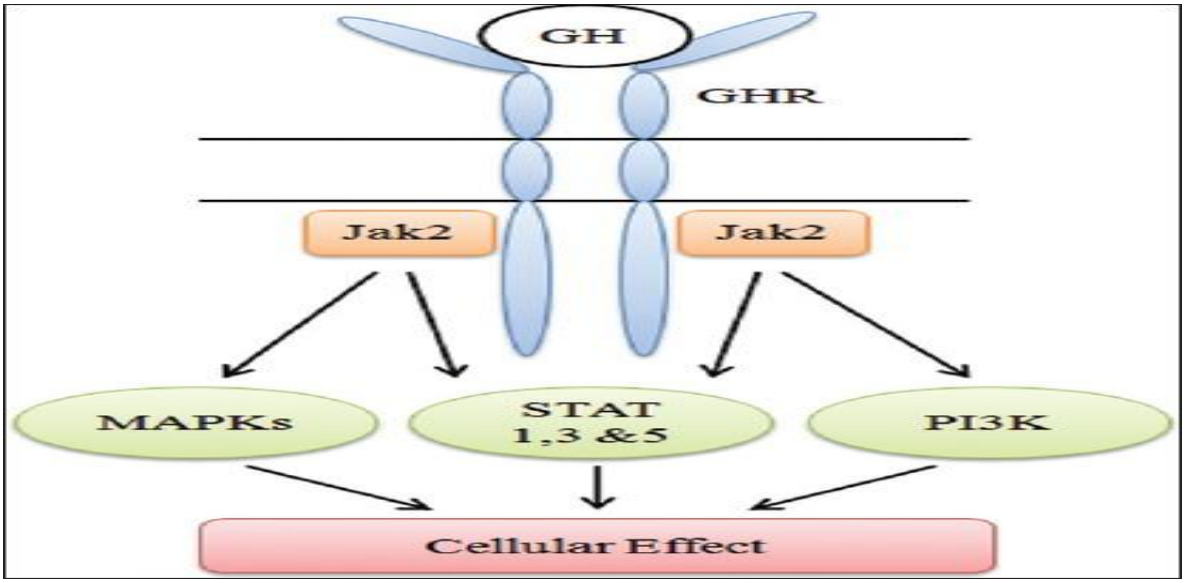


Figure 2.5: Growth hormone signal transduction pathway (Koopman*et al*, 2016).

Regulation of growth hormone

Secretion of growth hormone (GH) in the pituitary is regulated by the neurosecretory nuclei of the hypothalamus. These cells release the peptides Growth hormone releasing hormone (GHRH) or somatocrinin and Growth hormone-inhibiting hormone (GHIH) or somatostatin into the hypophyseal portal venous blood surrounding the pituitary gland. Growth Hormone release in the pituitary is primarily determined by the balance of these two peptides, which in turn is affected by many physiological stimulators (e.g., exercise, nutrition, sleep) and inhibitors (e.g., free fatty acids) of GH secretion (Leung *et al*., 2002). Surges of secretion during the day occur at 3 to 5 hour intervals. The plasma concentrations of GH during these peaks may range from 5-45 ng/ml. A number of factors are known to affect GH secretion,

such as age, sex, diet, exercise, stress and other hormones. Young adolescents secrete GH at the rate of about 700 µg per day, while healthy adults secrete GH at the rate of about 400 µg per day. Sleep deprivation generally suppresses GH release, particularly after early adulthood (Serrana*et al*., 2015).

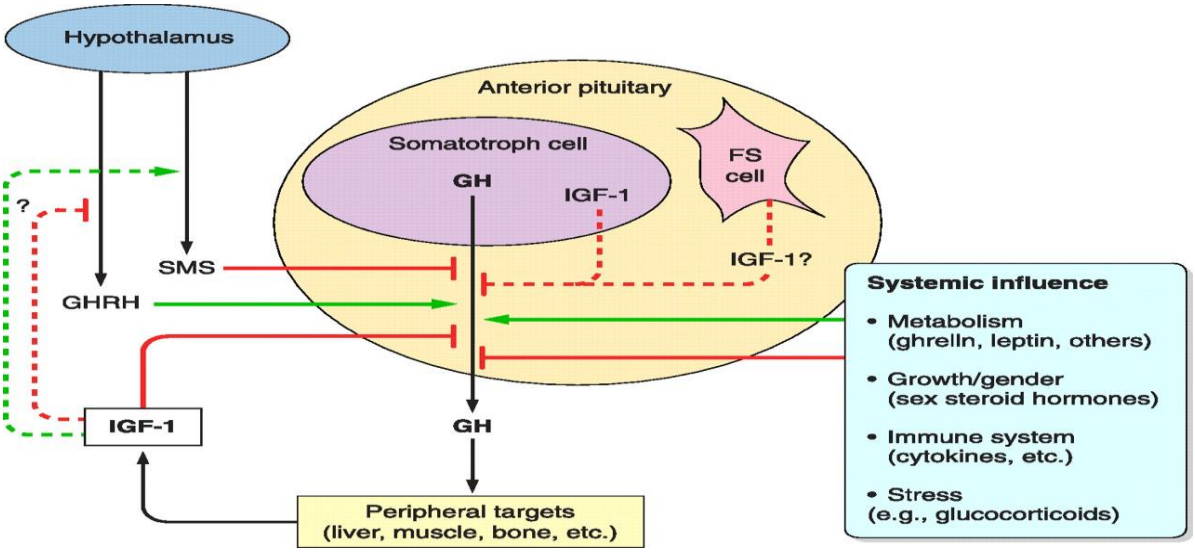


Figure 2.6: Regulation of growth hormone (Serrana *et al.*, 2015).

2.1.2 Oxytocin and milk ejection reflex

In addition to the role of oxytocin in the stimulation of uterine contractions during labour and delivery, oxytocin continues to play an important role in lactation after birth. Stimulation of the nipple by suckling sends a signal via the spinal cord to the hypothalamus to induce the release of oxytocin from the pituitary into circulation (Neumann *et al*., 1996). The oxytocin then binds to oxytocin receptors (OTRs) on the smooth muscle cells of alveoli in the mammary glands, causing them to contract and eject milk into the ducts and cisterns in what is referred to as the milk ejection reflex. Just as OTRs in the uterus become more sensitive to oxytocin prior to birth, the sensitivity of OTRs in the mammary glands also increases leading up to delivery to make the mother more physiologically responsive to

circulating oxytocin (Ebner*et al*., 2016). Furthermore, by binding to OTRs on prolactin-releasing cells in the anterior pituitary gland, oxytocin also stimulates the release of prolactin, a hormone responsible for milk production (Jonas, 2009).

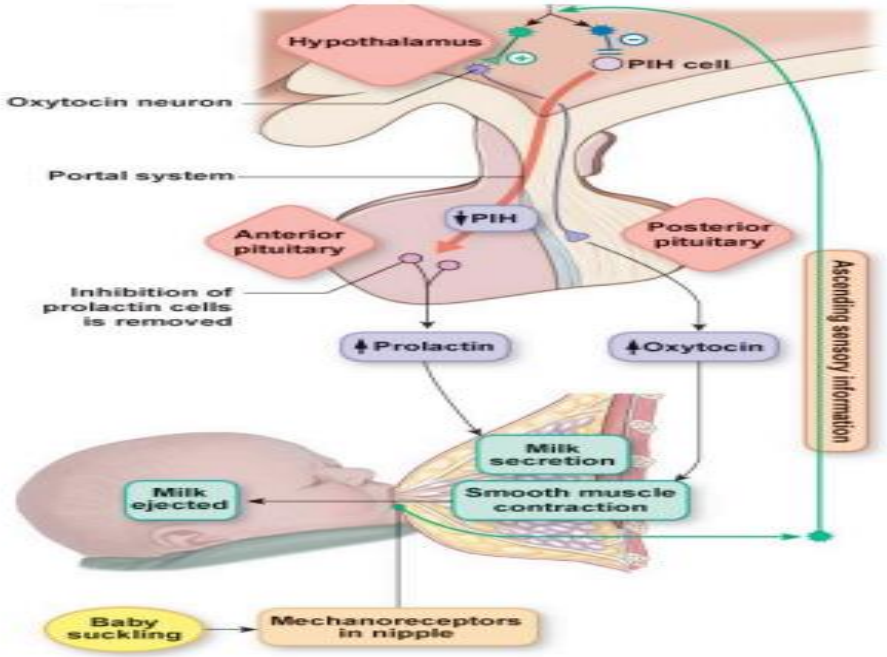


Figure 2.7: The milk ejection reflex(Silverthorn *et al*., 2013)

2.1.2.1Oxytocin

Oxytocin is synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) and axonally transported to the neural lobe where it is briefly stored until released from terminals. Moreover, short portal vessels connecting neural lobe and the inner zone of the anterior lobe also provide a potential route for delivering oxytocin to the adenohypophysis(Love, 2014). It is secreted into the hypophyseal portal blood in 10-15 times higher concentrations than found in the peripheral circulation, and high-affinity receptors resembling the uterine oxytocin receptors are present in the anterior lobe (Love, 2014).

Oxytocin is also released directly in the central nervous system from the cell bodies and dendrites of oxytocin-producing neurons to act as a neuromodulator in areas of the brain containing oxytocin receptors, such as the amygdala and hippocampus (Von Bohlen *et al*., 2002; Jonas, 2009). Oxytocin release is controlled by a positive feedback mechanism; for example, oxytocin release in the PVN stimulates further release of oxytocin by neurons in the same region (Tenga*et al.*, 2013).

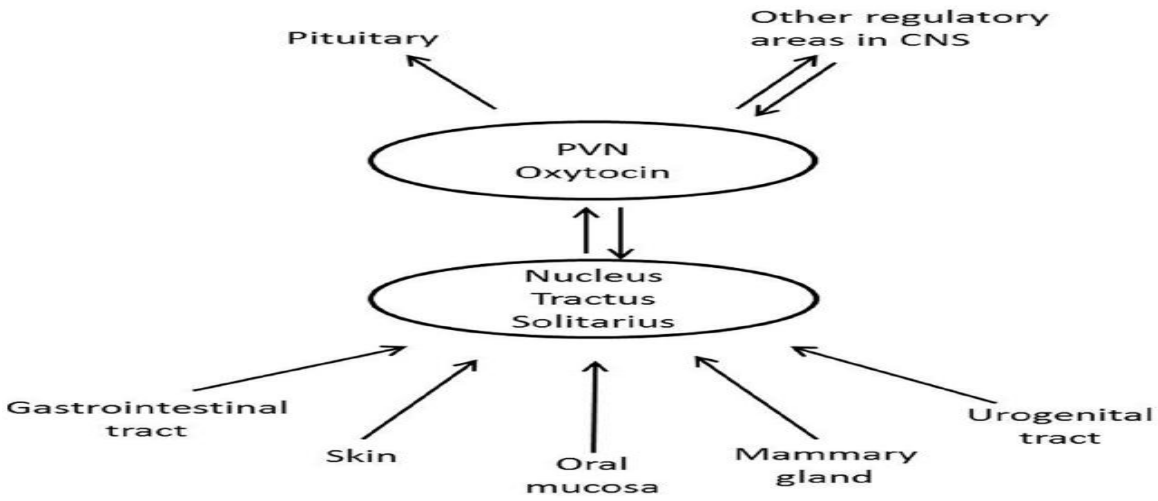


Figure 2.8: Oxytocin release from hypothalamus (Silverthorn *et al*., 2013).

2.1.2.2 Oxytocin receptor activation

Once oxytocin is released, it binds to oxytocin receptors (OTRs) located in neural tissue in the central nervous system and in the myoepithelial (smooth muscle) cells of the uterus and mammary glands in the peripheral system (Leng *et al.*, 2008). The OTRs are G-coupled receptors, and the binding of oxytocin to OTRs on these myoepithelial cells causes a rise in intracellular calcium levels to facilitate contraction of the smooth muscle tissue. Oxytocin has only one receptor, which belongs to the rhodopsin-type class I G-protein coupled receptor (GPCR) super family (David *et al.*, 2014; McQuaid *et al.*, 2016).

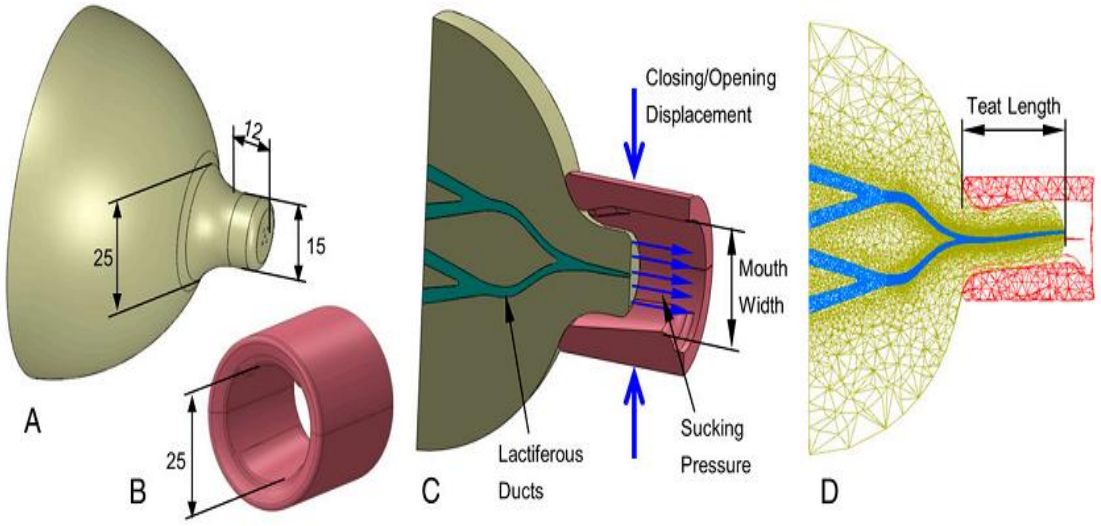


Figure 2.9: Biomechanics of milk ejection (Alekseev and Ilyn, 2015). Scale: mm.

2.1.2.3Role of Oxytocin in irregular Ca^{2+} oscillations

Using a novel, 3D imaging strategy, live oxytocin-induced alveolar unit contractions in the mammary gland was visualized, and demonstrated that milk is ejected by way of pulsatile contractions of these alveolar units. In mammary glands of ORAI1 knockout mice, these contractions are infrequent and poorly coordinated. Oxytocin was shown to also induce a

large transient release of stored Ca^{2+}in mammary myoepithelial cells followed by slow, irregular Ca^{2+} oscillations. These oscillations, and not the initial Ca^{2+} transient, are mediated exclusively by ORAI1 and are absolutely required for milk ejection and pup survival, an observation that redefines the signaling processes responsible for milk ejection. These findings clearly demonstrate that Ca^{2+} is not just a substrate for nutritional enrichment in mammals but is also a master regulator of the spatiotemporal signaling events underpinning mammary alveolar unit contraction (Kortus *et al*., 2016).

2.2 Hypogalactia

Total lactation failure was defined as either a total absence of milk flow or secretion of just a few drops of breast milk following suckling for at least 7 days. Partial lactation failure was defined as either inadequate milk output or the need for supplemental feedings to sustain growth.Low milk supply is the most commonly reported reason for early discontinuation of breastfeeding (Odom *et al*., 2013). The age, parity, education, socioeconomic status, religion, family structure, and urban versus rural status of mothers all have effect on lactation and its failure. More so, the perception of not having enough milk often leads to infrequent suckling, leading to a true reduction in production of breast milk (Mathur *et al.*, 2009). It was reported also that mothers of infants who are unwell and require supportive care after birth, such as preterm infants, are particularly vulnerable to experiencing low milk supply (Zuppa *et al*., 2010).

2.2.1 Causes of hypogalactia

The most common cause of hypogalactia is an insufficient secretion of prolactin from the pituitary gland. The D_2 receptor antagonists are used to correct this insufficiency by

blocking D_2 receptors in the anterior pituitary, which in turn disinhibits prolactin release (Sim *et al.*, 2015). Other causes include;

i. Lymphocytic hypophysitis: inflammation of the pituitary gland due to autoimmunity
ii. Sheehan's syndrome: postpartum hypopituitarism due to ischemic necrosis from blood loss and hypovolemia during and after child birth.
iii. Adult growth hormone deficiency: hypopituitarism in general can impair secretory activities of the pituitary gland such as prolactin and growth hormone, which in turn results in hypogalactia (Robert *et al.*, 2009).
iv. Other causes include insufficient glandular tissue of the breasts or breast hypoplasia, caused by lack of normal mammary gland growth and maturation during puberty or pregnancy (Karen, 2014).

2.2.2 Assessment of milk adequacy

When milk is not sufficient (< 20 gm/day) the infant does not feel satisfied after feeding, cries a lot, takes very long feedings, and does not gain weight properly. The number of wet diapers a day (less than six to eight) and infrequent bowel movements, with small dry or hard stools, are an indirect indicators of low intake of milk. Other signs of low breast milk in the first week of life include; weight loss greater than 10% of the birth weight, not increasing birth weight up to 2 weeks of life, decreased urine output in 24 hours, and absence of yellow stools in the first week with clinical signs of dehydration. An infant's milk ingestion is accurately measured by test weighing, which entails weighing the infant before and after breastfeeding without changing clothes or accessories between the two weighings (Kent *et al.*, 2012).

2.2.3 Strategies for management of hypogalactia

Although the problem of hypogalactia looks simple, it is rather a more difficult situation. In addition to motivation, encouragement, moral support and the use of galactogogues, the use of the following may be needed:

i. Nipple stimulation exercises by nipple stroking, massaging the breast, and rolling the nipple between thumb and the index finger.
ii. Frequent suckling, at least 8–10 times a day, each session lasting 10–15 minutes for each breast.

Drop and drip method may be employed if the infant fails to suckle for 8–10 minutes. The method involves expressing some breast milk or top milk in a cup and gradually pouring it over as drops over the breast. As the drops slide over the nipple down into infant's mouth, he is stimulated to suckle at the breast (Wan *et al*, 2008).

2.3 Galactogogues

Galactogogues are synthetic or plant molecules used to induce, maintain and increase milk production (Mortel and Mehta, 2013); mediating the process through interaction between physical and physiological factors.The most important factors are hormones such as PRL and GH. However, somatotropin, cortisol, insulin, leptin, estrogen, progesterone and oxytocin also play important roles as galactogogues (Forinash*et al.*, 2012).

2.3.1 Drugs affecting lactation (synthetic galactogogues)

Among the synthetic molecules used to increase lactation are the dopamine antagonists: metoclopramide and domperidone;antipsychotics: sulpiride and chlorpromazine; and hormone synthetic analogs such as oxytocin, thyroxine and medroxyprogesterone (Sim *et al*., 2014).

2.3.1.1 Metoclopramide

This drug was originally commercialized in Europe as an antipsychotic and later in the US as a gastro-kinetic agent that increases gastrointestinal motility. Its first reported use as a galactogogue was in 1975. It has also been evaluated in many clinical trials (Anderson and Valdes, 2007). It's reported side effects in humans include anxiety, insomnia, severe depression and seizure. Infants that consumed milk from treated mother had intestinal discomfort (Zuppa *et al*., 2010). A dose of 10 mg administered by oral route three times daily for 10 days increases milk production (Ingram *et al*., 2012).

2.3.1.2 Risperidone

Risperidone is a benzisoxazole derivative, and has a very strong affinity for serotonin (5-$HT_{2A}$and 5-HT_7) and dopamine D_2 receptors (its affinity for D_3 and D_4 receptors is three times lower) (Ereshefsky and Lacombe, 1993; Leysen *et al*., 1998). It is rapidly absorbed after oral administration, with peak plasma concentrations being reached in about one hour; its oral bioavailability is about 70-85 %. It mainly undergoes 9-hydroxylation in the liver that yields the active 9-hydroxy risperidone metabolite (9-OH-RSP), a step that is mainly catalysed by CYP2D6 and, to a lesser extent, CYP3A4; alicyclic dehydroxylation and oxidative N-dealkylation are minor metabolic pathways (Mannens *et al*., 1993).

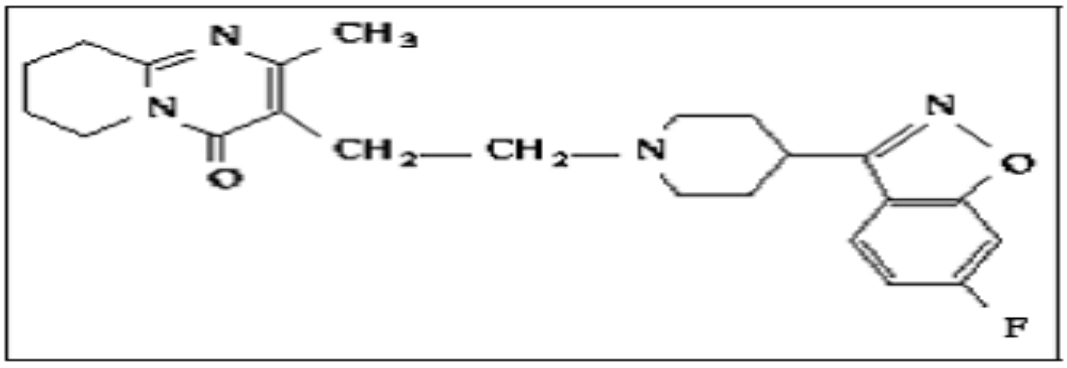


Figure 2.10: Chemical structure of risperidone (Lysen *et al*., 1998).

The mean half-life of risperidone is three hours in fast metabolizers (the majority of population), and 22 hours in poor metabolizers; the mean half life of the active moiety is almost constant at about 22 hours in both groups. Steady state levels are reached within five days of treatment. Risperidone and its metabolite are 89% and 77% bound to plasma proteins (Lysen *et al*., 1998).

2.3.1.3 Olanzapine

Olanzapine (OLZ) is a thienobenzodiazepine derivate structurally similar to clozapine that is effective in treating schizophrenia and acute manic episodes, and in preventing the recurrence of bipolar disorders (McCormack and Wiseman, 2004). Treatment with OLZ is associated with a higher risk of weight gain and, more extensively, metabolic syndrome than other typical and atypical antipsychotics (Duggan *et al*., 2005; Lambert *et al*., 2005). It also has a low propensity to cause extrapyramidal effects or sustained increases in prolactin levels (Lieberman *et al*., 2003).

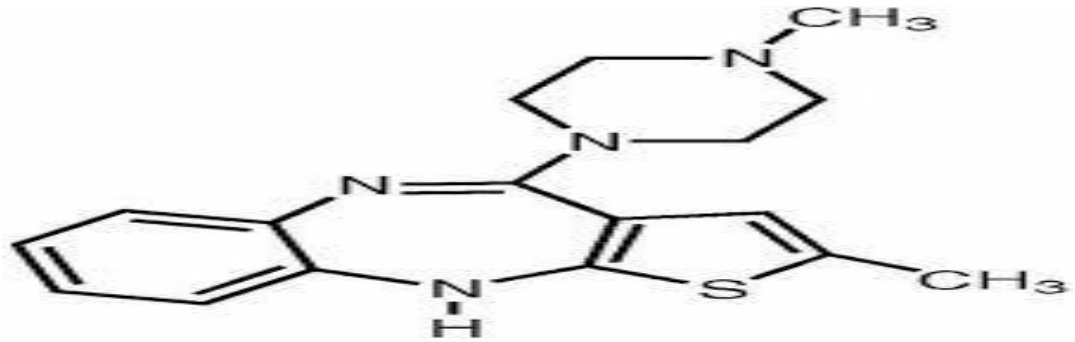


Figure 2.11: Chemical structure of olanzapine (Lieberman *et al*., 2003).

Olanzapine shares higher affinity to 5-HT2$_A$ receptors than D_2 receptors (high 5-HT2$_A$/D_2 ratio). In comparison to the other atypical antipsychotics, olanzapine presents high affinity for serotoninergic receptors. Approximately 85 % of an oral OLZ dose is absorbed but, as

about 40 % is inactivated by first-pass hepatic metabolism, its oral bioavailability is about 60 %. Olanzapine has a mean half-life in healthy individuals of 33 hours (range 21-54 hours) with peak plasma concentrations reached within six hours. The drug is approximately 93 % bound to plasma proteins, mainly albumin (90 %) and alpha 1-acid glycoprotein (77 %) (Callaghan *et al*., 1999).

2.3.2 Some plants that stimulate milk production (herbal galactogogues)

Many herbs are used to stimulate milk production**;** most of them have long histories oftraditional use, mainly in stimulating milk production in animals. *Hibiscus sabdariffa l.* has lactogenic activity with favorable enhancement ability in increasing serum prolactin level.Mechanism of action of *Hibiscus sabdariffa l.* has been reported to be through dopaminereceptors as dopamine antagonist (Bako*et al*, 2013). *Acacia nilotica* ssp *adansonii* alsostimulate milk production in lactating woman by stimulating the synthesis and release ofprolactin and enhancing lobuloalveolar development (Lompo-Ouedraogo*et al.* 2004).*Fogeniculum vulgare* (fennel) has been show3n to increase milk production in goats. This herbhas been used for a very long time as a galactagogue in humans (Wynn and Fougere,2007). *Galega officinalis* (goat‘s Rue) is also used to increase milk yield in cows (Bailey andDay, 2004). Raspberry leaf (*Rubus idaeus* L. [Rosaceae]) is believed to stimulate lactationand enrich breast milk by restoring the body‘s vitamins and minerals (Gladstar, 1993).

2.3.3*Momordica balsamina* L.

Momordica balsamina L.(Cucurbitaceae)is a climber or trailer with stems attaining 4-5mm in length (Welman, 2004; Thakur *et al.*, 2009; Madala *et al.*, 2016). Itis locally known as Balsam apple (English), *"Garahuni"*(Hausa), *"Akbon-ndewe"* (Igbo) and *"Ejirin"* (Yoruba). It isalso known as*mohodu* (Sotho); *nkaka*(Thonga) etc.

Researchers, governments and other organizations charge with food and nutritional standards show great concern on the nutritional status of general population more especially children, pregnant and lactating mothers in habiting the developing countries (Andersen *et al.*, 2003; Seena *etal.*, 2005).

The Phytochemicals secreening from the fruit of *Momordicabalsamina* L. are resins, alkaloid, flavonoid, anthroquinine,glycosides, steroids, terpenes, cardiac glycoside, saponinsand carbohydrate (Bot *et al.*, 2007).The seed of *Momordica balsamina* L. is known to contain glycosides, saponins and steroids (Karumi and bobboi, 1999).



Figure 2.12: *Momordica balsamina* L. (Wellman, 2004).

Momordica balsamina L.is awonder plant for neutraceutical sciences. The leaves are important source of nutrients having 17 amino acids (Hassan and Umar, 2006), adequate mineral composition like potassium, magnesium, phosphorus, calcium, sodium, zinc, manganese and iron contributes towards combating the problem of micronutrient deficiencies in soil and high value of protein and fat with low fibre content (Flyman and Anthony, 2007). The extract of various parts of this plant showed anti-HIV, antiviral, anti-inflammatory, shigellocidal,anti-diarrhoeal, antiseptic, antibacterial, and antimicrobial properties (Tommasi *et al.*, 1995; Iwalokun *et al.*, 2001; Karumi *et al.*, 2003; Jigam *et al.*, 2004; Otimenyin *et al.*, 2005; Otimenyin *et al.*, 2008, Vente *et al.*, 2008), as shown in figure 2.13;

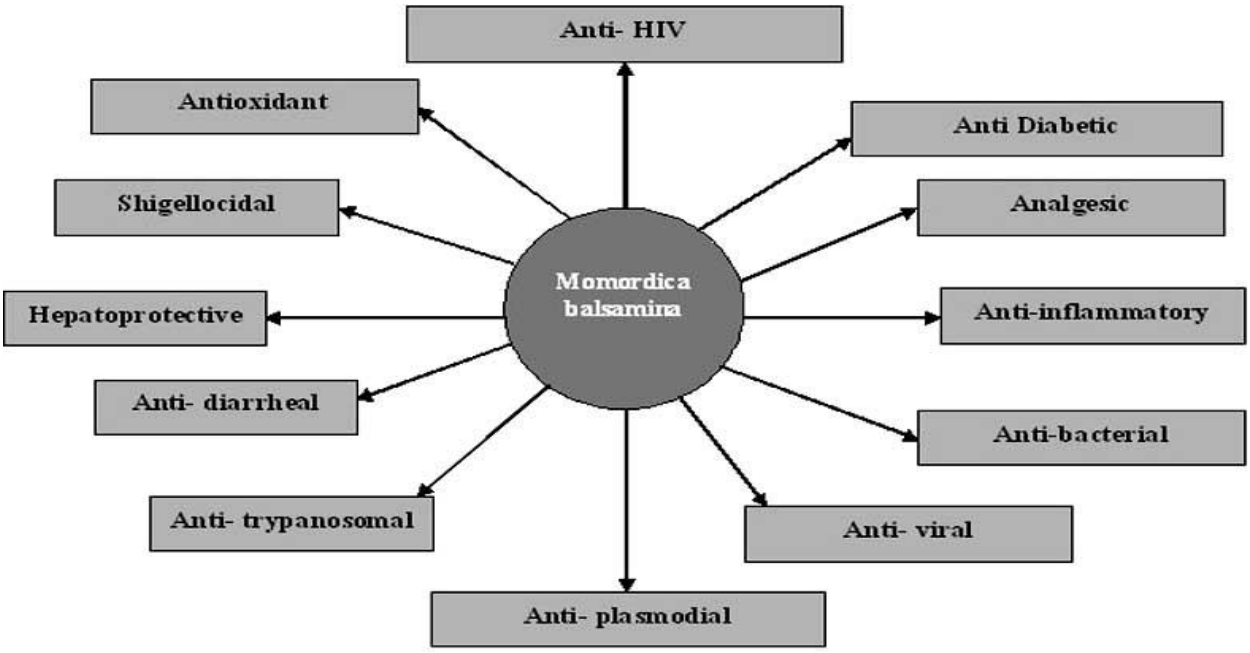


Figure 2.13:Medicinal importance of *Momordica balsamina* L. (Thakur *et al.*, 2009).

The whole plant extract has insecticidal properties (Dalziel, 1958). The leaves and fruits were observed to have hypoglycemic effects in rats (Karumi and bobboi, 1999; Karumi *et*

al., 1999). The leaves were found to be highly hemolytic and hepatotoxic in wistar rats. Furthermore, the fruits were observed to be toxic to various organs and tissues of rats in very high dose (Shetima *et al.,* 2001).

2.4 Free Radicals and Antioxidants

Free radicals, also known simply as radicals, are organic molecules responsible for aging,tissue damage, and possibly some diseases. These molecules are very unstable, therefore,they look to bond with other molecules, destroying their vigor and perpetuating thedetrimental process. Reactive oxygen species (ROS) and oxygen free radicals (OFR) canchange several intracellular processes; as a result they induce mutagenesis or impairmentof biological systems that, in turn, can affect cell survival. The most familiar OFR are thesinglet oxygen, hydroxyl radical, and the superoxide anion, all of which are usuallypresent in the cell arising from normalmetabolic oxidative processes which can increasein some conditions (Alessandra da Silva *et al.,* 2015). Among these oxygen radicals, hydroxyl radical isthe most reactive and induces severe damage to the adjacent biomolecules. The DNA is a major target of free radical induced damage. Under physiologicalconditions, the constant and endogenous rate of production of free radicals may lead to aminimal damage in DNA. However, if this production increases, oxygen radicals may attackDNA at either the sugar (deoxyribose) or the base, giving rise to a large number of products (Huang *et al.,* 2015).

Free radicals are known to initiate lipid peroxidation that results in potential toxiccompounds being produced and/or the destruction of cell membranes leading to cell death(Senthil *et al*, 2004). Tissue damage resulting from the imbalance between reactive oxygenspecies generating and scavenging systems (oxidative stress) has been implicated in

thepathology of a number of disorders, such as atherosclerosis, ischemia-reperfusion injury,cancer, malaria, diabetes mellitus, inflammatory joint disease, asthma, cardiovascular diseases,cataracts, immune system decline; and neurodegenerative diseases andageing processes (Young and Woodside, 2001). The harmful action of free radicals can,result in the death of milk secretary cells and thus lower or stop milk production.Mammalian cells have complex defense mechanisms for radical detoxification (Senthil *et al*, 2004).

Oxidative stress is imposed on cells as a result of one of three factors:

i. An increase in oxidant generation

ii. A decrease in antioxidant protection

iii. A failure to repair oxidative damage.

Reactive chemical intermediates derived from various substances have been invoked as agents in many toxicological mechanisms. Oxygen free radicals are thought to be the causative agents in a wide variety of diseases and degenerative states. Yet immune surveillance produces these reactive molecules to destroy foreign pathogens(Yoneda *et al.*, 2017). The primary mechanism that form these reactive intermediates is often referred to as a Fenton-type reaction, which is the catalytic reaction between hydrogen peroxide and redox active metals (such as ferrous cations) to produce powerful oxidants that can oxidisea wide number of important biomolecules:

i. $\bullet O_2^- + H_2O_2 \rightarrow \bullet OH + HO^- + O_2$

ii. $Fe^{3+} + \bullet O_2^- \rightarrow Fe^{2+} + O_2$

iii. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$

The Haber-Weiss reaction generates oxygen radicals from hydrogen peroxide and super-oxide, however the reaction is very slow. Reaction (i) can be catalysed by the presence of Fe^{3+} to a much faster set of reactions (ii) and (iii), known as the Fenton reaction. The resulting oxidant, the hydroxyl radical, is the most powerful oxidant known and is so reactive it has a lifetime of only 10^{-9} seconds. These oxidation reactions are thought to be the genesis of many diseases (Ames, 1993).

2.4.1 Antioxidant defence system

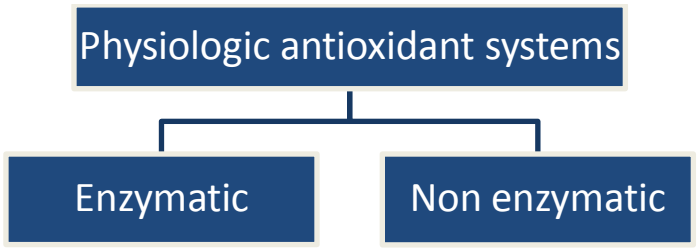


Figure 2.14: Antioxidant defence system (Al-Gubory *et al*., 2010).

2.4.1.1 Enzymatic anti-oxidants

Superoxide dismutase

Superoxide dismutase (SOD)is the antioxidant enzyme that catalyses the dismutation (a reaction between two identical molecules in which one is reduced and the other oxidized) of thehighly reactive superoxide anion to O_2 and to theless reactive species H_2O_2. The hydrogen Peroxide can be destroyed by catalase(CAT) or glutathione peroxidase(GPx) reactions (Teixeira *et al*., 1998).

$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ (this reaction is catalysed by SOD)

In humans there are three (3) forms of SOD which include;

i. Cytosolic Cu/Zn-SOD.

ii. Mitochondrial Mn-SOD.

iii. Extracellular SOD (EC-SOD) (contains copper and zinc).

Catalase

Catalase (CAT) reacts very efficiently with $H_2O_2$to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity (Imai *et al.,* 1998).

$$2\,H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$ (Imai *et al.,* 1998).

In animals, hydrogen peroxide is detoxified by CAT and by GPx. Catalase protects cells from hydrogen peroxide generated within them. Eventhough CAT is not essential for some cell typesunder normal conditions, it plays an important rolein the acquisition of tolerance to oxidative stress inthe adaptive response of cells (Thitimuta *et al.,* 2017).

Glutathione peroxidase

The selenium-containing peroxidase, glutathioneperoxidase (GPx) contains a single seleno-cysteine (Sec) residue in each of the four identicalsubunits, which is essential for enzyme activity (De Haan *et al*., 1998). The GPx catalyses the reduction of hydroperoxides using glutathione(GSH) thereby protecting mammalian cells against oxidative damage. In fact, glutathione metabolism is one of the most essential anti-oxidative defense mechanisms (Imai *et al*., 1998). Equation of glutathione reaction

$$ROOH + 2GSH \xrightarrow{GPX} ROH + GSSG + H_2O$$ (Imai *et al.,* 1998).

There are five GPX isoenzymes found in mammals. Although their expression is ubiquitous, the levels of each isoform vary depending on the tissue type.

These include;

i. Cytosolic glutathione peroxidase ($_{C}$GPxor GPX1).

ii. Mitochondrial glutathione peroxidase.

iii. Phospholipid hydroperoxide glutathione peroxidase (PHGPX or GPX4).

iv. Extracellular glutathione peroxidase (GPX3 or GPXP).

v. Glutathione peroxidase (GPX5).

Cytosolic and mitochondrial glutathione peroxidase (cGPX or GPX1) reduces fatty acid hydroperoxides and hydrogen peroxide at the expense of glutathione. The GPX1 and the phospholipid hydroperoxide glutathione peroxidase (PHGPX or GPX4) are found in most tissues. GPX4 is located in both the cytosol and the membrane fraction. The PHGPX can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins (Imai *et al*., 1998). The GPX1 is predominantly present in erythrocytes, kidney, and liver, while GPX4 is highly expressed in renal epithelial cells and testes. Cytosolic GPX2 or GPX-G1, and extracellular GPX3 or GPX-P are poorly detected in most tissues except for the gastrointestinal tract and kidney, respectively. Recently, a new member, GPX5, expressed specifically in mouse epididymis, is interestingly selenium independent (Han *et al.,* 2016; Augusta-obara *et al.,* 2017).

2.4.1.2Non-enzymatic antioxidants

The non-enzymatic antioxidants consist of dietary supplements and synthetic antioxidants such as vitamin C, GSH, taurine, hypotaurine, vitamin E, Zn, selenium (Se), beta-carotene, and carotene (Sharma and Argawal, 2004). Vitamin C (ascorbic acid) is a known redox catalyst that can reduce and neutralize ROS. Its reduced form is maintained through reactions with GSH and can be catalyzed by protein disulfide isomerase and glutaredoxins.Glutathione is a peptide found in most forms of aerobic life as it is made in the cytosol from cysteine, glutamate, and glycine. It is also the major non-enzymatic antioxidant found in oocytes and embryos. Its antioxidant properties stem from the thiol group of its cysteine component, which is a reducing agent that allows it to be reversibly oxidized and reduced to its stable form (Behrman *et al*., 2001).

Vitamin E (α-tocopherol) is a lipid soluble vitamin with antioxidant activity. It consists of eight tocopherols and tocotrienols. It plays a major role in antioxidant activities because it reacts with lipid radicals produced during lipid peroxidation. This reaction produces oxidized α-tocopheroxyl radicals that can be transformed back to the active reduced form by reacting with other antioxidants like ascorbate, retinol, or ubiquinol.The hormone melatonin is an antioxidant that, unlike vitamins C and E and GSH, is produced by the human body. In contrast to other antioxidants, however, melatonin cannot undergo redox cycling. Once it is oxidized,melatonin is unable to return to its reduced state because it forms stable end-products after the reactionoccurs.Transferrin and ferritin, both iron-binding proteins, play a role in antioxidant defense by preventingthe catalyzation of free radicals through chelation (Shkolnik *et al*., 2011; Shih*et al*, 2011). Nutrients such as Se, Cu, and Zn are required for the activity of some antioxidant enzymes, although they have no antioxidant action themselves (Shkolnik *et al*., 2011).

2.5Liver Enzymes

The liver is the primary site of drug metabolism; followed by other tissues, such as kidney, brain, skin, blood, lung and gastrointestinal mucosa. The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea. In conjunction with the spleen it is involved in the destruction of finished red blood cells and the reclamation of their constituents. It is responsible for synthesizing and secreting bile (Pessayre *et al*., 2010). It maintains a stable blood glucose level by taking up and storing glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis) and forming glucose from non-carbohydrate sources such as amino acids (gluconeogenesis).

Abnormal liver enzyme levels may signal liver damage or alteration in bile flow. Liver enzyme alteration may be either the accompanying biochemical picture in a patient with symptoms suggestive of liver disease or an isolated, unexpected finding in a patient who has undergone a wide range of laboratory tests for a non-hepatic disease. Measurement of liver enzymes remains the most practical tool to diagnose liver damage which includes mainly alanine aminotransferase (ALT), an enzyme present in hepatocytes, and alkaline phosphatase (ALP), an enzyme in the cells lining the biliary ducts of the liver (Jessica *et al.*, 2017).

2.5.1 Alkaline phosphatase

Alkaline phosphatase (ALP)is an enzyme that transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissues, such as the placenta,

kidneys, intestines, or from leukocytes (Fishman, 1990). Alkaline phosphatase is present in mucosal epithelia of small intestine, proximal convoluted tubule of the kidney, bone, liver and placenta. It performs lipid transportation in the intestine and calcification in bone. The serum ALP activity is mainly from the liver with 50% contributed by bone (Mauro *et al*., 2006). Normal serum ALP is 41 to 133U/L. The half-life of ALP in the circulation is about 1 week (Dufour *et al*., 2000), and drug-induced liver injury may present with a cholestatic pattern (preferential increase in ALP or ALT/ALP ratio < 2), although the degree of ALP alteration is variable and may be accompanied by hyperbilirubinemia (Velayudham and Farrell, 2003).

2.5.2 Alanine amino transferase

Alanine amino transferase (ALT)is found in kidney, heart, muscle and greater concentration in liver compared with other tissues of the body. It is purely cytoplasmic in origin and catalyzing transamination reactions(Mauro *et al*., 2006). Normal serum ALT is 7-56 U/ L. Any type of liver cell injury can reasonably increase ALT levels. Elevated values up to 300 U/L are measured nonspecific. Despite the association between greatly elevated ALT levels and its specificity to hepatocellular diseases, the absolute peak of the ALT elevation does not correlate with the extent of liver cell damage (Kallei *et al*., 1964). Moreover increased ALT level was associated with reduced insulin sensitivity, adiponectin and glucose tolerance as well as increased free fatty acids and triglycerides (James *et al*., 2006).

2.5.3 Aspartate amino transferase

Aspartate amino transferase (AST)catalyze transamination reactions. It exists in two different isoenzyme forms which are genetically distinct, the mitochondrial and

cytoplasmic form. It is found in highest concentration in heart compared with other tissues of the body such as liver, skeletal muscle and kidney (Mauro *et al*., 2006). Normal serum AST is 0 to 35U/L, and elevated mitochondrial AST is seen in extensive tissue necrosis during myocardial infarction and also in chronic liver diseases like liver tissue degeneration and necrosis (Diana, 2007). About 80% of AST activity of the liver is contributed by the mitochondrial isoenzyme, whereas most of the circulating AST activity in normal people is derived from the cytosolic isoenzyme. However the ratio of mitochondrial AST to total AST activity has diagnostic importance in identifying the liver cell necrotic type condition and alcoholic hepatitis (Panteghini *et al*., 1983).

2.5.4 Main types of drug-induced liver injury

Drugs can cause liver injury in several ways, but three main types are usually dose-dependent (or intrinsic) toxicity, dose-independent (or idiosyncratic metabolic) toxicity and drug allergy (or idiosyncratic immunological). Intrinsic toxicity is predictable and often caused by high concentrations of parent drug and/or a metabolite when the liver`s defense systems against toxic damage are overwhelmed (Adams *et al*., 2010).

2.5.5 Main mechanisms of drug-induced liver injury

The liver has a central role as a detoxifying organ towards xenobiotics and chemicals. However, biotransformation to less toxic substances, actually involve production of molecules that can induce liver injury through various pathways. Important mechanisms involved in non-allergic drug-induced hepatic injury are divided into:

a. Drug metabolism and reactive metabolite formation.
b. Covalent binding of reactive metabolites.
c. Reactive oxygen species generation.

d. Activation of signal transduction pathways that modulate cell death or survival.

e. Mitochondrial damage.

f. Immunological mechanism.

2.5.5.1 Drug metabolism and reactive metabolite formation

Biotransformation of lipophilic xenobiotics to more hydrophilic substances before excretion in urine or bile is an important function of the liver. The metabolites can, via Phase II enzymes be conjugated to hydrophilic molecules before excretion. The reason why reactive metabolites can induce damage through idiosyncratic liver injury is based on a complex interplay between several factors. Age, sex, liver function and disease, environmental (enzyme induction and inhibition) and genetic (polymorphism) can influence the level or function of CYP-450, and thereby production of reactive metabolites. Inherent toxicity of these, their distribution and accumulation within the organ, cells and sub-cellular organelles together with the status of defense systems are likely to be of additional importance (Grattagliano *et al*., 2009).

2.5.5.2 Covalent binding of reactive metabolites

Bio-activation of drugs through Phase I reactions can produce electrophilic metabolites with intrinsic chemical reactivity toward several cellular macromolecules. An important pathway for bio-activation of many acidic nonsteroidal anti-inflammatory drugs (NSAIDs) involves formation of reactive acylglucuronides. Thus, Phase II reactions of bioactivation, usually thought of as a detoxifying process, can also produce reactive metabolites

associated with hepatotoxicity. Furthermore, covalent modification of cellular components can initiate both toxic and mechanisms of liver injury (Kemper, 2008).

2.5.5.3 Reactive oxygen species generation

Metabolic activation of drugs can involve production of reactive oxygen species through chemical modification of cellular macromolecules. Reactive oxygen species can initiate protein and lipid peroxidation, and deplete antioxidant defences like reduced glutathione (GSH), and modify sulphydryl (SH) groups on cellular components. The subsequent increased cellular stress and uncontrolled increase in cytosolic Ca^{2+} concentration stimulate Ca^{2+} -activated degradative enzymes with further cellular injury. Disruption of membranes of cells, sub-cellular organelles including mitochondria, can affect energy production and further disrupt ionic homeostasis, and initiate cell death pathways (Han *et al*., 2010).

2.5.5.4 Activation of signal transduction pathwaysthat mediates cell death or survival

The stress in hepatocytes induced by reactive metabolites, including reactive oxygen species, can activate several signal transduction pathways. Several endogenous signal substances modify signal transduction including various cytokines. The pathways activate pro death or survival proteins that determine the fate of the cell (Han *et al*., 2010). Important for liver injury is c-Jun N-terminal protein kinases (JNK). JNK can be activated with many stresses including reactive oxygen species, and represents a common mechanism of cellular injury associated with several diseases (Han *et al*., 2010).

2.5.5.5 Mitochondrial damage

Mitochondria have a central role in liver injury. Cytotoxic drugs or metabolites can attack the organelle directly or through activation of signal transduction pathways. Important

pathological mechanisms are mitochondrial permeability transition (MPT) through opening of MPT pore in the membranes of the organelle, and activation of signals, death receptors and pro-apototic pathways. As a result acute necrosis, apoptosis and autophagic cell death can occur (Kass, 2006). Mitochondrial dysfunction is involved in most forms of drug-induced liver injury. Valproic acid can inhibit mitochondrial fatty oxidation, uncouple mitochondrial respiration and induce MTP (Yengkhom*et al*., 2017).

2.5.5.6 Immunological mechanisms

Various types of immune cells, including lymphocytes, reside in the liver (Adams *et al.*, 2010). Furthermore, antidrug antibodies and auto antibodies can be detected during liver injury (Liu and Kaplowitz, 2002). A drug-modified protein formed due to reactions described previously (reactive metabolite formation and covalent binding) can initiate either classic immune responses against the drug or an autoimmune response against a modified protein(Adams *et al*., 2010). The classic immune response is characterized with short time period to onset of symptoms and a memory component, while the autoimmune response develops after a long time period with no memory. The different courses are based on interaction with different T helper cells, but involvement of B cells and other immune cells could be of importance (Adams *et al*., 2010)

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

The following materials: Saw dust, feeds, clean water, cotton wool,disposable gloves, mortar and pestle were used for the experiment.

3.1.1 Chemical and drugs

Metoclopramide hydrochloride tablets 10 mg; Jiangsu Pengyao Pharmaceutical Inc. China, Manufacturing Licence No.: B131205, NAFDAC Reg. No.: 04-6476, chloroform was purchased from Steve Moore Chemical stores Zaria, disinfectant (Dettol) and methylated spirit were purchased from the Pharmacy store of the Ahmadu Bello University Teaching Hospital (ABUTH) Shika; located about 8 km away from Samaru at Latitude 112^{o} 12'' N and Longitude 07^{o} 37'' E, Zaria-Kaduna State, Nigeria.

3.1.2Instruments and devices

Enzyme-linked immune-absorbent assay (ELISA) kit for Prolactin [(No. GA-E0504RT)], growth hormone [(No. GA-E0512RT)], oxytocin [(No. GA-E0521RT)], Catalase [(No. E-EL-R2356)], Glutathione peroxidase [(No. BC0056)], Superoxide dismutase [(No. E-EL-R2346)]and Malondialdehyde [(No. GA-E0204RT)]were purchased from GenAsia Biotech Co. Ltd. Dissection Kits, 120 Standard bottle, 2 ml pipette and Mortar, Bench Centrifuge Hawksley [(RegNo: 891481 Ser. No. 07. 4. 26.®)],Weighing balance [(Salter, HoMedics Group Ltd. Production no. IB-1066-1011-03)], Oral cannula, Dissection board, 5ml syringe, stainless steel metal cages, cannula, Aneasthetic chamber.

3.1.3 Plant collection

The leaves and stems of *Momordica balsamina* L. were collected from swampy areas in Sabon Gari Local Government Area of Kaduna State, Nigeria in June 2016. The plant was identified at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University Zaria, with a voucher number of 1139 by Malam Namadi Sunusi.

3.1.4Extraction of *Momordica balsamina* L.

The aqueous extraction was done in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. The *Momordica balsamina* L. was dried under shed and pounded into powder using mortar and pestle. Five hundred (500 g) of the powderedwas weighed into a clean bowl and 2.5 L of hot distilled water was poured in the bowl and covered. This was allowed to infuse for 3 hr and then cooled at roomtemperature. The mixture was sieved with a clean muslin cloth. The filtrate was allowed to stand for 1-2 hours. It was then decanted gently and evaporated to dryness on water bath at 70^{o}C with percentage yield of 9.06%. A green-brownish semi-solid sticky residue of about 45.3gwas obtained and was kept in air-tight container at room temperature.

3.1.5Experimental Animal management

A total of thirty (30) female and twenty (20) male Wistar rats weighing between 180-240g were purchased from the Animal house of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria.Twenty five (25) female Wistar rats were used for the lactogenic study, thirteen (13) male and female Wistar rats were used for the acute toxicity studyand twelve (12) male Wistar rats were used for the mating protocol. The rats were housed in the Animal house of the Department of Human Physiologyunder standard laboratory condition with 12 h light/dark cycle.Their cages were cleaned on regular bases and they were fed on commercial feeds with tap water *ad libitum.* The animal management and experimental protocol was carried out in conformity with Animal Welfare ethics of the European Union Research Animal Welfare Protocol (2015). The research was approved by the Ahmadu Bello University Committee on Animal Use and Care

(ABUCAUC) from the Ahmadu Bello University Zaria, Kaduna, Nigeria(Approval No.: **ABUCAUC/2017/017**).

3.2Methodology

3.2.1 Acute toxicity study

The lethal doses (LD_{50}) of the plant extract were determined according to the method of Lorke using 13 adult Wistarrats of both sexes. In the first phasethe rats were divided into 3 groups of 3 rats each and weretreated with the aqueous extract of the plant at doses of 10,100 and 1000 mg/kg body weight intraperitoneally. The animals were placed under observationfor 24 hours for any sign of toxicity and mortality.

In the second phase, 4 rats were divided into 4 groups of 1 rat each and were alsotreated with the aqueous extract at doses of 1000, 1600,2900 and 5000 mg/kg bodyweight (i.p). The animals were observed for behavior as well as mortality. The LD_{50} was calculated as the geometric mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality.

$LD_{50}= \sqrt{D0}/D100$

Where: D0 = Maximum dose producing 0% mortality

D100 = Minimum dose producing 100% mortality.

3.2.2 Preliminary phytochemical screening

The aqueous extract of *Momordica balsamina*L. was subjected to preliminary phytochemical screening, toidentify the chemical constituents. The phytochemical screening of *Momordica balsamina* L. extract was conducted in Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria. The

screeningwas then analyzed as described by Odebiyi and Sofowara, (1978) and Harbone (1973).

3.2.3 Experimental design

The twenty five (25) female Wistar rats weighing between 180-240g were mated with apparently healthy mature male Wistar rats at the Animal House of the Department of Human Physiology.The number of pups per dam was culled to 4 immediately following parturition and the day of parturition was designated as Day 1 of lactation. After birth the 25lactating Wistar rats were randomly selected and divided into five (5) groups of five (5) rats each and treated as follows:

I. Control group (n=5); (1 ml/kg normal saline)

II. Metoclopramide-treated group (n=5); (5 mg/kg body weight, Quedraogo *et al*., 2004; Bako *et al*., 2013).

III. *Momordica balsamina* L. treated group (n=5); (200 mg/kg body weight).

IV. *Momordica balsamina* L. treated group (n=5); (400 mg/kg body weight).

V. *Momordica balsamina* L. treated group (n=5); (800 mg/kg body weight).

The dams were given the extract and the drug via oral route of administration daily for the period of ten days, starting from day 4 to day 13 of lactation.

3.2.4 Determination of the effect ofaqueous extract of *Momordica balsamina* L.on milk yield in lactating Wistar rats.

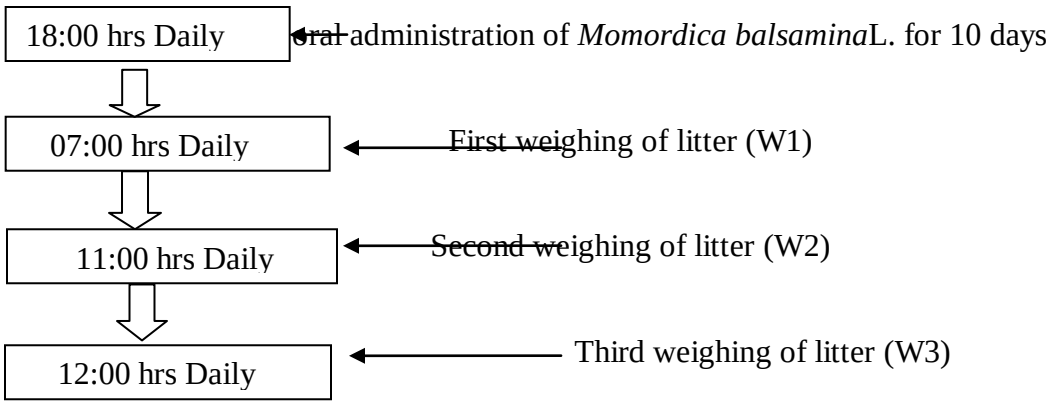
Milk production for each group was estimated at 18 hours after gavage for a period of ten days starting from day 5 to day 14 of lactation. The pups were weighed every day during the study period at 07:00 h and their weight recorded as (w1), and then isolated from their dam for a period of 4 hours (Sampson and Jansen, 1984; Ann and Linzell, 2003). At 11:00

h, the pups were weighed again and the weight recorded as (w2); and then re-united with their dam and allowed to feed for 1 hour and their weight taken again at 12:00 h and recorded as (w3). Milk yield 18 hours after gavage was estimated as w3-w2 with a correction for weight loss due to metabolic processes using the formula as follows:

Correction for weight loss =*(w2-w1)/4* (Sampson and Jansen, 1984).

This value was then multiplied by the number of suckling hours per day and added to the suckling gain, daily. Daily weight gain of pups was calculated from the pup weight at w2. (Sampson and Jansen, 1984;Alexandre *et al*., 2002; Ouedrago *et al*., 2004).The dams and pups were weighed using an electronic balance daily (Metter P3®) accurate to 0·01 g.

MILK YIELD DETERMINATION PROTOCOL



3.2.5 Evaluation of the effect of aqueous extract of*Momordica balsamina* L. on serum prolactin, growth hormone and oxytocin in lactating Wistar rats.

On day 14th of lactation, the lactating rats wereanaesthetized using ketamine (5 mg/kg) and placed on the dissecting board (in a supine position) with the abdominal region facing upwards. The paws were pinned down with pins on the board and the abdominal cavity exposed using a pair of scissors. The diaphragm was cut open and the heart accessed using a 5 ml syringe to collect blood (Bako *et al*., 2013). The blood sample was then discharged from the syringe into test tubes and centrifuged (2000 x g for 10 minutes) to obtain the

serum. The labeled sera were analyzed for prolactin, growth hormone,oxytocin, oxidative stress biomarkers using theappropriate ELISA kitand liver enzymesat the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria.

3.2.5.1 Prolactin assay

Prolactin assay was carried out using the prolactin rat ELISA kit according to the manufacturer's instructions. The prolactin species specific enzyme-linked immunosorbent assay (ELISA) kit in microplate was designed for the quantitative evaluation of rat prolactin. The sera sample was pipetted into an antibody coated microplate, and allowed to incubate for two (2) hours during which the endogenous prolactin in the sample bind to the antibodies fixed on the inner surface of the wells. After the addition of a second polyclonal horseradish peroxidase-labelled antibody, a sandwich complex which consisted of two antibodies and the rat prolactin was formed. The excess enzyme conjugate was washed out and a chromogenic substrate, tetra-methyl benzidine (TMB) was added to the wells and allowed for 30 minutes incubation, during which the substrate was converted to a coloured end product (blue) by the fixed enzyme. The enzyme reaction was inhibited by dispensing hydrochloric acid as the stop solution. The optical density of the colour solution was measured using a microplate reader at 450 nm. A standard curve was obtained by plotting the concentration of standard versus the absorbance. The PRL concentration of the specimens and controls run concurrently with standards. The lowest detectable level of prolactin with this test was 0.6 ng/mL, with a range of 6.64-23.26 ng/mL for males and 13.7-23.3 ng/mL for females (Dombrowicz *et al*., 1992).

3.2.5.2 Growth hormone assay

The growth hormone analysis was carried out according to the manufacturer's instructions, using the growth hormone ELISA kit. The MICRO-ELISA growth hormone test was based on the principle of a solid phase enzyme linked immunosorbent assay. The sample sera were allowed to react simultaneously with the coated and conjugated antibodies which resulted in the rat growth hormone molecule being sandwiched between the solid phase and enzyme linked antibodies. It was then incubated for 45 minutes at room temperature and the unbound enzyme washed out. Hydrogen peroxide and tetra-methylbenzidine (TMB) were added to the well and incubated for 30 minutes at room temperature which resulted in the formation of a blue colour. 1.0 N of H_2S0_4 was added to stop the reaction which converted the colour to yellow. The intensity of the yellow colour was directly proportional to the concentration of rat growth hormone in the sera.

3.2.5.3Oxytocin assay

Oxytocin analysis was carried out using the oxytocin rat ELISA kit according to the manufacturer's instructions. The oxytocin species specific enzyme-linked immunosorbent assay (ELISA) kit in microplate was designed for the quantitative evaluation of rat oxytocin. The sera sample was pipetted into an antibody coated microplate, and allowed for two (2) hours during which the endogenous oxytocin in the sample bound to the antibodies fixed on the inner surface of the wells. After the addition of a second polyclonal horseradish peroxidase-labelled antibody, a sandwich complex which consisted of two antibodies and the rat oxytocin was formed. The excess enzyme conjugate was washed out and a chromogenic substrate, tetra-methyl benzidine (TMB) was added to the wells and allowed for 30 minutes incubation, during which the substrate was converted to a coloured

end product (blue) by the fixed enzyme. The enzyme reaction was inhibited by dispensing hydrochloric acid as the stop solution. The optical density of the colour solution was measured using a microplate reader at 450 nm. A standard curve was obtained by plotting the concentration of standard versus the absorbance. The oxytocin concentration of the specimens and controls run concurrently with standards. The lowest detectable level of oxytocin with this test was 5 ng/mL.

3.2.6 Evaluation of the effect of aqueous extract of *Momordica balsamina* L. on biomarkers of oxidative stress in lactating Wistar rats.

The ability of the *Momordica balsamina* L. extract to affectantioxidant enzymes activitywasevaluated by determining the activity of the following antioxidant enzymes:

3.2.6.1 Assay of superoxide dismutase activity

The assay for the superoxide dismutase (SOD**)**activity was carried out using rat superoxide dimutase ELISA Kit according to manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for SOD was pre-coated onto a microplate. Standards and samples (blood serum) were pipetted into the wells, gently mixed, covered with a closure plate membrane and incubated for 30 mins at 37ºC. This allowed any SOD present to be bound by the immobilized antibody. After removing from the incubator, the wells were washed with a prepared 'wash solution'; the microplate was inverted and thereafter blot-dried by hitting onto an absorbent paper to remove the moisture. An enzyme-linkedmonoclonal antibody specific for SOD was added to the wells, sealed and, again incubated for 30 mins at 37ºC. Following a wash to remove any unbound antibody-enzyme reagent, substrate solutions (tetramethyl benzidine and hydrogen peroxide) were added to the wells and a colour developed in proportion to the

amount of SOD bound in the initial step. This was covered and incubated for 15 mins at 37ºC.The colour development was stopped by addition of sulfuric acid 'stop solution' and the intensity of the colour measured with a microplate reader.

3.2.6.2 Assay of catalase activity

The assay for the catalase (CAT**)**activity was carried out using rat catalaseELISA Kit according to manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CAT was pre-coated onto a microplate. Standards and samples (blood serum) were pipetted into the wells, gently mixed, covered with a closure plate membrane and incubated for 30 mins at 37ºC. This allowed any CAT present to be bound by the immobilized antibody. After removing from the incubator, the wells were washed with a prepared 'wash solution'; the microplate was inverted and thereafter blot-dried by hitting onto an absorbent paper to remove the moisture. An enzyme-linked monoclonal antibody specific for CAT was added to the wells, sealed and, again incubated for 30 mins at 37ºC. Following a wash to remove any unbound antibody-enzyme reagent, substrate solutions (tetramethyl benzidine and hydrogen peroxide) were added to the wells and a colour developed in proportion to the amount of CAT bound in the initial step. This was covered and incubated for 15 mins at 37ºC. The colour development was stopped by addition of sulfuric acid 'stop solution' and the intensity of the colour measured with a microplate reader.

3.2.6.3 Assay of glutathione peroxidase activity

The assay for the glutathione peroxidase activity was carried out using rat glutathione peroxidase (GPx**)**ELISA Kit according to manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific

for GPx was pre-coated onto a microplate. Standards and samples (blood serum) were pipetted into the wells, gently mixed, covered with a closure plate membrane and incubated for 30 mins at 37ºC. This allowed any GPx present to be bound by the immobilized antibody. After removing from the incubator, the wells were washed with a prepared 'wash solution' buffer; the microplate was inverted and thereafter blot-dried by hitting onto an absorbent paper to remove the moisture. An enzyme-linked monoclonal antibody specific for GPx was added to the wells, sealed and, again incubated for 30 mins at 37ºC after which it was washed and blot-dried to remove any unbound antibody-enzyme reagent. Substrate solutions (tetramethyl benzidine and hydrogen peroxide) were added to the wells and a colour developed in proportion to the amount of GPx bound in the initial step. This was covered and incubated for 15 mins at 37ºC. The colour development was stopped by addition of sulfuric acid 'stop solution' and the intensity of the colour measured with a microplate reader.

3.2.6.4 Estimation of lipid peroxidation (malondialdehyde)

Lipid peroxidation in the blood samples was estimated spectrophotometrically as thiobarbituric acid reactive substances (TBARS). A principal component of TBARS is malondialdehyde (MDA), a product of lipid peroxidation.The assay for malondialdehyde was carried out using malondialdehyde ELISA kit according tothe manufacturer's instruction. This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific for MDA has been pre-coated onto a microplate. A competitive inhibition reaction was launched between biotin labeled MDA and unlabeled MDA/samples (sera) with the pre-coated antibody specific for MDA. After incubation the unbound conjugate was washed off. Next, avidin conjugated toHorseradish Peroxidase

(HRP) was added to each microplate well and incubated. The amount of bound HRP conjugate was taken to be inversely proportional to the concentration of MDA in the sample. After addition of substrate solution, the intensity of colour developed was measured using a microplate reader and the reading taken to be inversely proportional to the concentration of MDA in the sample.

3.2.7 Evaluation of the effect of aqueous extract of *Momordica balsamina* L. on liver enzymes in lactating Wistar rats.

3.2.7.1 Determination of serum alkaline phosphatase

This was estimated according to the method of Bowers and Mc Comb (1966). Briefly 0.5 mL of the reagent was added to 0.05 mL of the samples then mixed and incubated at 37ºC for 10 minutes. The change in the absorbance of the sample was measured per minute spectrophotometerically at wavelength of 590 nm.

3.2.7.2 Determination of serum aspartate aminotransferase

This was estimated by the method described by Bergmeyer and Walefeld (1978). Briefly 1000 µL of the reagent was added to 100 µL of the sera and then mixed and incubated at 37 ºC for 1 minute. The change in the absorbance of the samples was measured per minute spectrophotometerically at wavelength of 590 nm.

3.2.7.3 Determination of serum alanine aminotransferase

This was estimated by the method described by Bergmeyer and Walefeld (1978). Briefly 1000 µL of the reagent was added to 100 µL of the sera and then mixed and incubated at 37 ºC for 1 minute. The change in the absorbance of the samples was measured per minute spectrophotometerically at wavelength of 590 nm.

3.2.8Evaluation of the effect of aqueous extract *Momordica balsamina* L. on the histology of liverand mammary gland

At the end of the experiment liver and mammary gland tissues from all the groups were harvested using a pair of scissors and a forceps and then weighed; and thereafter deposited in a labelled test tube containing a physiological solution(10 % formal saline).

3.2.8.1 Histology of the liver and mammary gland

The method of Hematoxylin and Eosin staining technique was used for the histological preparation. The histological preparation and interpretation was carried out by Mr. A. Yaro (laboratory scientist) of the Department of Histopathology, Ahmadu Bello University Teaching Hospital Shika, Zaria-Kaduna. The liver and mammary tissues section were placed on glass slides and hydratedin descending grades of alcohol from 100%, 95%, 90% and finally 70% (Laporta *et al*., 2013). Each of the steps lasted for three (3) minutes and the tissues were washed in a running tap water and stained afterward with haematoxylin for twenty five (25) minutes, washed with water and then differentiated in acid alcohol. The tissue slides were then counter stained with eosin and blued in Scott water. Afterward, the tissue slides were hydrated in ascending grades of alcohol and cleared in xylene for three (3) changes in five (5) minutes each. The tissue slides were then mounted with cover slips using a mounting media. There after, the slides were viewed under a light microscope and the photomicrographs taken.

3.3 Statistical Analysis

Data obtained were expressed as mean ± standard error of mean and analysis of variance was done using one way (ANOVA), followed by *Tukey's post hoc* test using SPSS version 23.0 (2015).Values of $p<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute Toxicity StudyofAqueous Extract *Momordica balsamina* L.

Table 4.1: Results of acute toxicity study, showing doses of *Mormodica balsamina* L.extract

Phase One

Group (N=3)	Dose (mg/kg) body weight	Deaths	Percentage mortality (%)
1	10	0/3	0
2	100	0/3	0
3	1000	0/3	0

Phase Two

Group (N=1)	Dose (mg/kg) body weight	Deaths	Percentage mortality (%)
1	1600	0/1	0
2	2900	0/1	0
3	5000	0/1	0

There was a little sign of toxicity but no mortality recorded in all phases. The $LD_{50}$was estimated to be above 5000 mg/kg.

4.2Preliminary Phytochemicals Screening ofAqueousExtract of *Momordica balsamina*L.

The result on Phytochemicals constituents in *Momordicabalsamina*L. showed presenceof alkaloids, flavonoids, tannins, cardiac glycosides, saponinsand carbohydrates.

Table 4.2:Phytochemical constituents of aqueous extract*Momordica balsamina*L.

Compound	Present
Tannins	+
Flavonoids	+
Alkaloids	+
Anthraquinone	–
Saponin	++
Carbohydrate	++
Cardiac Glycosides	+

++= Present in appreciable quantity.
+ = Trace.
- = Absent.

4.3 Effect of Aqueous Extract of *Momordica balsamina* L.on Milk Yield 18 Hours after Administration

There was a significant increase ($p<0.05$) in mean milk yield between the MBL 400 mg/kg and metoclopramide treated groups when compared with the control; (1.44 ± 0.20 vs 0.62 ± 0.15) and (2.08 ± 0.17 vs 0.62 ± 0.15), respectively. However no significant difference was observed between MBL 200 mg/kg & 800 mg/kg treated groups when compared to control group (Figure 4.1).

The highest level of daily milk yield was seen in the 400 mg/kg treated group on day 9 of the treatment (3.0 ± 0.45). However metoclopramide treated group showed high daily milk yield on day 4 of the treatment (2.8 ± 0.74).

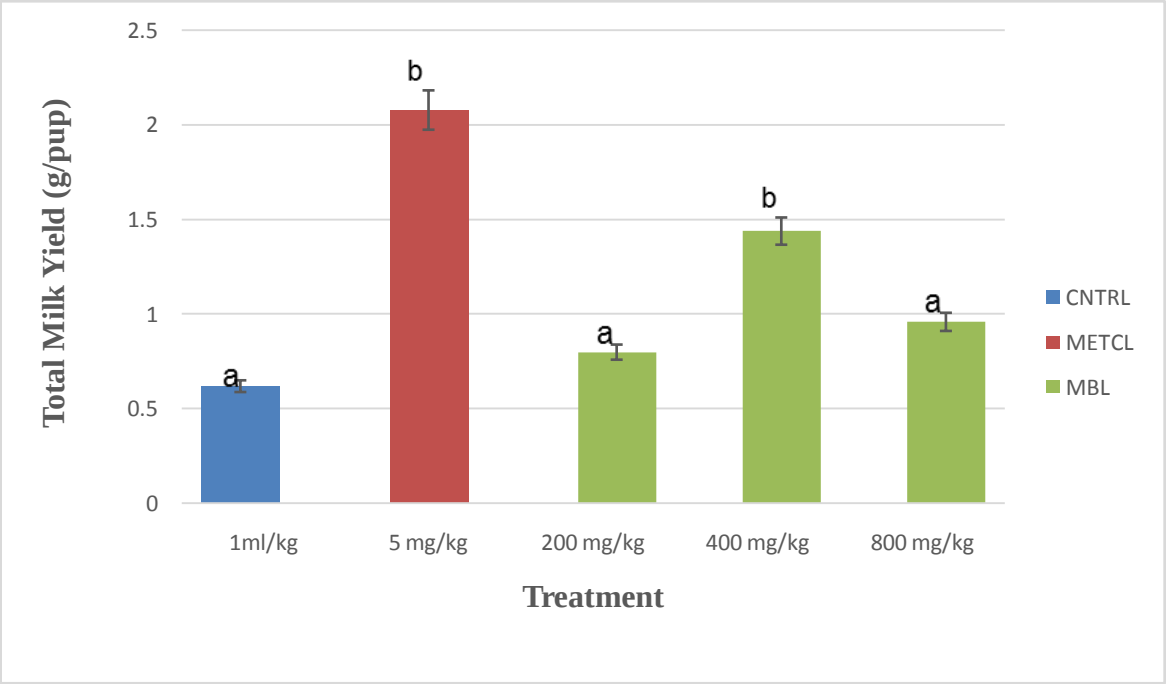


Figure 4.1: Mean milk yield in lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a**(*p<0.05)*.

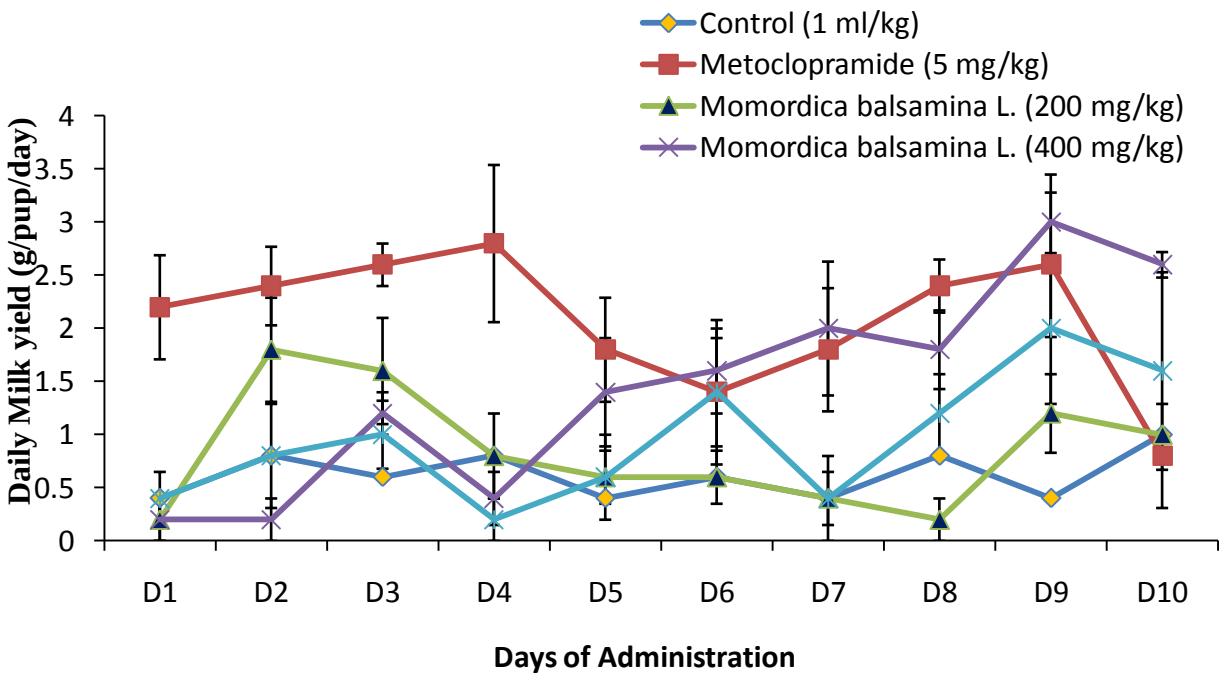


Figure 4.2: Daily milk yield in rats treated with metoclopramide and aqueous extract of *Momordica balsamina* L.(MBL) 18 hours after administration.

4.4 Effect of Aqueous Extract of *Momordica balsamina* L.on Serum Prolactin, Growth Hormone and Oxytocin in Lactating Wistar Rats

The serum level of prolactin was significantly *($p<0.05$)* lower in the group treated with MBL 800 mg/kg group compared to the control (28.66 ± 1.60 vs 38.06 ± 2.04) and to other treated groups metoclopramide (43.14 ± 0.84 vs 28.66 ± 1.60), MBL 200 mg/kg (41.20 ± 0.58 vs 28.66 ± 1.60), MBL 400 mg/kg (42.50 ± 1.39 vs 28.66 ± 1.60) (Figure 4.3)

There was a significantly higher growth hormone level *($p<0.05$)* in all the MBL and metoclopramide treated groups when compared to the control; metoclopramide treated (12.46 ± 0.03 vs 9.10 ± 0.33), MBL 200 mg/kg (12.08 ± 0.20 vs 9.10 ± 0.33), MBL 400 mg/kg (13.26 ± 0.32 vs 9.10 ± 0.33) and MBL 800 mg/kg (13.64 ± 0.80 vs 9.10 ± 0.33)(Figure 4.4).

The serum level of oxytocinwassignificantly higher*($p<0.05$)* in the MBL treated groups when compared to the control group; MBL 200 mg/kg (404.80 ± 1.71 vs 309.80 ± 8054), MBL 400 mg/kg (384.60 ± 6.33) and MBL 800 mg/kg (412.40 ± 7.67 vs 309.80 ± 8.54) (Figure 4.5). The oxytocin levels in the MBL treated groups were also significantly higher *($p<0.05$)* when compared to the metoclopramide treated group (320.00 ± 5.26).

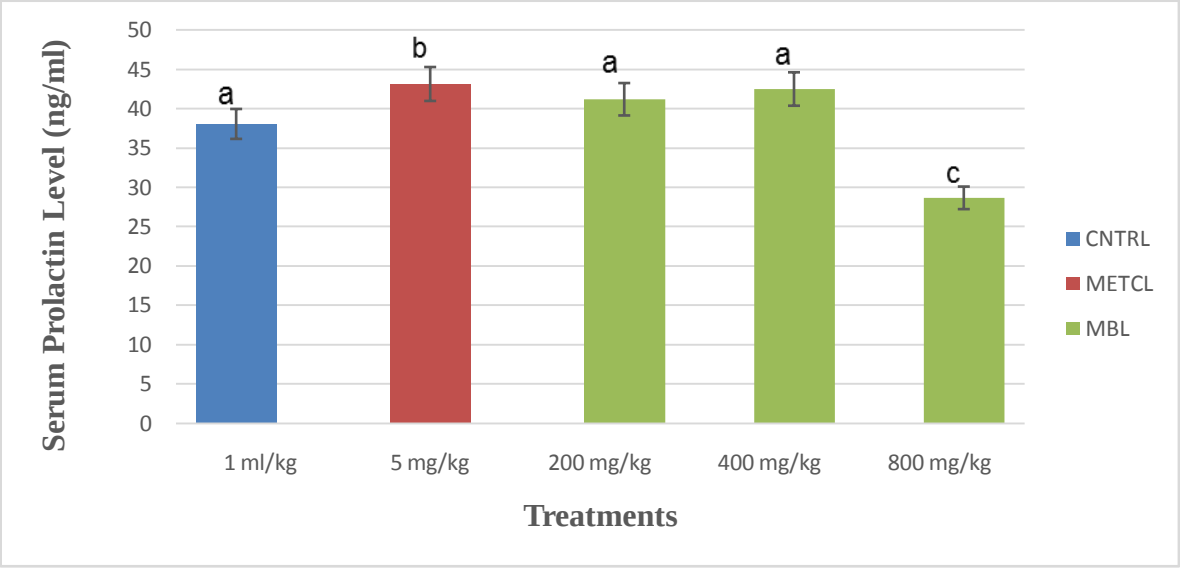


Figure 4.3: Serum prolactin levels in lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a.** Superscripts **c** indicate significant difference when compared with **a**(*p<0.05)*.

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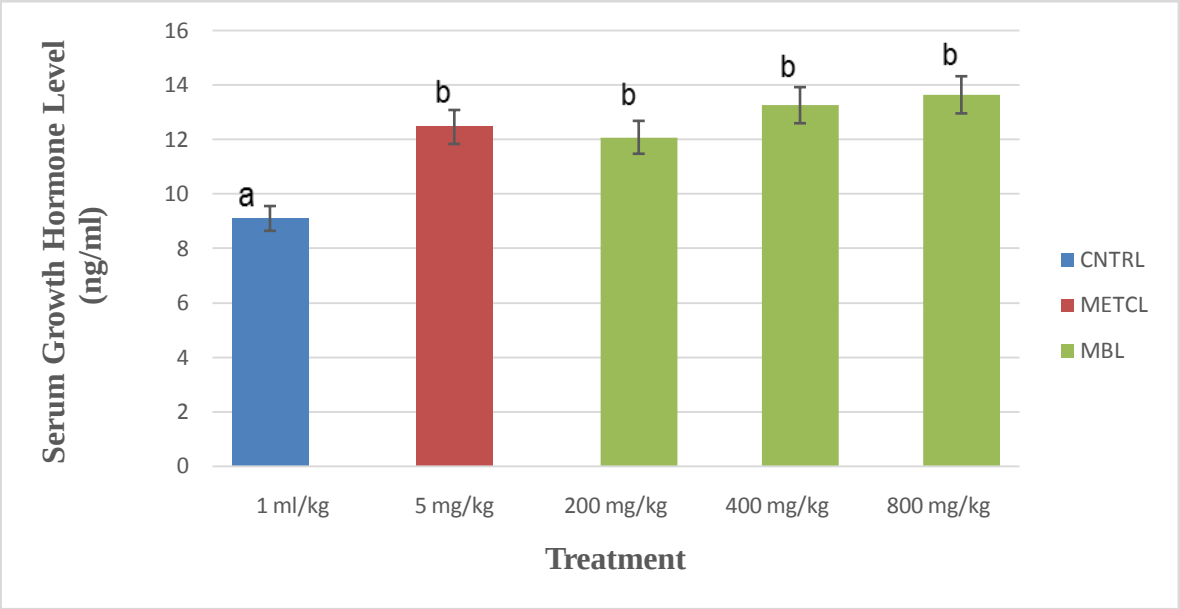


Figure 4.4: Serum growth hormone levels in lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.;Superscripts **b** indicate significant difference when compared with **a***(p<0.05)*.

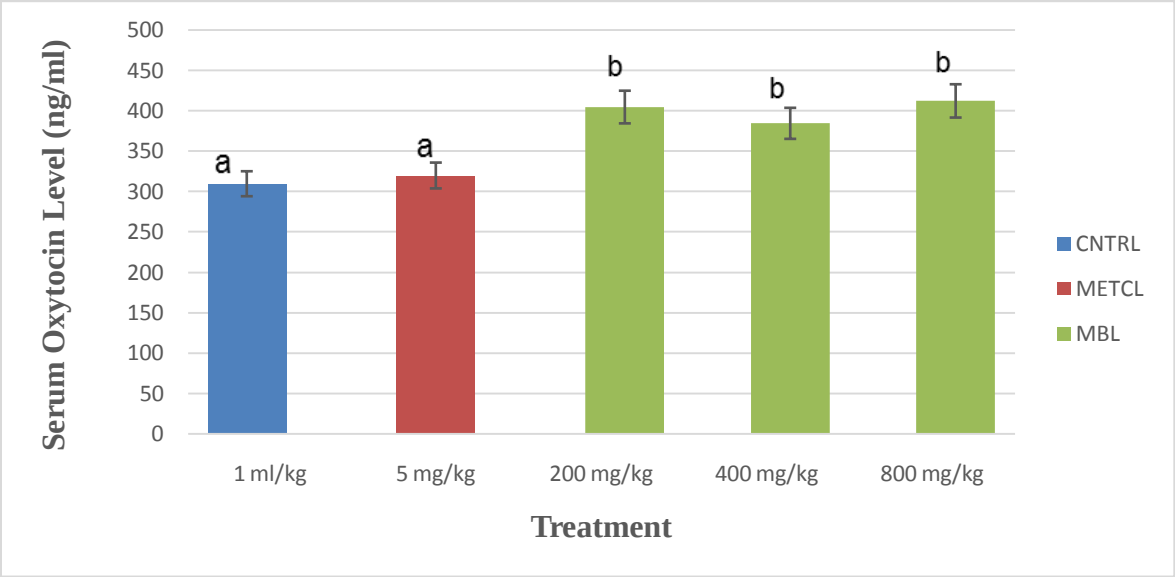


Figure 4.5: Serum oxytocin levels in lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a**(*p<0.05)*.

4.5 Effect of Aqueous Extract of *Momordica balsamina* L.on Oxidative Biomarkers Lipid Peroxidation in Lactating Wistar Rats

There was significantly higher*(p<0.05)* lipid peroxidation as indicated by the serum malondialdehyde (MDA) comcentration (conc.) in all the treated groups when compared to the control group as follows: Metoclopramide treated (1.40 ± 0.05 vs 1.14 ± 0.04) and (1.40 ± 0.05 vs 1.14 ± 0.05, 1.62 ± 0.04 vs 1.14 ± 0.05, 1.82 ± 0.06 vs 1.14 ± 0.05) for MBL 200, 400 and 800 mg/kg respectively (Figure 4.6).

The result showed a significantly *(p<0.05)* lower serum SOD activity in MBL 400 and MBL 800 mg/kg treated groups when compared to the control (1.48 ± 0.08 vs 2.16 ± 0.10) and (1.54 ± 0.07 vs 2.16 ± 0.10), respectively (Figure 4.7).

No significant difference was observed in the activity of serum GPx between the control (48.60 ± 2.20) and the MBL treated groups: 400 and 200 mg/kg (49.60 ± 1.44; 48.60 ± 1.20). However, serum Gpx activity was significantly*(p<0.05)* lower in the MBL 800 mg/kg treated group when compared to the control treated group (44.20 ± 1.90 vs 48.60 ± 2.20) (Figure 4.8).

The serum catalase activitywas significantly lower *(p<0.05)* in MBL 800 mg/kg when compared to control (40.80 ± 1.24 vs 50.40 ± 1.17), metoclopramide (40.80 ± 1.24 vs 46.40 ± 1.72) and MBL 200 mg\kg (40.80 ± 1.24 vs 46.60 ± 0.92) respectively (Figure 4.9).

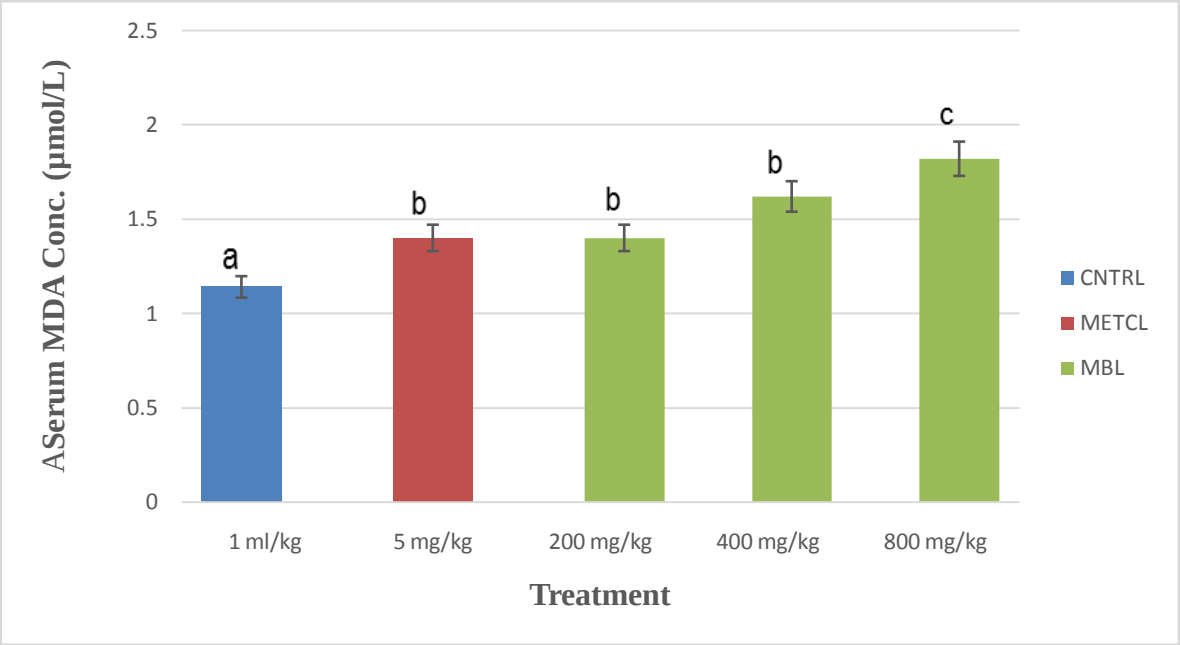


Figure 4.6: Malondialdehyde (MDA) concentration (conc.) in the serum of lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.;Superscripts **b** indicate significant difference when compared with **a.** Superscripts **c** indicate significant difference when compared with **b***(p<0.05)*.

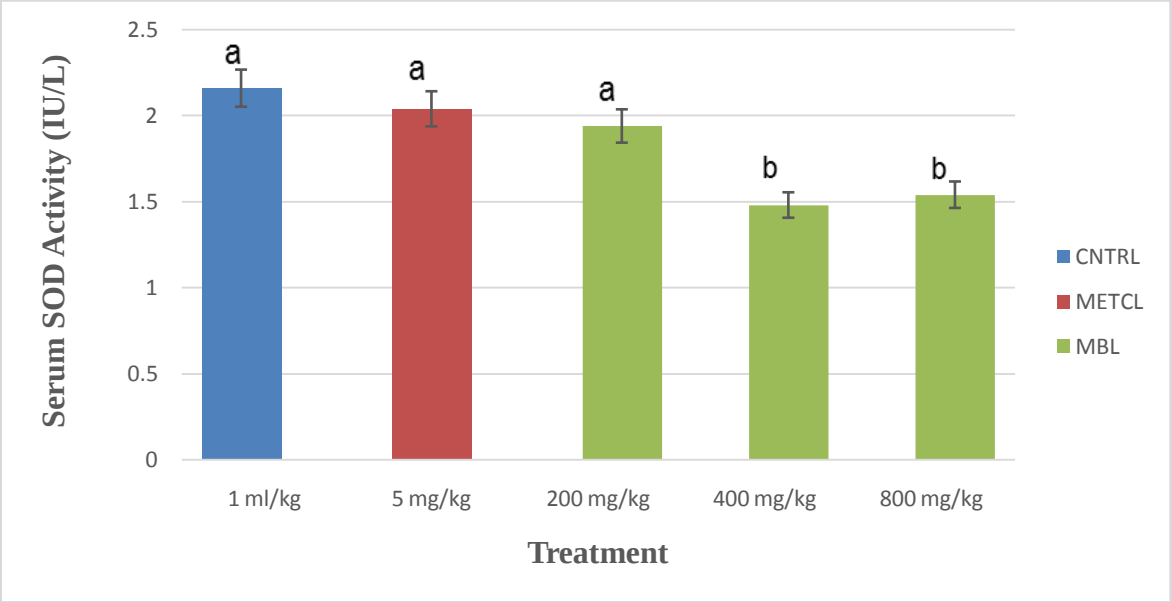


Figure 4.7: Superoxide dismutase (SOD) activity in the serum of lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a***(p<0.05)*.

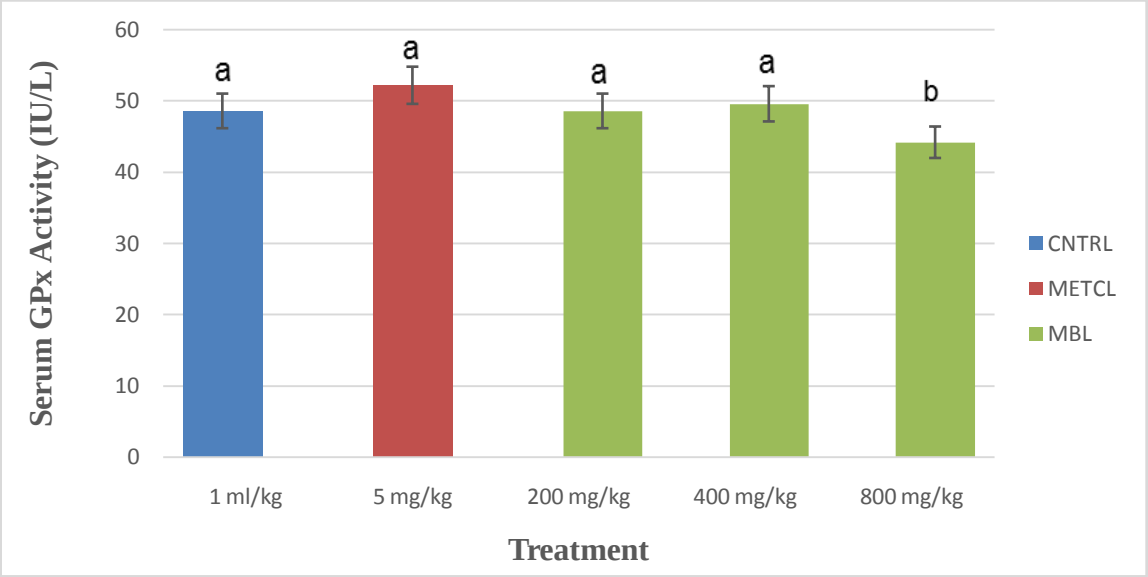


Figure 4.8: Glutathione peroxidase (GPx) activity in the serum of lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a***(p<0.05)*.

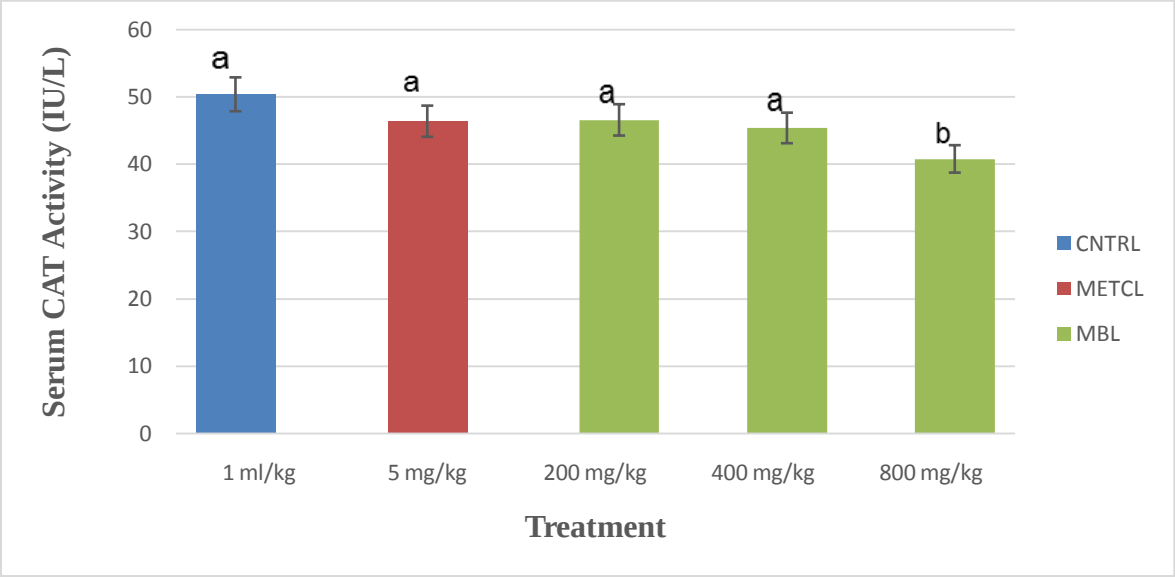


Figure 4.9: Catalase (CAT) activity in the serum of lactating Wistar rats treated with metoclopramide and *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a**(*p<0.05)*.

4.6 Effect of Aqueous Extract of *Momordica balsamina* L.on Liver Enzymes in Lactating Wistar Rats.

There was a significantly higher*(p<0.05)*serum levels of alanine amino tranferase(ALT) in the treated groups when compared to the control as follows: Metoclopramide group (73.40 ± 1.78 vs 62.40 ± 1.22) and (76.60 ± 2.20 vs 62.40 ± 1.22; 97.00 ± 2.88 vs 62.40 ± 1.22; 127.00 ± 4.30 vs 62.40 ± 1.22) for MBL 200, 400 and 800 mg/kg respectively (Figure 4.10). The relatively higher level of serum ALT in the treatment groups was dose-dependent.

The serum aspartate amino transferase (AST) levels in the 400 mg/kg (89.40 ± 3.37) and 800 mg/kg (119.00 ± 1.14) treated groups being significantly higher*(p<0.05)* compared to the control group (60.40 ± 0.68) (Figure 4.11).

The serum alkaline phosphatase (ALP) levels being significantly higher *(p<0.05)* in the all the treated groups; 200 mg/kg (128.20 ± 3.09), 400 mg/kg (132.40 ± 2.16), 800 mg/kg (140.40 ± 1.99) and metoclopramide (129.60 ± 3.44) when compared with the control group (90.20 ± 2.80) (Figure 4.12).

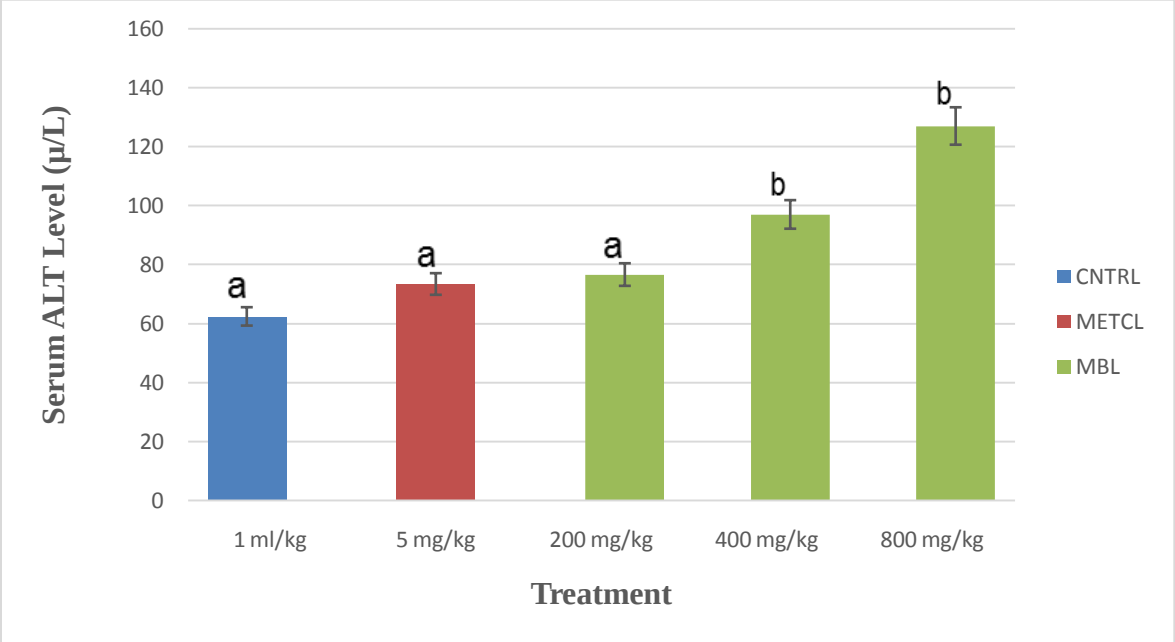


Figure 4.10: Serum alanine amino tranferase (ALT) levels in lactating Wistar rats treated with *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a***(p<0.05)*.

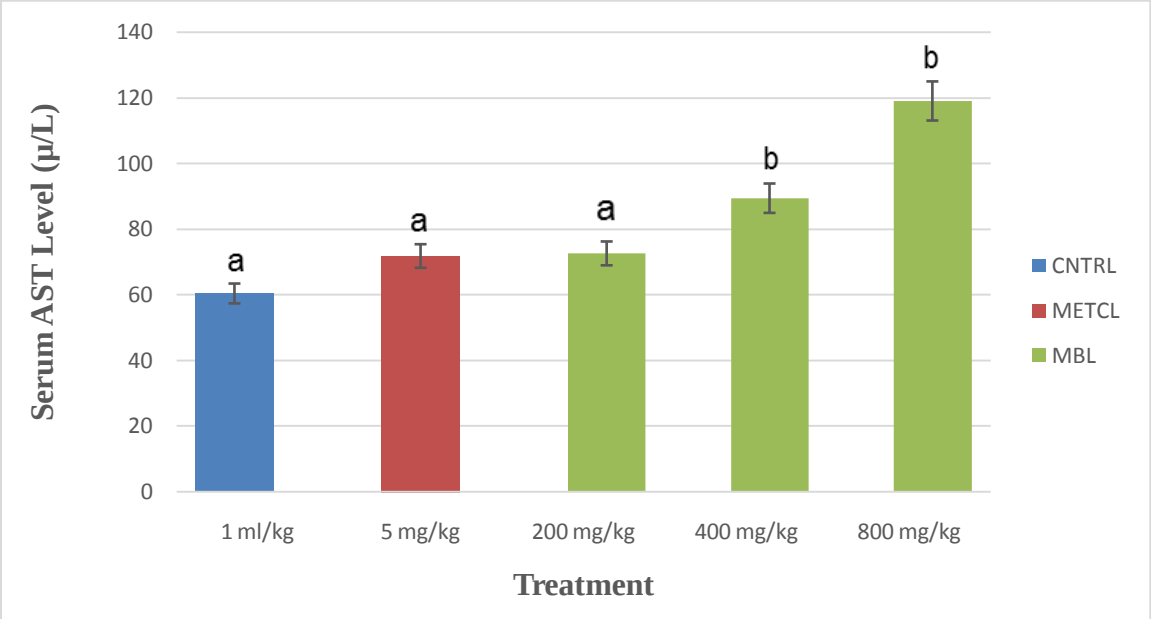


Figure 4.11: Serum aspartate amino transferase (AST) levels in lactating Wistar rats treated with *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a**(*p<0.05)*.

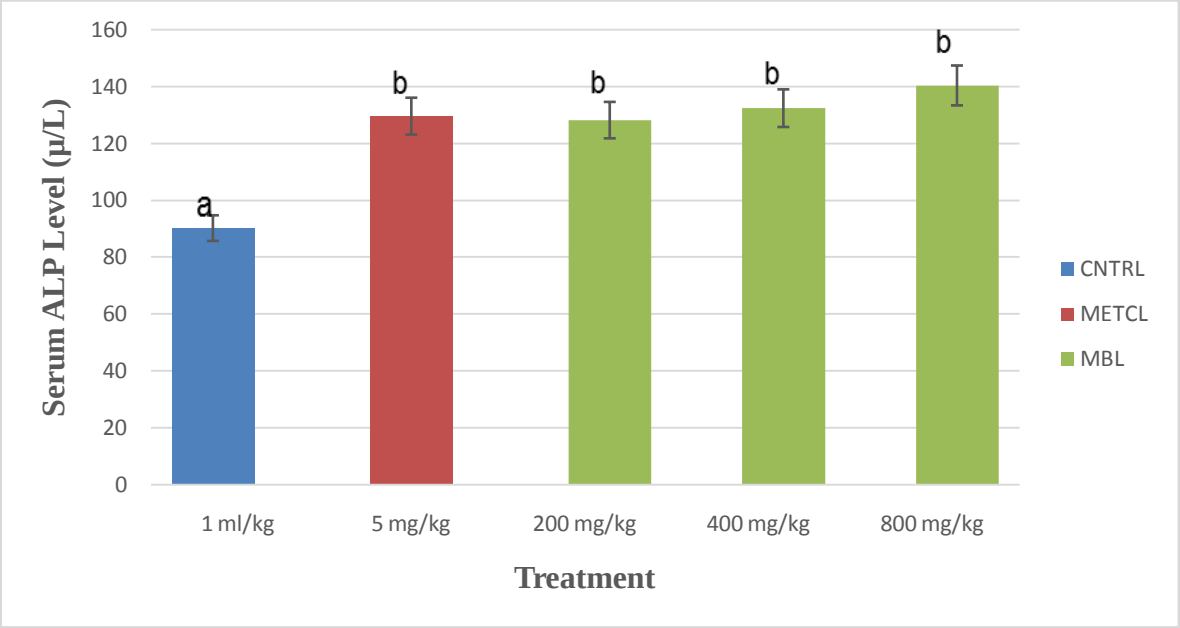


Figure 4.12: Serum alkaline phosphatase (ALP) levels in lactating Wistar rats treated with *Momordica balsamina* L.; Superscripts **b** indicate significant difference when compared with **a**(*$p<0.05$*).

4.7 Effect of Aqueous Extract of *Momordica balsamina* L.on the Histology of the Mammary gland and Liver.

A. Plate I: Photomicrograph section of the mammary tissue of control group rats showing large lobules with relatively small interlobular connective tissues.

B. Plate II: Photomicrograph section of the mammary tissue of metoclopramide (5 mg/kg) treated rats showing a similar breast cytoarchitecture with control rats; but with a slightly more alveoli distention.

C. Plate III: Photomicrograph section of the mammary tissue of MBL (200 mg/kg) treated rats showing a similar breast cytoarchitecture with control rats; but with a slightly more alveoli distention.

D. Plate IV: Photomicrograph section of the mammary tissue of MBL (400 mg/kg) treated rats showing enlarge and dilated acini, few containing amorphose eosinophilic secretion, with alveoli distention indicating the presence of milk was observed.

E. Plate V: Photomicrograph section of the mammary tissue of MBL (800 mg/kg) treated rats showing the breast cytoarchitecture like connective tissue (CN), Adipocyte (AD), and large lobules (ID).

A. Plate VI: Photomicrograph section of the liver tissue of control group rats showing normal central vein, hepatocellular integrity, kupfer cells and sinusoidal dilations.

B. Plate VII: Photomicrograph section of the liver tissue of metoclopramide (5 mg/kg) treated rats showing normal Central Vein (CV), Hepatocyte (H), with scanty microvascular fatty droplet.

C. Plate VIII: Photomicrograph section of the liver tissue of MBL (200 mg/kg) treated rats showing normal Central Vein (CV), Hepatocyte (H) similar with that of the control group with some dilated sinusoid (HS).

D. Plate IX: Photomicrograph section of the liver tissue of MBL (400 mg/kg) treated rats showing normal Central Vein (CV), Hepatocyte (H) similar with that of the control group with some dilated sinusoid (HS).

E. Plate X: Photomicrograph section of the liver tissue of MBL (800 mg/kg) treated rats showing the Portal Triad (P), Macrovscula fatty droplet (MD) and bi-nucleated Hepatocyte.

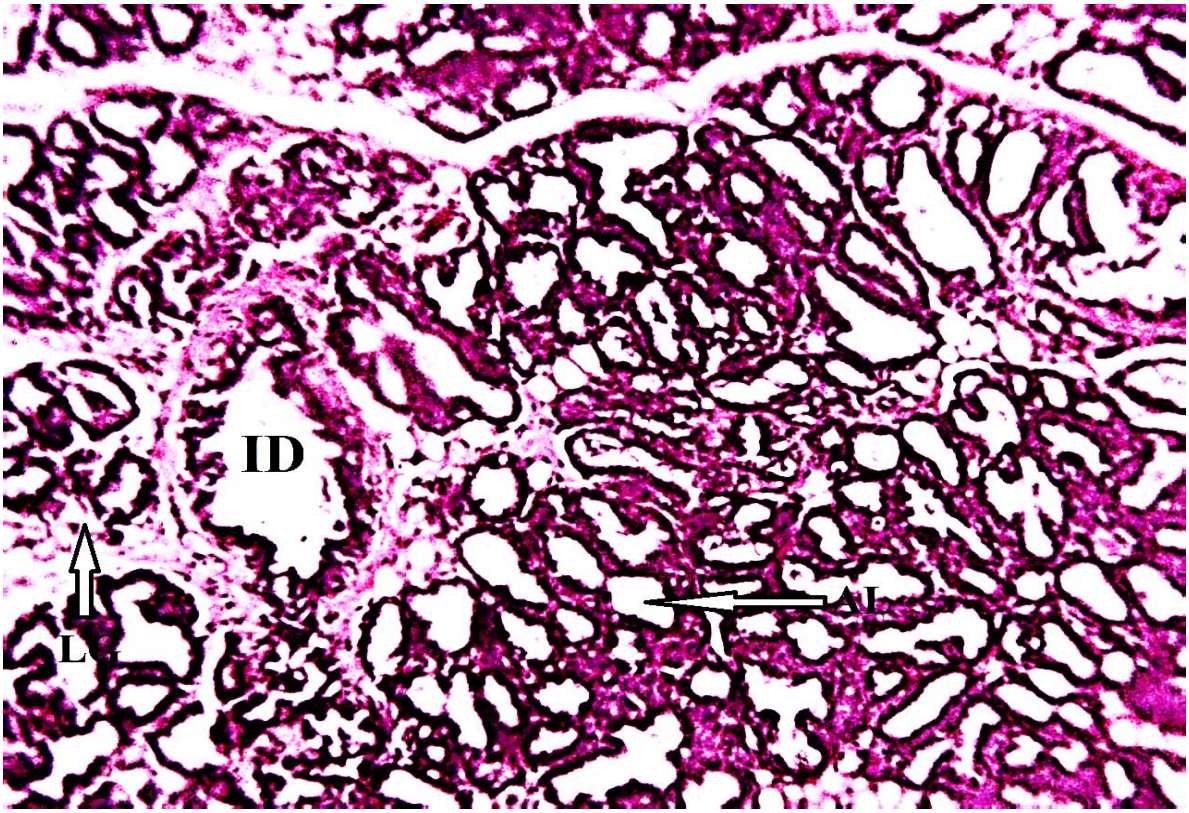


Plate I: A micrograph of mammary tissue of control rats showing the large lobules (ID) and a relative small interlobular connective tissue (L). (H and E Stain, X100).

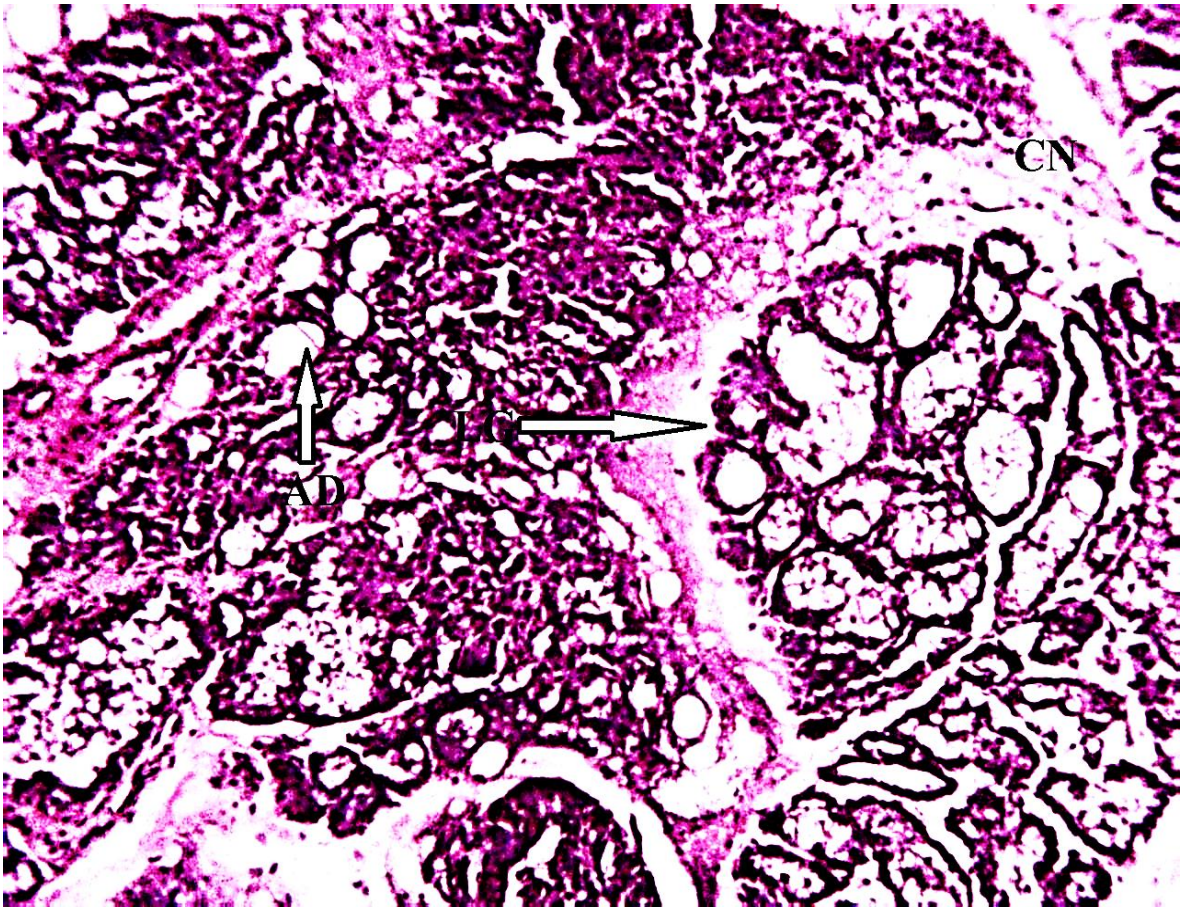


Plate II: A micrograph of mammary tissue of rats treated with metoclopramide (5mg/kg) showing the normal breast cytoarchitecture similar with that of the control group (H and E stain, X 100).

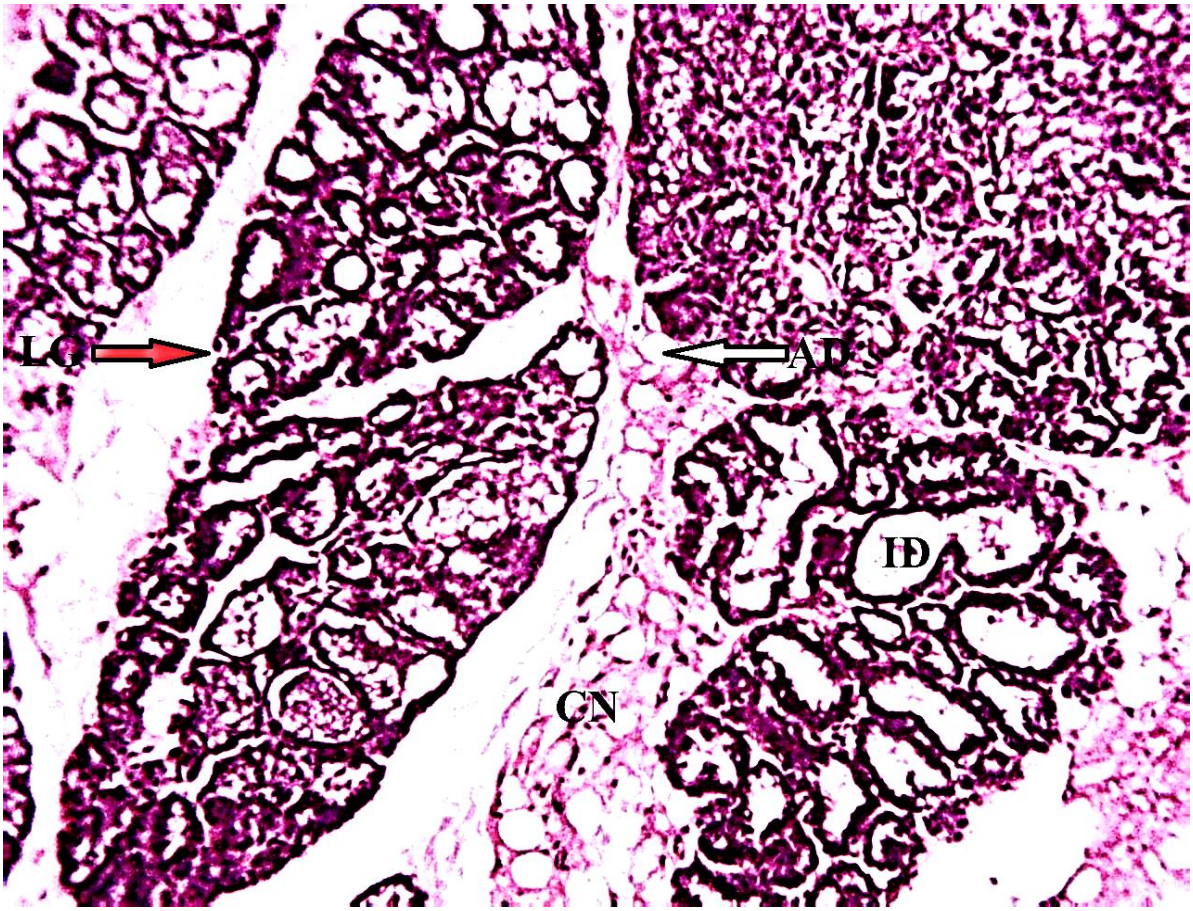


Plate III: A micrograph of mammary tissue of rats treated with *Momordica balsamina* L. (200 mg/kg) showing the normal breast cytoarchitecture similar with that of the control group (H and E stain, X 100).

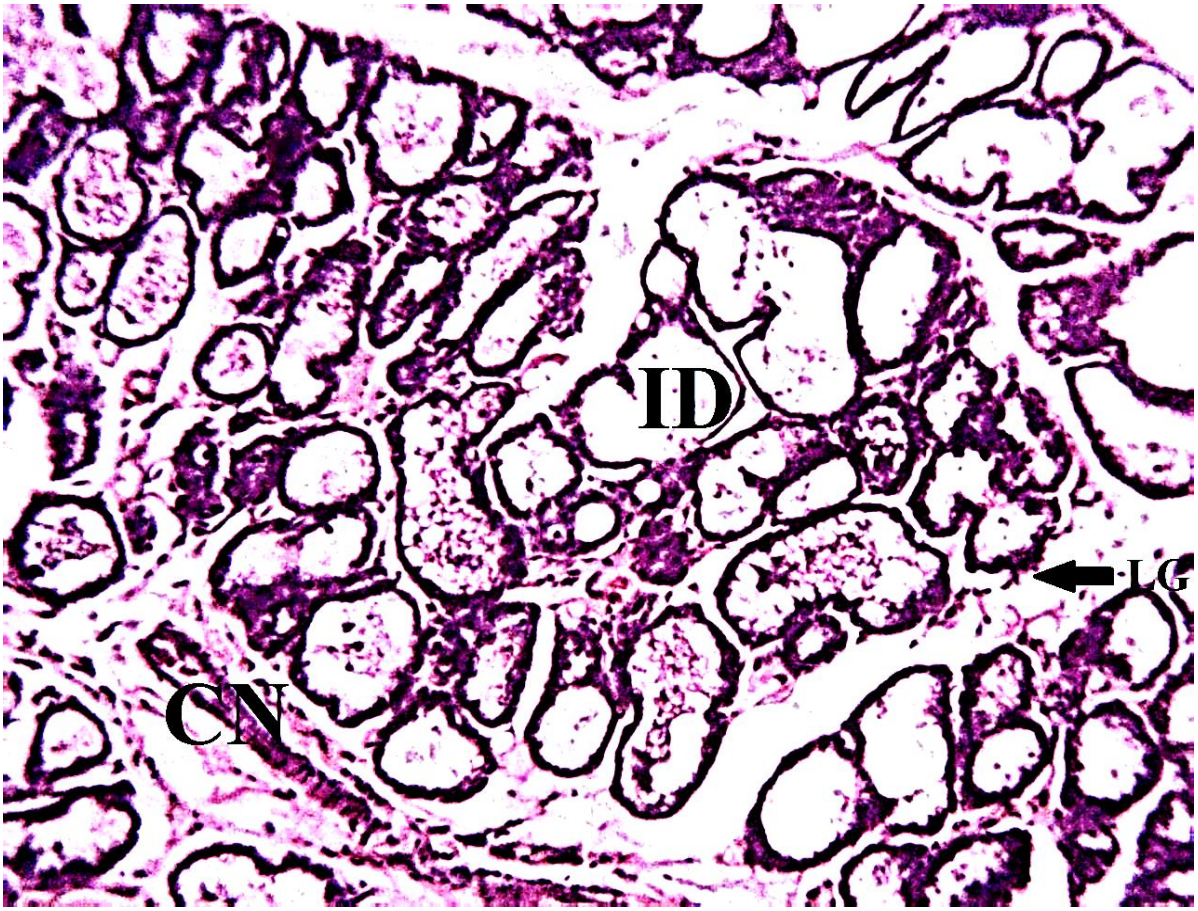


Plate IV: A micrograph of mammary tissue of rats treated with *Momordica balsamina* L. (400 mg/kg) showing enlarge and dilated acini, few containing amorphose eosinophilic secretion (H and E stain, X 100).

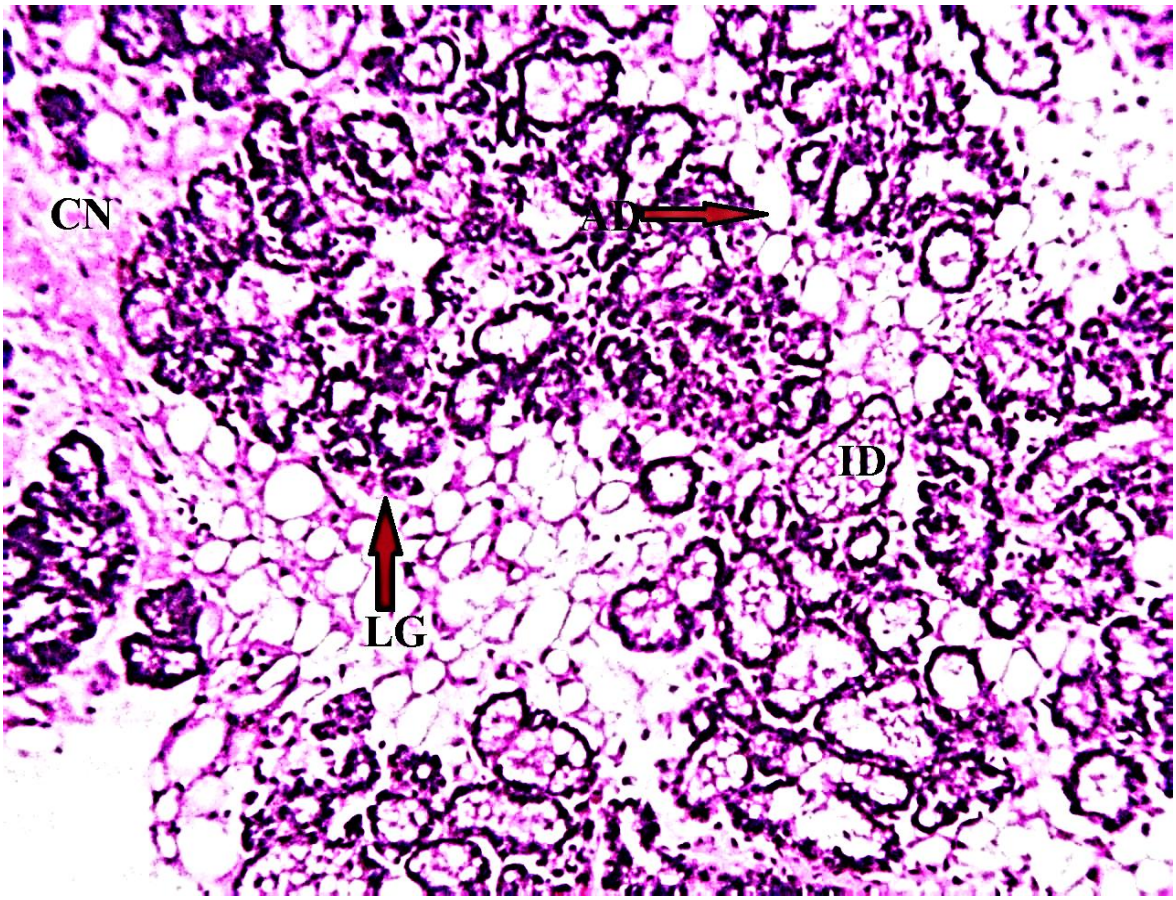


Plate V: A micrograph of mammary tissue of rats treated with *Momordica balsamina* L. (800 mg/kg) showing the breast cytoarchitecture like connective tissue (CN), adipocyte (AD), and large lobules (ID) (H and E stain, X 100).

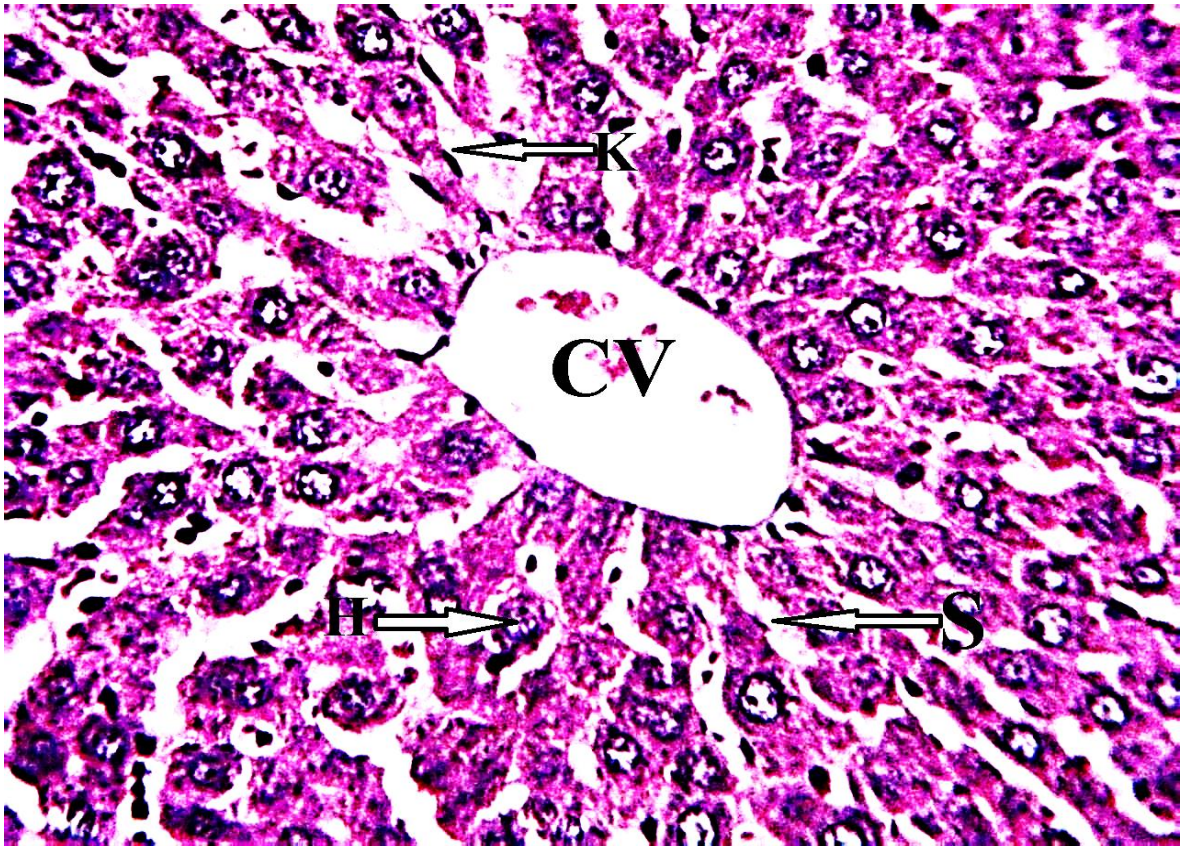


Plate VI: Amicrograph of liver of control rats showing normal central vein (CV), hepatocyte (H), kupffer cells (K) and sinusoid (S) (H and E Stain, X250).

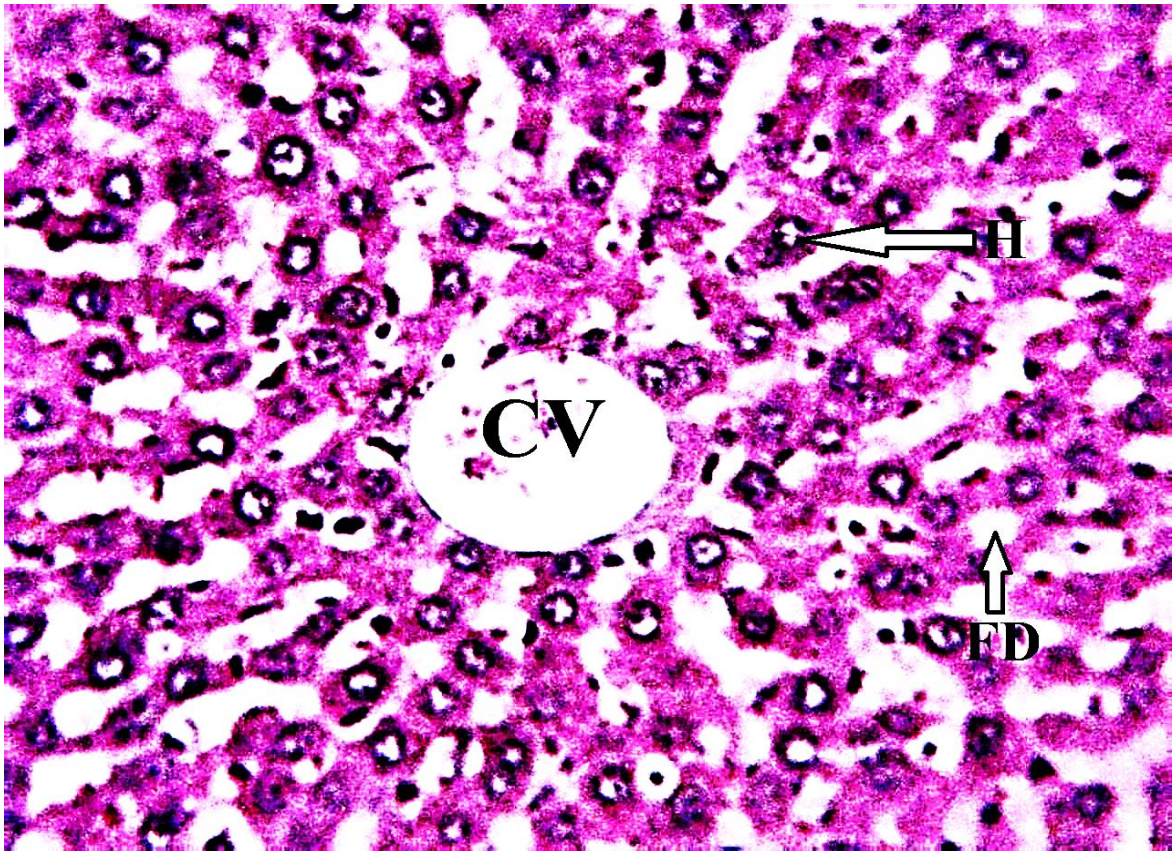


Plate VII: A micrograph of liver of rats treated with metoclopramide (5mg/kg) showing normal central vein (CV), hepatocyte (H), with scanty microvascular fatty droplet. (H and E Stain, X250).

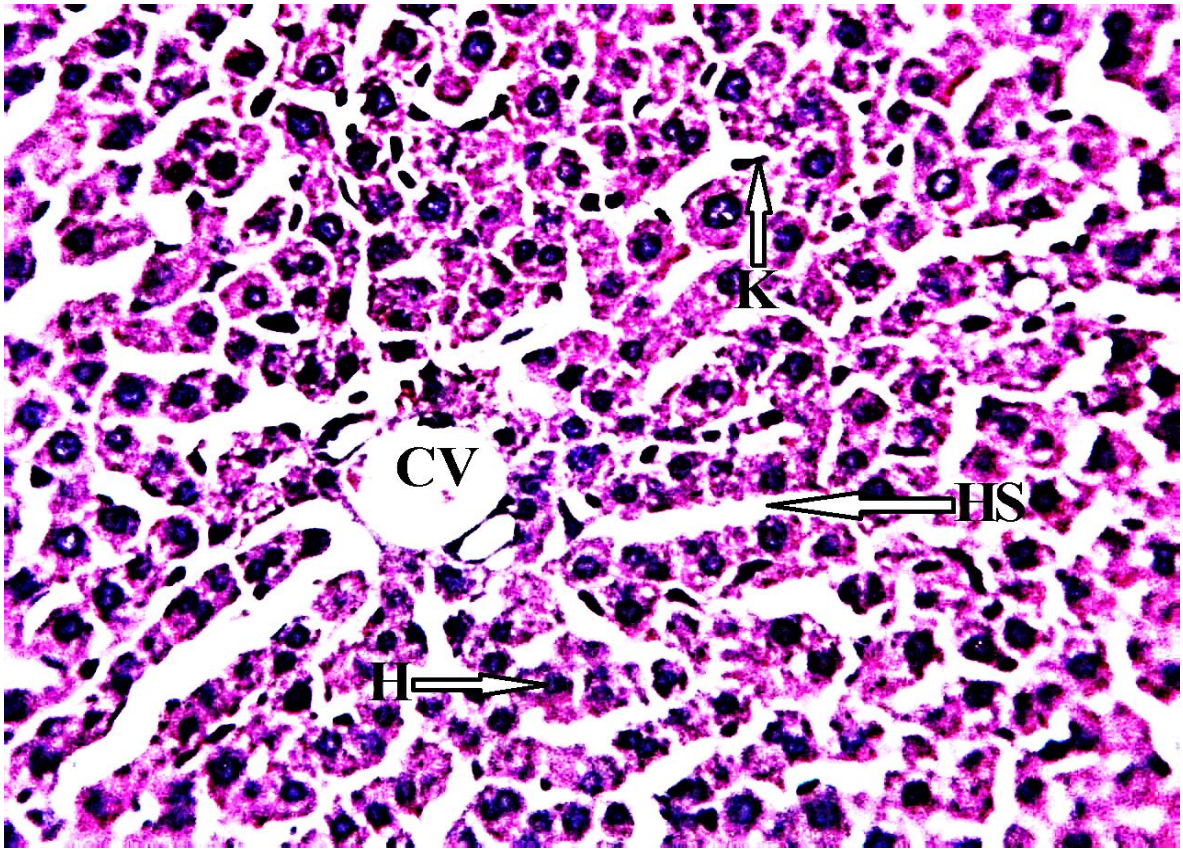


Plate VIII: A micrograph of liver of rats treated with *Momordica balsamina* L. (200 mg/kg) showing normal central vein (CV), hepatocyte (H) similar with that of the control group with some dilated sinusoid (HS). (H and E Stain, X250).

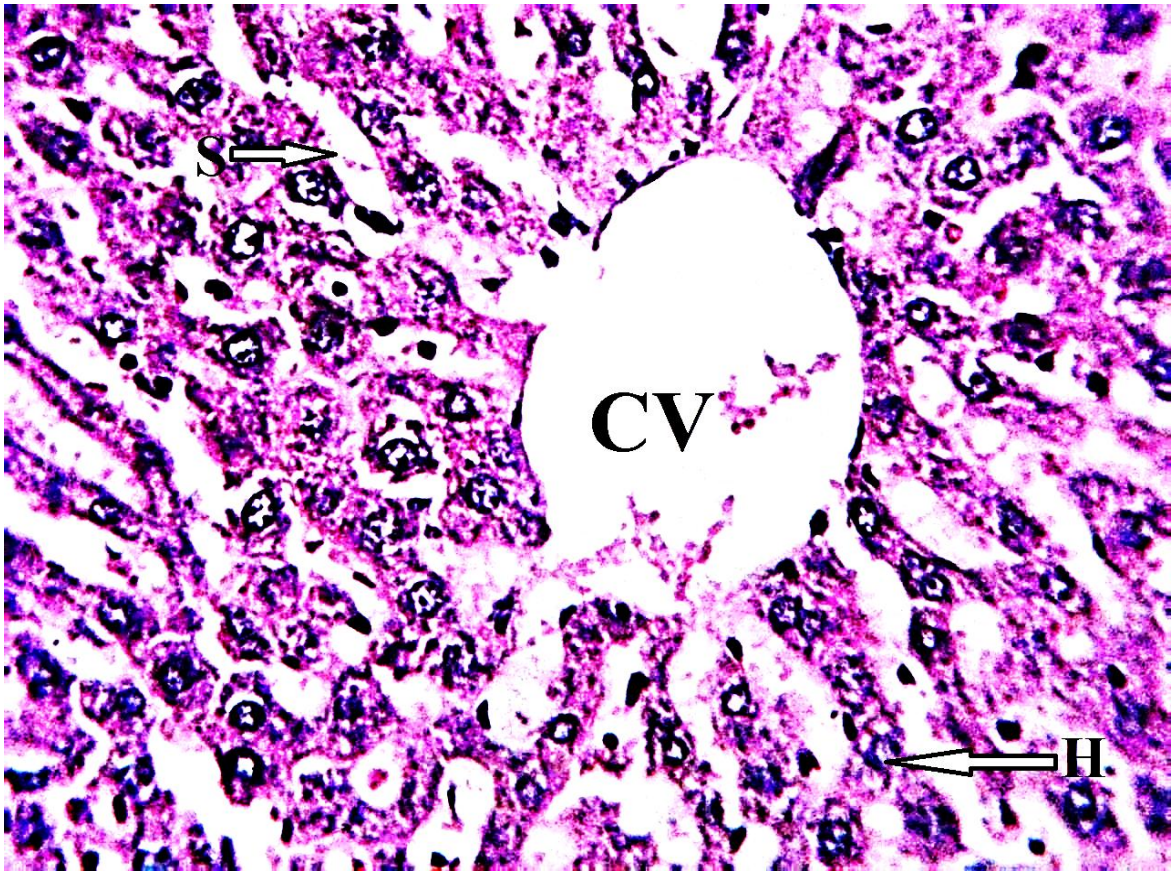


Plate IX: A micrograph of liver of rats treated with *Momordica balsamina* L. (400 mg/kg) showing normal central Vein (CV), hepatocyte (H) similar with that of the control group with some dilated sinusoid (HS). (H and E Stain, X250).

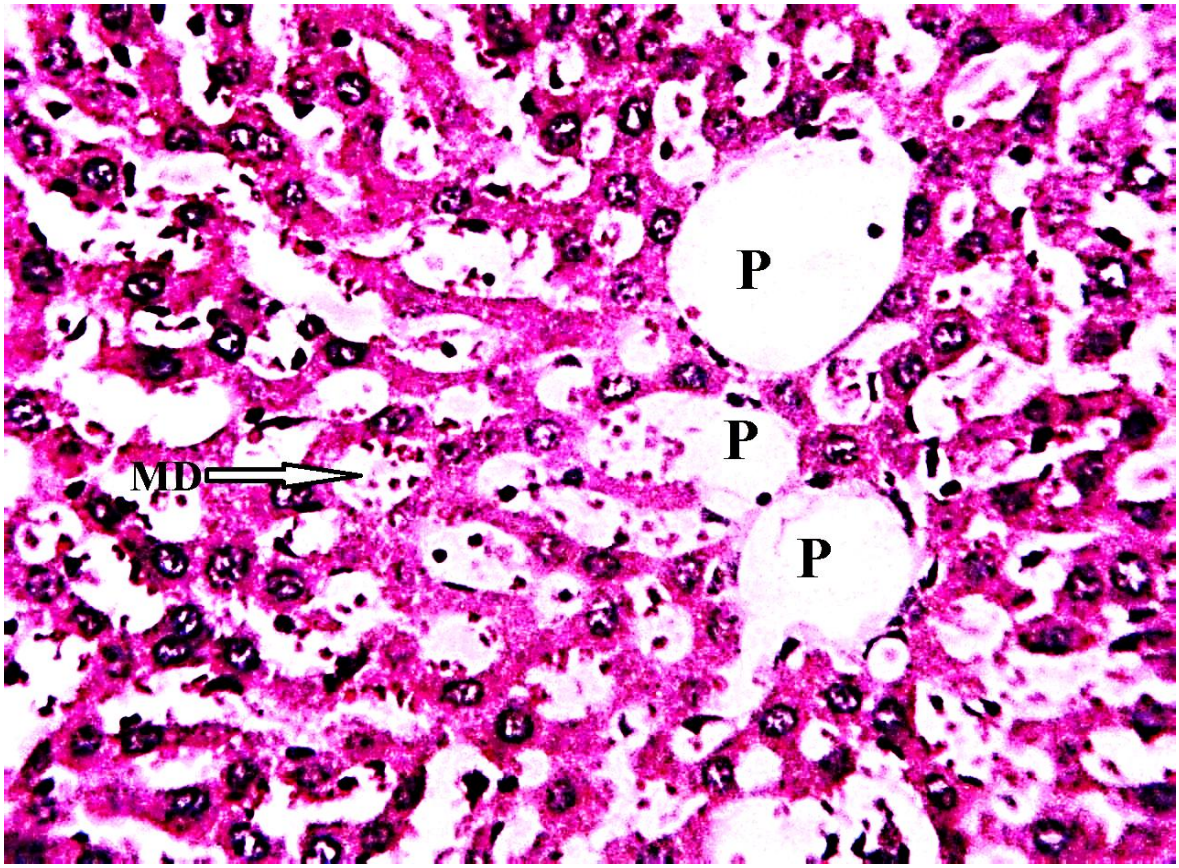


Plate X: A micrograph of liver of rats treated with *Momordica balsamina* L. (800 mg/kg) showing the portal Triad (P), macrovscular fatty droplet (MD) and bi-nucleated hepatocyte. (H and E Stain, X250).

CHAPTER FIVE

5.0 DISCUSSION

Herbs have been used since the beginning of time to aid in many different ailments. Milk production has been enhanced and even corrected by the use of certain herbs (Gabay, 2002; Micheal, 2008). *Momordica balsamina* L. is one of the widely used plantsfor the treatment of hypogalactia in Northern Nigeria.

5.1 Acute Toxicity Study of Aqueous Extract of *Momordica balsamina* L.

The result of acute toxicity study showed that the aqueous extract of MBL is relatively safe above 5000mg/kg.Although there was a little sign of toxicity but no mortality recorded in all phases**.** This is not surprising because the plant has been consumed as food by man and other animals for ages without any deleterious effect (Hassan and Umar, 2006). This conforms with findings of Bako *et al.*,(2013) who reported that the $LD_{50}$of ethyl-acetate fraction of *Hibiscus sabdariffa* L., another galactogenic plant was found to be above 5000 mg/kg.

5.2 Preliminary Phytochemicals Screening ofAqueousExtract of *Momordica balsamina*L.

The presence of flavonoids, tannins, saponins, alkaloids, cardiac glycosides and carbohydrate in the aqueous extract of MBLagrees with the findings of Akinniyi *et al.*,(1986) and Bot *et al.*,(2007). Due to the fact that most polar compounds will dissolve in a polar solvent of extraction (Mahmood *et al.*,2012),it can then be concluded that the compounds contain in the aqueous extract of MBL are polar compounds. The presence of saponins and tannins in the aqueous extract of MBL indicates that atleast one or more of these polar compounds have galactopoietic effect.

5.3 Lactogenic Effects of Aqueous Extract of *Momordica balsamina* L. on Milk Yield

From this study it was observed that MBL 400 mg/kg group showed a significant increase in milk yield when compared with the control group. This might be due to the significantly higher GH and oxytocin levels as well as the increased in growth and function of the mammary tissue observed in this group. However, there was no significant difference in serum prolactin level between the MBL 400 mg/kg and control. This finding conform with the findings of Simelane *et al.*,(2012) who showed that aqueous extract of *Guinnera perpensa* L. has lactogenic effect but did not significantly influence the levels of prolactin. Growth hormone has been shown to maintain both the structural integrity and functional activity of the mammary epithelium during lactation in rodents (Tucker, 2000; Akers, 2006). Oxytocin causes the contraction of myoepithelial cells for milk ejection during suckling (Love, 2014).

In the daily milk yield; the gradual day by day increase in milk production at 400 mg/kg could be due to long term effect of growth hormone in causing the development of mammary tissue. It takes longer period of time for growth hormoneto enhancedevelopment and proliferation of mammary tissue. Therefore, prolactin produces fast effect on milk yield than growth hormone.Metoclopramide started acting with agreat momentum from day 1 due to it effects on prolactin secretion. Highest daily milk yield was observed on day 9 of 400 mg/kg MBL treated group. This is in conformity with the findings of Al-Saady *et al.*, (1997); Al-Saidi *et al.*, (2006); Simelane *et al.*, (2012); Mahmood *et al.*, (2012) and Badgujar and Bandivdekar, (2015) who identified that development of lobular alveolar cells and increase in growth and function of the mammary gland enhance lactation. In contrast however to Lompo-Ouedraogo*et al.*, (2004); Okasha *et al.*, (2008); Sahoo *et al.*,

(2016), studies demonstrateslactogenic activity of some plant extracts by increasing prolactin level in the lactating Wistar rat.

In the present study, the histology of the mammary gland of the MBL treatment group showed larger lobules with alveoli distention indicating the presence of milk with a more profound activity seen at higher doses of MBL 400 mg/kg and 800 mg/kg, repectively. This results could have been due to the effect of the increased activity of serum GH in the treated groups when compared to the control group, resulting in increased in milk yield. This result agrees with that of Cai *et al*. (2017), who reported that the distension of alveoli and mitotic presence as a pro-factor for increased secretory activity and milk synthesis of mammary epithelial cells. The obvious minor distortion in the cytoarchitecture of the mammary gland and lower milk production at MBL 800 mg/kg, might be due to mild toxic effect of the MBL on various organs of the body at higher dose (MBL 800 mg/kg), (Shettima *et al.*,2001).

5.4Effects of Aqueous Extract of *Momordica balsamina* L. on Serum Level of Prolactin, Growth Hormone and Oxytocin.

The lower serum level of prolactin at higher doses of MBL extracts might be related to dopamine receptor activation. Through adirect effect on anterior pituitary lactotrophs, dopamine inhibits basal secretory tone of the cell. Itaccomplishes this by binding to D2 receptors expressed on the lactotroph cell membrane, which causes areduction in prolactin hormone exocytosis and gene expression by inhibiting cAMP/PKA signaling through Gi-mediatedinactivation of adenylyl cyclase (Binart, 2016). This result could infer that MBL at a higher dosage has inhibitory activity on lactotrophic cells of the pituitary gland, hence the decrease in serum prolactin level. It may also be due to a possible excitatory activity on the

doperminergic cells along the tubero-infundibular axis resulting in an antagonistic activity of dopamine on the lactotrophic cells of the hypophysis (Freeman *et al.,* 2000).This indicatethat a higher concentration of MBL extract lowered the level of prolactin secretion. This result is in concordance with work of Simelane *et al.*,(2012). However, it is contrary to the findings of Ushiroyama *et al.*,(2007); Okasha *et al.*,(2008); Luangpirom *et al.*,(2013) and Gaya *et al.*,(2014) who reported a dose-dependent lactogenic activity of some plant extracts by increasing prolactin level.

The increase in serum growth hormone level was found to be dose dependent. This may be due to a possible agonist activity of MBL on 5HT (5-hydroxy tryptamine), resulting in increased GH secretion from pituitary somatotrophs; or it could be due to an excitatory effect of MBL on somatotropic cells of the adenohypophysis (Musumeci *et al*., 2013).

The serum level oxytocin hormone was greater in all the MBL treatment group. This might be due to a stimulatory effect of the MBL on synthesis of oxytocin from the hypothalamus or its secretion from the posterior pituitary gland. This is in conformity with the findings of Soka *et al.*,(2011). However, the significant increase in serum oxytocin level did not translate into a significant increase in milk production, at high dose (800 mg/kg) in this study. This could be due to the fatty infiltration of the mammary tissue or excessive lipid peroxidation observed at higher doses of MBL in this study.

5.5 Effects of Aqueous Extract of *Momordica balsamina* L. on Oxidative Stress Biomarkers and Lipid Peroxidation

Aqueous extract of MBL in this study caused higher degree of lipid peroxidation in lactating wistar rats, as indicated by a significantly higher serum concentration of malondialdehyde (MDA).One of the most oftenused biomarker of oxidative damage on

lipidcell membrane bilayer is MDA, a major lipid peroxidation product (Hussein *et al.*, 2014; Sanilkumar and Muthu, 2013). The MDA as a marker of lipid peroxidation occurs in areas where polyunsaturated fatty acids side chains are prevalent. These chains react with O_2 creating the peroxyl radical, which can obtain H^+ from another fatty acid, creating a continuous chain reaction (Ning-ning, 2015).

The aqueous extract of MBL at 800 mg/kgshowed lower antioxidant enzymes activity: superoxide dismutase (SOD),glutathione peroxidase (GPx)and catalase (CAT) in lactating animals. This may suggest a pro-oxidant activity of MBL at higher doses, where it could cause lipid peroxidation as well asdecreased antioxidant enzymes activity in lactating animals. The relatively lower SOD, GPx and CAT at the 800 mg/kg, may also suggest an increased utilization of the antioxidant enzymes to ameliorate oxidative stressthat leads to depletion of the enzymes as reported by Ibtissem *et al.*, (2017).Therefore MBL at 800 mg/kg tends to has both less antioxidant property and less lactogenic effects. The possibility ofpro-oxidant activity of MBL at higher doses could be due to the fact that it contains a bitter principle, momordicin (Watt and Breyer-Brand wijk, 1962); and possession of insecticidal property (Dalziel, 1958). However, Van wyk and Gericke, (2000) and Akula and Odhav, (2008)had reported an antioxidant effect of MBL in Wistar rat.

5.6 Effects of Aqueous Extract of *Momordica balsamina* L. on Liver Enzymes

The aqueous extract of *Momordica balsamina* L. showed a potential for hepatotoxicity at higher doses in this study. It increases serum liver enzymes, which is indicative of liver damage (Feroz Khan *et al.*, 2009; Roy*et al.*, 2009 Reddy *et al.*, 2017). This finding is in harmony with earlier researches where it was reported that some herbal medicinal products are associated with a spectrum of hepatotoxicity events. Increased activity of liver enzymes

are suggestive of the degree and type of liver damage (Edoardo, 2005; Taheri *et al.*, 2012). The hepatotoxic effect of the MBLwas dose dependent with higher liver enzymes level at 800 mg/kg. The effect of MBL on liver damage could be due it bitter constituents,as it contains momordin (Dalziel, 1958; Bokhari and Ahmed, 1980).This might causefree radicals damage to cell membranes of the hepatocytes, which cause the liver enzymes which are normally located inside the cell cytosol to leak into systemic blood circulation (Azim*et al.,* 2017). This observation is in concordance with the findings of Shettima *et al.*, (2001) who reported that the leaves and fruits of MBL extract,produce hepatotoxicity and also induce toxicity in various organs and tissues in Wistar rats at high doses.However, it is contrary to the findings of Alqasoumi and Al-Dosari (2009); who showed that ethanolic extract ofMBL have protective effects for both biochemical markers assessment and histological appearance of hepatocytes.However, the diference between the mild hepatic damage observed in our study and the findings of the former authors may be due to differences on the dose of MBL used.

The histopathological picture of the organs can corroborate with the biochemical changes accounting for the functional disruptions in the activity of the organs due to cellular damage (Nayak *et al*., 1996). In this study the MBL treatment groups showed progressive hepatocellular damage with increase in dose at 200 mg/kg, 400 mg/kg and 800 mg/kg, respectively. This finding is in agreement with the findings of Shetima *et al*.,(2001). And contratry to the findings of Alqasoumi and Al-Dosari (2009) who reported ameliorative effect of MBL extract cadmium induced liver damage.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

This study was designed to evaluate the effectof *Momordica balsamina* L. on milk yield, lactogenic hormones and oxidative stress parameters in lactating Wistar rats.The findings of the study were summarized as follows:

i. The acute toxicity study of the MBL extract showed that the extract is safe for consumption with LD_{50} above 5000 mg/kg.

ii. *Momordica balsamina* L. given orally at 400 mg/kg significantly increased milk yield at 18 hours after gavage. Mean milk yield for the period of 10 days per dam was found to be 1.44 ± 0.20 g/pups/day (1.5 ml)*($p< 0.05$)*. Mean milk yield per pup per day at 18 hours after gavage is 0.36 ± 0.12 g/pup/day (0.4 ml). The highest daily milk yield per dam is 3.00 ± 0.45g (3.1 ml)which occurred on day 9.

iii. *Momordica balsamina* L. at 800 mg/kg MBL decreased serum prolactin levels compared to control rats (28.66 ± 1.60 ng/ml vs 38.06 ± 2.04 ng/ml, *$p<0.05$)*.

iv. However, aqueous extract of MBL at 200 mg/kg, 400 mg/kg and 800 mg/kg increased serum growth hormone levels compared to control rats (12.08 ± 0.20 ng/ml, 13.26 ± 0.32 ng/ml and 13.64 ± 0.80 ng/ml vs 9.10 ± 0.33, *$p<0.05$*).

v. *Momordica balsamina* L. at 200 mg/kg, 400 mg/kg and 800 mg/kg increased serum oxytocin levels compared to control rats (404.80 ± 1.71 ng/ml, 384.60 ± 6.30 ng/ml and 412.40 ± 7.67 ng/ml vs 309.80 ± 8.54, *$p<0.05$*).

vi. Therewas a significant (*$p<0.05$*) dose dependent increase in serum MDAlevels among the treatment groups and control rats(1.14 ± 0.04 µmol/L, 1.40 ± 0.05

µmol/L, 1.40 ± 0.05 µmol/L, 1.62 ± 0.04 µmol/L, 1.82 ± 0.06 µmol/L; respectively for control, MBL 200 mg/kg, MBL 400 mg/kg and MBL 800 mg/kg groups.

vii. *Momordica balsamina* L. at 400 mg/kg and 800 mg/kg caused a significant decrease in serum SODlevels compared to control rats (1.48 ± 0.08 IU/L, 1.54 ± 0.07 IU/L vs 2.16 ±0.07 IU/L, $p<0.05$). While MBL at 800 mg/kg caused a significant decrease in serum levels of GPX (44.20 ± 1.90 IU/L vs 48.60 ± 2.20 IU/L)and CAT (40.80 ± 1.24 IU/L vs 50.40 ± 1.17 IU/L, $p<0.05$) compared to control rats respectively.

viii. The MBL extract caused a significant ($p<0.05$) dose dependent increase in serum liver enzymes compared to control rats: ALT (µ/L): 62.40 ± 1.22, 76.60 ± 2.20, 97.00 ± 2.88 and 127.00 ± 4.30;ALP (µ/L): 90.20 ± 2.80, 128.20 ± 3.09, 132.40 ± 2.16 and 140.40 ± 1.99; for control, MBL 200 mg/kg, MBL 400 mg/kg and MBL 800 mg/kg, respectively. While,AST (µ/L) values were: 60.40 ± 0.68, 89.40 ± 3.37 and 119.00 ± 1.14; for control, MBL 400 mg/kg and MBL 800 mg/kg, respectively.

ix. Histologically, MBL 400 mg/kg showed dilated mammary acini filledwith milk, while MBL 800 mg/kg showedsmaller/shrinking mammary acini compared to control and metoclopramide treated rats.

x. Histologicallyalso, mild fatty infiltration was observed in the liver of MBL 800 mg/kg treated rats compared to the other groups.

6.2 Conclusion

The aqueous extract of *Momordica balsamina* L.caused increased milk yield through increased lobular alveoli development and secretory activity of the mammary alveoli and increased serum levels of growth hormone and oxytocin. However, it caused a significant lowering of serum prolactinlevel at MBL 800 mg/kg. It also showed a dose-dependent pro-oxidant activity and mild hepatotoxic effect at 800 mg/kg.

6.3Recommendations

i. *Mormodica balsamina* L. could be used as a galactogogue in nursing mothers at moderately low doses (≤400 mg/kg).
ii. Further study onlactogenic effect using different fractions of MBL should be conducted to ascertain the most potent component of the extract.
iii. Assessment of the lactogenic effect of the extract beyond 18 hours after gavage should be carried out to determine an optimum time and duration of efficacy.
iv. The active componentsshould be isolated and their various effects on lactogenic hormones and oxidative stress biomarkers should be fully investigated to ascertain the role of each in lactogenesis.
v. More extensive experiments on hepatotoxic and antioxidant effects of *Momordica balsamina* L. using different fractionsshould be conducted to ascertain both short and long-term toxicity andpro-oxidant effect,if any of the different fractions.

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APPENDICES

APPENDIX I: Total milk yield following 10 days of oral administration of normal saline metoclopramide and *Momordica balsamina* L.

Treatment Groups	Milk yield (g/pup)
Group I: Control (1 mg/kg Normal saline)	0.62 ± 0.15^{a}
Group II: Metoclopramide (5 mg/kg)	2.08 ± 0.17^{b}
Group III: MBL (200 mg/kg)	0.80 ± 0.10^{b}
Group IV: MBL (400 mg/kg)	1.44 ± 0.20^{a}
Group V: MBL (800 mg/kg)	0.96 ± 0.29^{b}

± = SEM; Superscripts **b** indicate significant difference when compared with **a***(p<0.05)*.

APPENDIX II: Daily milk yield following 10 days of oral administration of normal saline, metoclopramide and *Momordica balsamina* L.

	DAILY MILK YIELD OF TREATMENT GROUPS				
	Norm saline	**Metoclopramide**	**MBL**	**MBL**	**MBL**
No. of days	**1 ml/kg**	**5 mg/kg**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Day 1	0.40 ± 0.00	2.20 ± 0.49	0.20 ± 0.20	0.20 ± 0.20	0.40 ± 0.25
Day 2	0.80 ± 0.00	2.40 ± 0.37	1.80 ± 0.49	0.20 ± 0.20	0.80 ± 0.49
Day 3	0.60 ± 0.00	2.60 ± 0.20	1.60 ± 0.50	1.20 ± 0.20	1.00 ± 0.32
Day 4	0.80± 0.00	2.80 ± 0.74	0.80 ± 0.40	0.40 ± 0.25	0.20 ± 0.20
Day 5	0.40 ± 0.00	1.80 ± 0.49	0.60 ± 0.40	1.40 ± 0.51	0.60 ± 0.25
Day 60.60 ± 0.00	1.40 ± 0.51	0.60 ± 0.25	1.60 ± 0.40	1.40 ± 0.68	
Day 7	0.40 ± 0.00	1.80 ± 0.58	0.40 ± 0.40	2.00 ± 0.63	0.40 ± 0.25
Day 8	0.80 ± 0.00	2.40 ± 0.25	0.20 ± 0.20	1.80 ± 0.37	1.20 ± 0.37
Day 9	0.40 ± 0.00	2.60 ± 0.68	1.20 ± 0.37	3.00 ± 0.45	2.00 ± 0.71
Day 10	1.00 ± 0.00	0.80 ± 0.49	1.00 ± 0.00	2.60 ± 0.12	1.60 ± 0.93

± = SEM; Means with different superscripts within columns are statistically significant *($p < 0.05$)*.

APPENDIX III: Serum prolactin levels following 10 days of oral administration of normal saline, metoclopramide and *Momordica balsamina* L.

Treatment Groups	Prolactin (ng/ml)
Group I: Control (1 ml/kg Normal saline)	38.06± 2.04[a]
Group II: Metoclopramide (5 mg/kg)	43.14± 0.84[b]
Group III: MBL (200 mg/kg)	41.20 ± 0.58[a]
Group IV: MBL (400 mg/kg)	42.50 ± 1.39[a]
Group V: MBL (800 mg/kg)	28.66± 1.60[c]

± = SEM; Superscripts **b** indicate significant difference when compared with **a,c** indicate significant difference when compared with **b***(p<0.05)*.

APPENDIX IV: Serum growth hormone levels following 10 days of oral administration of normal saline, metoclopramide and *Momordica balsamina* L.

Treatment Groups	Growth hormone(ng/ml)
Group I: Control (1 mg/kg Normal saline)	9.10 ± 0.33^{a}
Group II: Metoclopramide (5 mg/kg)	12.46 ± 0.33^{b}
Group III: MBL (200 mg/kg)	12.08 ± 0.20^{b}
Group IV: MBL (400 mg/kg)	13.26 ± 0.32^{b}
Group V: MBL (800 mg/kg)	13.64 ± 0.80^{b}

± = SEM; Superscripts **b** indicate significant difference when compared with **a***(p<0.05)*.

APPENDIX V: Serum oxytocin levels following 10 days of oral administration of normal saline, metoclopramide and *Momordica balsamina* L.

Treatment Groups	Oxytocin(ng/ml)
Group I: Control (1 mg/kg Normal saline)	309.80 ± 8.54[a]
Group II: Metoclopramide (5 mg/kg)	320.00 ± 5.26[a]
Group III: MBL (200 mg/kg)	404.80 ± 1.71[b]
Group IV: MBL (400 mg/kg)	384.60 ± 6.33[b]
Group V: MBL (800 mg/kg)	412.40 ± 7.67[b]

± = SEM; Superscripts **b** indicate significant difference when compared with **a***(p<0.05)*.

APPENDIX VI: Biomarkers of oxidative stress following 10 days of oral administration of normal saline, metoclopramide and *Momordica balsamina* L.

Treatment Groups	SOD (IU/L)	CAT (IU/L)	GPx (IU/L)	MDA (µmol/L)
Control (1 mg/kg)	2.16± 0.07[a]	50.40 ± 1.17[a]	48.60 ± 2.20[a]	1.14 ± 0.04[a]
Metoc (5 mg/kg)	2.04 ± 0.05[a]	46.40 ± 1.72[a]	52.20 ± 1.63[a]	1.40 ± 0.05[b]
MBL (200 mg/kg)	1.94 ± 0.05[a]	46.60 ± 0.90[a]	48.60 ± 1.20[a]	1.40 ± 0.05[b]
MBL (400 mg/kg)	1.48 ± 0.08[b]	45.40 ± 1.21[a]	49.60 ± 1.44[a]	1.62 ± 0.04[c]
MBL (800 mg/kg)	1.54 ± 0.07[b]	40.80 ± 1.24[b]	44.20 ± 1.90[b]	1.82 ± 0.06[c]

± = SEM; Superscripts **b** indicate significant difference when compared with **a,c** indicate significant difference when compared with **b***(p<0.05)*. SOD= Super oxide dismutase, CAT= Catalase, GPx= Glutathione peroxidase and MDA= Malondialdehyde.

APPENDIX VII: Serum liver enzymes levels for 10 days of oral administration of normal saline, metoclopramide and *Momordica balsamina* L.

Treatment Groups	ALT(µ/L)	AST(µ/L)	ALP(µ/L)
Control (1 mg/kg Normal saline)	62.40 ± 1.22^{a}	60.40 ± 0.68^{a}	90.20 ± 2.80^{a}
Metoclopramide (5 mg/kg)	73.40 ± 1.78^{b}	71.80 ± 1.83^{a}	129.60 ± 3.44^{b}
MBL (200 mg/kg)	76.60 ± 2.20^{b}	72.60 ± 2.89^{a}	128.20 ± 3.09^{b}
MBL (400 mg/kg)	97.00 ± 2.88^{c}	89.40 ± 3.37^{b}	132.40 ± 2.16^{b}
MBL (800 mg/kg)	127.00 ± 4.30^{c}	119.00 ± 1.14^{b}	140.40 ± 1.99^{b}

± = SEM; Superscripts **b** indicate significant difference when compared with **a,c** indicate significant difference when compared with **b***($p<0.05$)*. ALT= Alanine amino transferase, AST= Aspartate amino transferase, ALP= Alkaline phosphatase.