EFFECT OF VITAMIN B6 SUPPLEMENTS ON GLUCOSE TOLERANCE, BLOOD HEMOGLOBIN LEVEL AND BODY WEIGHT IN FEMALE MICE TAKING COMBINED ORAL CONTRACEPTIVE

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A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY OF SOKOINE UNIVERSITY OF AGRICULTURE MOROGORO, TANZANIA

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ABSTRACT

An increasing number of women worldwide are using some form of contraceptive drugs for family planning including combined oral contraceptives (COC). However, the use of COC has been associated with some adverse effects such as anemia and impaired energy metabolism in some users. A cross-sectional study was, therefore, carried out to investigate the effect of vitamin B6 supplementation on plasma glucose tolerance, hemoglobin levels and body weight using experimental mice model. Forty five adult female mice at their reproductive age (6 - 12 weeks) were randomly divided into three groups of 15 mice each. G1 served as a control group and received maize basal diet, G2 received 0.061 mg and G3 received 0.061 mg COC together with 0.028 g vitamin B6 at the same time for 56 consecutive days. Glucose tolerance test was taken and hemoglobin levels were tested weekly for 56 days period of the study. Measurement of body weight was done before and after treatment. Results obtained showed that vitamin B6 has significant effect on blood glucose intolerance and hemoglobin level (P < 0.05) and body weight changes (P < 0.05) caused by COC between treated groups. From the study it is recommended that the use of COC by women of reproductive age should be accompanied by concurrent use of vitamin B6 to reduce the effects associated with oral contraceptive.
DECLARATION

I, Regina Rudovick Komba, do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any institution.

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Regina Komba  Date
(MSc Biochemistry Candidate)

The above declaration is confirmed

______________________  ______________________
Prof. Robert. A. Max  Date
(Supervisor)
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DEDICATION

To my beloved parents, mother, Ann Yungu and father, Rudovick Komba for building up my strong and concrete background. My beloved husband Crispin B. Mombe, our lovely sons Benedict, Bruno and Lewis without forgetting my young sister Mary and young brothers Emanuel and Rudovick for their endless support and encouragement during my studies.
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<table>
<thead>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARU</td>
<td>Animal Research Unit</td>
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<tr>
<td>ATP</td>
<td>Adenosine tri-phosphate</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
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<tr>
<td>COC</td>
<td>Combined oral contraceptive</td>
</tr>
<tr>
<td>CVMS</td>
<td>College of Veterinary and Medical Sciences</td>
</tr>
<tr>
<td>EE</td>
<td>Ethinylestradiol</td>
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<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GTT</td>
<td>Glucose tolerance test</td>
</tr>
<tr>
<td>HB</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HED</td>
<td>Human equivalent dose</td>
</tr>
<tr>
<td>IUD</td>
<td>Intrauterine device</td>
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<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
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<tr>
<td>LNG</td>
<td>Levonorgestrel</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PLP</td>
<td>Pyridoxal 5-phosphate</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package of the social sciences</td>
</tr>
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<td>SUA</td>
<td>Sokoine University of Agriculture</td>
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</table>
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Millions of women in the world today use family planning methods (Mosher et al., 2004). These are used in family planning for economic reasons, social and political influences. Tanzania also is among the countries in the world today that use family planning methods (PSU, 1997). Family planning or birth control is any method, medicine or device used to prevent pregnancy. This is the way of preventing pregnancy before it begins. Mode of action depends on type of birth control used. There are several birth control methods including hormonal, barrier, condoms and intrauterine devices (IUD). Others include natural methods such as abstinence (Everett et al., 2000).

Hormonal methods are contraceptive methods that act on endocrine system to prevent pregnancy. They contain synthesized female hormones (estrogen and progestin). There are several hormonal birth control methods including; pills, patch and injection. Hormonal methods are either in combined form (estrogen and progestin) or contain progestin alone. Combined contraceptives (COC) exist in various formulations depending on the amount of estrogen and progestin they contain. Pills method is a very popular method, with around 100 millions of women in the world today using it (De Irala et al., 2011). These hormonal pills may provide equal amount of estrogen and progestin (monophasic) or provide different amount of these artificial hormones (multiphasic) in a month (Hall et al., 2012).

The use of combined contraceptives results into side effects which include, breast tenderness, headaches, weight changes, mood changes/swings and abnormal menstruation. Others are glucose impairment and raising insulin level in blood; there is
also increased risk of cancer and blood clotting. Risks have been reported to vary depending on combination of contraceptive and the type used (Roach et al., 2015). Oral method of contraceptives (pills) interferes with carbohydrate and amino acid metabolisms of the body. They stimulate secretion of enzymes causing deficiency of vitamin B6 which is an important cofactor for those enzymes (Spellacy, 1969; Kalkhoff, 1975; Adugna et al., 2004).

The use of some contraceptive have been shown to increase the severity of some diseases like heart disease, renal disease, diabetes, hypertension, cancer, asthma and sickle cell anemia (Spellacy, 1969; Okada et al., 1999). Scientists’ use low doses of estrogen in the combination and advice people to use uncombined oral contraceptive to reduce side effects caused by combined oral contraceptive. This study aims to find out the effect of vitamin B6 supplement on the glucose impairment, hemoglobin level in blood and body weight caused by combined monophasic hormonal contraceptives.

1.2 Problem Statement and Justification of the Study

About sixty four percent (64%) of married or women of reproductive age (15 – 44 years) worldwide are using some form of contraception (Hall et al., 2012). The number of women using COC is increasing and expected to reach 800 million by the year 2030. About 17% of these women use combined oral contraceptive (COC) in the world today (Hall et al., 2012). Different formulations of COCs are used but the leading ones are the one with monophasic Ethinylestradiol (EE) and Levonorgestrel (LNG) others are of Ethinylestradiol with Norgetimate and EE and Desogestrel (Godsland et al., 1990; PSU, 1997; Bjarnadottir et al., 2002; Hall et al., 2012). These combinations have adverse effects to users including; impaired carbohydrate metabolism, headaches, breast tenderness (Rosenberg, 1991; Gaussem et al., 2011). The efforts done to reduce these side effect are not much enough since their not conversant and available to users.
Also they have been reported to cause side effect to users. Thus there is a need to find out how to combat these side effects by using most available, conversant, simple and cheap methods.

The main objective of the study was therefore to investigate the effect of oral supplementation of vitamin B6 on glucose impairment, hemoglobin level in blood and body weight of female mice taking (monophasic Ethinylestradiol and Levonorgestrel). It is anticipated that findings from this study will be used to design a clinical trial using human subjects.

1.3 Objectives

1.3.1 Main objective
To investigate the effect of vitamin B6 on glucose tolerance, hemoglobin levels and body weight of mice taking monophasic combined (Ethinylestradiol/Levonorgestrel) oral contraceptives (COC).

1.3.2 Specific objectives

i. To determine the effect of the combined oral contraceptive on body’s ability to clear glucose (using blood glucose tolerance test)

ii. To determine the effect of the combined oral contraceptive on hemoglobin level

iii. To determine the effect of combined oral contraceptive on body weight

iv. To determine the effect of combined oral contraceptives with vitamin B6 supplement on hemoglobin level, body weight and body’s ability to clear glucose
1.4 Research Hypothesis

a) \( H_0 \): Vitamin B6 has no effect on ability of body to clear glucose caused by combined oral contraceptives

\( H_1 \): Vitamin B6 has effect on body ability to clear glucose caused by combined oral contraceptives

b) \( H_0 \): Vitamin B6 has no effect on hemoglobin level caused by combined oral contraceptives

\( H_1 \): Vitamin B6 has effect on hemoglobin level caused by combined oral contraceptives

c) \( H_0 \): Vitamin B6 has no effect on body weight changes caused by combined oral contraceptives

\( H_1 \): Vitamin B6 has effect on body weight changes caused by combined oral contraceptives
2.0 LITERATURE REVIEW

2.1 Hormonal Birth Control Methods

These are the birth control methods that act on the endocrine system. They work by mimicking the natural hormonal women body to prevent pregnancy. They include pills, injections, patch, vagina ring and implants. They can be combined (estrogen and progestin) or uncombined (progestin) contraceptives (Lidegaard et al., 2009; Hall et al., 2012). Some of contraceptive provides same amount of estrogen and progestin each month (monophasic) and others provide different amount of estrogen and progestin each month (multiphasic) (Hall et al., 2012). Among types of combined hormonal contraceptives, pills and injections are the most popular ones. They take about 18% of all hormonal contraceptives used in the world today (Trussel et al., 2007).

Most of women in the reproductive age (about 100 million women worldwide) use combination oral contraceptives (Mosher et al., 2004; Trussel et al., 2007) which come in packets of 21, 28, or 91 tablets. Each category has to be taken as instructed by the pharmacist or doctor (Speroff et al., 2005). Combined hormonal oral contraceptives exist in different formulations. Formulations differ from one another in the amount of estrogen and progestin they contain and in the way of using them. The common used formulations in Tanzania are monophasic 0.03 mg EE and 0.15 mg LNG, 0.03 mg EE and 0.3 mg Norgestrel, 0.03 mg EE and 0.15 mg Desogestrel (PSU, 1997). Among these three formulations the one containing 0.03 mg EE and 0.15 mg LNG has shown to be used by most women compared to the other two (Spona et al., 1996; Gaussem et al., 2011).
2.2 Monophasic Ethinylestradiol/Levonorgestrel and its Biochemistry

This is a pill that contains female hormones which prevent pregnancy. Ethinylestradiol and Levonorgestrel are synthetic estrogen and progesterone respectively. They work by inhibiting ovulation, fertilization and/or implantation of ovum (Spona et al., 1996). They inhibit ovulation by suppressing the secretion of gonadotropins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)) (Spona et al., 1996). Implantation is inhibited by reducing endometrial growth. This hormonal contraceptive is effective by about 99% when taken as instructed (Pincus et al., 1959; PSU, 1997). Apart from preventing pregnancy it is reported to reduce acne condition and they have ability of reducing the risk of developing endometrial and ovarian cancer (Leyden et al., 2002).

The contraceptives are reported to have many side effects to users including; coronary heart diseases (thromboembolism), hypertension, depression, liver diseases, raise insulin level, glucose impairment, abnormal uterine bleeding, constipation, yellowish discoloration, double vision, weight changes and severe headaches (Stadel, 1981; Oliver, 1983; Rosenberg, 1991; Archer et al., 1997; PSU, 1997; Bjarnadottir et al., 2002; Adugna et al., 2004). This contraceptive stimulates synthesis of enzymes that require vitamin B6 thus making the body experience the deficiency of this vitamin. Also it inhibits formation of neurotransmitter serotonin which reduces depression in individuals that use this medicine (Adugna et al., 2004; Rios-Avilla et al., 2014).

Efforts are done every day to find ways on how to reduce adverse effects of contraceptives by administering low doses of estrogen on combination (Archer et al., 1997). Scientists also advice people to use uncombined instead of combined oral contraceptive although this also is reported to have adverse effects (Godsland et al., 1988). Adverse effects can be reduced by changing the type of progestin or the methods of contraception (Hall et al., 2012).
2.3 Impaired glucose tolerance (IGT)

IGT means that blood glucose level is raised beyond the normal levels. The level is not high enough to warrants a diabetes diagnosis. This condition may be caused by many factors including the use of some drugs. There are some drugs that induce disorders in glucose tolerance (Pandit et al., 1993). Basing on dosage and time used a drug may induce hyper and hypoglycemia (Perlman et al., 1985). It is not prudent to monitor plasma glucose value when not possible to avoid prescription of medication with known effect on the carbohydrate metabolism.

Normal blood glucose level of human being is ranging from the 4-8 mmol/L. For individual to have IGT should have blood glucose level 7.8 mmol/L more or less than 11.1 mmol/L after 2 hours of oral glucose administration (Nolan et al., 2011). IGT have no symptoms, the individual may have this condition while are unaware of it. The increase of IGT increases the development of diabetes and cardiovascular diseases (Haffner et al., 1996; Reavan, 2012). IGT is diagnosed by using test known as oral glucose tolerance test (OGTT) (koletsky et al., 2007).

2.3.1 Oral glucose tolerance test (OGTT)

This is the standardized test used to diagnosed impaired glucose tolerance as the effect of carbohydrate metabolism (koletsky et al., 2007). The test is based on oral administration of glucose and subsequently following glucose plasma level over time.

This first was introduced as a diagnosis tool for diabetes, later studies focused on identifying glucose patterns. OGTT is particularly useful in characterizing the action of drugs which can be either detrimental or beneficial to individual’s glucose metabolism (koletsky et al., 2007).
2.4 Hemoglobin Level

This is a protein in red blood cell that carries oxygen throughout the body. Hemoglobin also is responsible for the shape of red blood cell, a disk like shape which helps them to move easily through blood vessels. The hemoglobin level in the body determines clinical significance (Braden et al., 2000). The normal hemoglobin level of adult female (human being) is 15.5 g/dL and male hemoglobin level is 14.5 g/dL (Braden et al., 2000). The hemoglobin level of female mice is 16.2 -16.8 g/dL (Fleischman et al., 1979).

The normal hemoglobin level varies with age, gender, drugs and health status of an individual. The formation of hemoglobin is an enzymatic controlled process which last for 17 days. Enzymes involved in formation of hemoglobin depend on vitamin B6 as the cofactor (Adugna et al., 2004). Determination of hemoglobin level of an individual is done by the automated machine called the hemoglobin-meter.

2.5 Body Weight

There is a link between body weight and health. The weight of an individual is required to relate with age and height of an individual (Guo et al., 1994). There are many factors that may affect weight of an individual includes pharmacological agents, genetic makeup, gender etc. Some drugs can produce weight gain and fat gain these include glucocorticoids and ant allergies (Baptista et al., 1987).

As the weight increases the body fails to defend itself against non transmitted diseases. Having excess weight may contribute too many different health problems likes diabetes, heart disease, strokes etc. Excess loss of weight may also results to metabolic disorders (Bae et al., 2008).
2.6 Vitamin B 6

This is water soluble vitamin obtained from food as pyridoxine. Active forms include pyridoxal phosphate and pyridoxamine 5 phosphate. When inside the body, pyridoxine is converted into an active form pyridoxal phosphate under the catalytic action of ATP dependent pyridoxal kinase and pyridoxal 5 phosphate oxidase (Di Salva et al., 2011). It exists in three forms: pyridoxamine, pyridoxine and pyridoxal phosphate (Anderson et al., 1974). The vitamin is obtained from muscle meat, egg yolk, corn, wheat, milk and liver.

This vitamin acts as the co enzyme to more than 140 enzymatic reactions (Di Salva et al., 2011). For human beings, the recommended daily intake amount in an adult is 1.4-2.2 mg/d and for children is 0.3-0.4 mg/d (Bender, 1989; Adugna et al., 2004). The recommended amount of vitamin B6 intake per day for an adult mouse is 1 mg (Baumann and Miller, 1945; Nutrients requirement of laboratory rat, 1995).

2.6.1 Vitamin B 6 and metabolism

The vitamin acts as the cofactor of many enzymatic reactions like transamination, racemization, elimination and gluconeogenesis. It works via formation of Schiff’s base between amino acids and the coenzymes. This gives planarity of the structure which is essential for catalytic reactions to occur (Adugna et al., 2004). The pyridine ring of the co enzyme acts as the electrophile; its nitrogen acts as the electron sink which draws electrons from neighboring amino acids and stabilizes the carbon ion intermediate.

This vitamin is also essential for synthesis of neurotransmitter serotonin, noradrenalin, component of sphingolipids necessary for the myelin and the heme formation (Adugna et al., 2004). Its use is reported to reduce diabetic complications, cognitive aging, and
coronary heart disease. It reduces oxygen radicals and it modifies mitochondrial functions (Kannan et al., 2004). Intake of this vitamin reduces the risk of colorectal cancer in women (Rimm et al., 1998). The clinical signs for vitamin deficiency include anorexia, nausea, titleness, cheilosis, conjunctivitis, glossitis, seborrhoeic dermatitis, convulsions and polyneuritis (Vitter et al., 1953; Molony et al., 1954). This vitamin deficiency has been reported to be teratogenic (skeletal, neural defects and splenic hypoplasia) to animal (Davis et al., 1970).

2.7 Knowledge Gap

Many studies have been done on how to reduce adverse effects caused by combined oral contraceptives, but no study has been reported to use glucose impairment measurement, hemoglobin level and body weight as among the indicators of adverse effects caused by Ethinylestradiol and Levonorgestrel combined contraceptives. Thus this study aimed at investigating the effects of vitamin B6 supplement on adverse effects (glucose impairment, hemoglobin level and body weight) caused by monophasic Ethinylestradiol and Levonorgestrel.
CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Study Site
The study was conducted at the College of Veterinary Medicine and Medical Sciences (CVMS), Sokoine University of Agriculture. Experimental mice were maintained at Small Animal Research Unit.

3.2 Experimental Animals and their Management
Forty five female albino mice at their reproductive age (6 – 12 weeks; 26 – 28 g bodyweight) were purchased from the small animal research unit and were maintained on maize grain basal diet with *ad libitum* drinking water. The animals were randomly assigned into three groups of 15 mice each and kept in three separate cages. Mice were left in the experimental environment for a 2-week acclimatization period and then weighed prior to the onset of the experiment.

3.3 Source of Test Materials
Vitamin B6 (Easy swallow tablets®, 21st century, USA) tablets with 100 mg per tablet, glucose powder (Power Glucose®, Mwanza, Tanzania) 80 g pack and combined oral contraceptive (Familia®, Jai Pharma Ltd, India) with 0.03 mg of EE and 0.15 mg of LNG per pill were purchased from a pharmacy in Morogoro town.

3.4 Preparation of Test Solutions
All test solutions were prepared using distilled water (from department of Physiology, Biochemistry, and pharmacology).
3.4.1 Preparation of glucose solution

The solution was prepared based on average bodyweight of mice in each group, whereby G1, G2 and G3 received 0.028, 0.028 and 0.028 g of glucose/mouse respectively.

3.4.2 Preparation of vitamin B 6 solution

Since recommended daily intake amount of vitamin B6 to mouse is 1 mg/kg body weight; each mouse in G3 received 0.028 g of vitamin B6 daily for two months.

3.4.3 Preparation of combined oral contraceptive solution

A COC dose per each mouse in each group was obtained using the formula according to Reagen-shaw et al. (2007). The following formula was used for calculation of dose translation based on body surface area:

\[
\text{HED (mg/kg)} = \frac{\text{Animal dose (mg/kg)}}{\text{Animal } Km} \times \frac{\text{Human } Km}{\text{Human } Km}
\]

Where;

\[
\begin{align*}
\text{HED} & = \text{Human equivalent dose} \\
\text{Human } Km & = 37 \\
\text{Mouse } Km & = 3 \\
\text{HED of COC} & = 0.18 \text{ mg}
\end{align*}
\]

After plugging in the data on formula, the animal dose of combined oral contraceptive was 0.0612 and 0.0621 mg COC per mouse for G2 and G3 respectively.
3.5 Treatments Allocations

G1 served as the control group and continued with the basal diet only. G2 received the basal diet plus an oral dose 0.061 mg COC/mouse daily for two months. G3 received the basal diet plus an oral dose 0.061 mg COC/mouse with 0.028 mg/mouse vitamin B6 for two months. Dosage calculations were based on average group body weight. The table below elaborates more.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment per group</th>
<th>Basal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>-</td>
<td>yes</td>
</tr>
<tr>
<td>G2</td>
<td>0.061</td>
<td>-</td>
</tr>
<tr>
<td>G3</td>
<td>0.061</td>
<td>0.028</td>
</tr>
</tbody>
</table>

3.6 Blood Sampling

Blood samples were collected for oral glucose tolerance test (OGTT) and hemoglobin level determination. Briefly, the mice were fastened for 5 - 6 hours before the first blood sample was taken and the second sample was obtained two hours following oral administration of glucose. A blood sample was obtained from tail tip as follows: the tail was disinfected using 70% ethanol cotton swabs (Neosafe®, UK); the tail was then rubbed to increase blood circulation before making a small incision using a sterile lancet. About 10 μL of blood was collected using calibrated transfer tube, i.e., 5 μL for GTT and 5 μl for Hb level (some mice required gentle squeezing to ooze the blood). The second sample was collected two hours after the oral glucose administration using the same procedures.
3.6.1 Oral glucose tolerance test. (OGTT)

OGTT was carried out using hemoglucometer (Easytouch®) GHb, Taiwan) and strips (Easytouch®) glucose strips, Taiwan). Briefly, a strip was inserted into the glucometer, and then a drop of blood from transfer tube was added to the tip of the strip and allowed to settle for 9 s before the reading was recorded in mg/dL.

3.6.2 Hemoglobin level measurement

Hemoglobin level determination was carried out using the hemoglucometer (Easytouch®) GHb, Taiwan) using different code and strips (Easytouch®) hemoglobin strips, Taiwan). Briefly, a strip was inserted into the hemoglucometer, and then a drop of blood from transfer tube was added to the tip of the strip and allowed to settle for 6 s before the reading was recorded in g/dL.

3.7 Body Weight Determination

Weight of each mouse in each group was carried out using beam balance. Briefly, the mouse was put in a source pan (with a known weight) of beam balance and then weight reading was recorded in grams.

3.8 Data Analysis

Data were organized using Microsoft Excel to determine descriptive statistics. The determination of statistical significance of the results and plotting of graphs were done using Microsoft excel program (2007).
CHAPTER FOUR

4.0 RESULTS

Results on oral glucose tolerance test are presented in Fig 1. Normally, if the level of blood sugar increases significantly following the oral administration of glucose, it is taken as an indication that the animal is incapable of clearing sugar from the blood stream. Results from the current study showed that G2 (COC alone) had reduced ability to clear blood glucose particularly on day 7, 28 and 42 on experiment. Mice in G3 (COC + B6) showed good ability in reducing blood sugar from day 7 throughout the experiment with exception of day 42. G1 (control) blood glucose levels were very similar to those of G3; whereby on day 42 the level was also higher than expected. Furthermore, most of the time during the experiment blood glucose tolerance in G3 was above that of G2 showing that vitamin B6 was effective in maintaining blood glucose.

Figure 1: Blood glucose level (mg/dL) of mice in the three groups before and after oral administration of glucose during tolerance test.
Effect of different treatments on hemoglobin levels are shown in Fig 2. Overall hemoglobin levels of mice in G1 and G3 increased steadily throughout the experimental period. In general, the average hemoglobin level of G1 was higher compared to the other two groups whereas for G3 the level was similar to that of G1. Hemoglobin level of mice in G2 was lower than the other two groups. The standard deviation for G1, G2, and G3 were 1.64, 2.02 and 1.91 respectively. The standard deviation of hemoglobin level for G2 was higher. Standard deviation of G3 was similar to that of G1.

Figure 2: Effect of COC and/or vitamin B6 on hemoglobin levels of experimental mice (means ± SD).
Weight of mice during the experiment was monitored and is presented in Fig 3. Initially, the average body weights of mice in all groups were very similar. The increments of average body weight after treatment in G1, G2 and G3 were 2.55, -4.46 and -0.93g respectively. After treatment, the average body weight of mice in control group (G1) was higher than those of the remaining two groups. At the end of the experiment, mice in G2 had lower average body weight compared to those in G1 and G3. There was statistical significance negative difference on body weight changes to mice in G2 (P < 0.05). This indicates that the decrease in body weight of mice in G2, after treatment was due to the use of COC and that concurrent administration of B 6 reverses this effect.

![Figure 3: Body weight of mice (g) in three groups before and after treatments.](image-url)
CHAPTER FIVE

5.0 DISCUSSION

Results obtained from this study have clearly indicated that administration of vitamin B6 to female mice of reproductive age receiving COC, had a significant positive effect in maintaining blood glucose level as measured by oral glucose tolerance test (OGTT). This effect was evident on day 14 and 42 of the experiment whereby G2 (COC alone) had significantly increased plasma glucose levels following oral glucose administration (Fig. 1). Although the effects seen on the other day of experiment (7, 28 and 56) should not be ignored since have biological significance. The effects of oral contraceptives on glucose tolerance impairment have also been reported in human subjects (Wynn et al., 1966; Philip, 1973). Not only that but also Vrbikova et al. (2005) found that the use of COC worsen glucose tolerance to healthy users. Oral contraceptives when taken they bind and transactivate the progesterone receptors modifying the half life of insulin and increase the insulin response to increased glucose. Oral contraceptives including COCs interfere with glucose metabolism in part by creating the decrease of insulin receptors at peripheral level, as the result it increase the insulin resistance causing reducing glucose utilization (glucose intolerance) in muscle and adipose tissues (wynn et al., 1979; Reavan et al., 1989; Godsland et al., 1992).

The use of OC has been associated with glucose intolerance and insulin sensitivity. This is why all OCs are not advised to women with histories of gestational diabetes or glucose intolerance (Reavan et al., 1989; Godsland et al., 1990; Godsland et al., 1992; Sitruk-Ware and Nath, 2013).
Administration of vitamin B6 to mice receiving daily COC doses (G3) counteracted the tendency to glucose tolerance impairment to levels similar to those of G1 (control). Similar results were also obtained by Bennick and Schreurs (1975); Adam et al. (1976); Spellacy et al. (1977), they found that vitamin B6 supplements improving glucose tolerance. The vitamin B6 effects are known to be mediated through its role as a cofactor pyridoxal phosphate (PLP) in transamination reactions involving transfer of amino groups. PLP functions as a co enzyme for glycogen phosphorylase an enzyme that catalyzes the release of glucose from stored glycogen. PLP is also a coenzyme for reactions that generate glucose from amino acids, a process known as gluconeogenesis (Leklem et al., 1999, Adugna et al., 2004). The PLP perform all these by stabilizing the intermediates products during the transamination process and gluconeogenesis process that are necessary for the carbohydrate metabolism (Adugna et al., 2004). PLP can catalyze transamination reactions that are essential for providing amino acids as a substrate for gluconeogenesis. Thus the use of vitamin B6 to COC users assists a lot in carbohydrate metabolism and body glucose balance in particular.

Adam et al. (1976) suggested that the effect of vitamin B6, on glucose tolerance in women taking oral contraceptive was due to increased formation of quilolinic acid. This acid is an inhibitor of phosphoenopyruvate carboxykinase one of the key enzymes of gluconeogenesis. Administration of vitamin B6 to COC users has shown to reduce the formation of quilolinic acid as the results improve glucose tolerance.

In the current study, it was also noted that blood glucose levels of mice in G3 after oral glucose administration were getting closer to the normal range (95 – 105 mg/dL) towards the end of the 56-day experiment (Rerup et al., 1966). This tendency suggests that administration of vitamin B6 should always go together with the use of COC. Suggestion
similar to this was put forward by Yu.Vysotsky et al. (2015) following his study in pharmacological effects of various drugs and found that effect of hormonal drugs becomes more effective if they are taken regularly for a given time.

Results obtained from this study have evidently indicated that administration of vitamin B6 to female mice receiving COC had a significant positive effect in formation of hemoglobin Fig 2. The use of COC has been found to cause the hemolytic crisis to users (Adugna et al., 2004). The COC cause deficiency of vitamin B6 in the body. Deficiency of vitamin B6 in the body prevents healthy red blood cell formation. Vitamin B6 deficiency impairs hemoglobin synthesis and lead to microcytic anaemia. This deficiency reduces function of co enzymes PLP depended red cell aminotransferase enzymes, alanine aminotransferase and aspartate aminotransferase which are important in amino acids metabolism and relatively hemoglobin formation (Adugna et al., 2004). Similar results were obtained by Toryila et al. (2014); Sajida et al. (2006) who found that there is significance decrease of hemoglobin level to OC users than others.

The administration of vitamin B6 to mice in G3 counteracted the effect of COC by significantly improving the hemoglobin levels of mice after the treatment period. Bioactive compound of vitamin B6 (PLP) is the essential coenzymes in heme formation during the hemoglobin formation (Adugna et al., 2004). PLP stabilizes the intermediate products during the enzymatic reaction on transamination. The hemoglobin level of mice in G3 was statistically significant higher (P < 0.05) than that of mice in G2.

A similar result was obtained by Bender and Totoe (1984) they found that the vitamin B6 administration reduces the side effects of glucocorticoid hormones to users. They also suggested that vitamin B6 might be useful in the treatment of tryptophan impairment.
In another study by Anderson et al. (1971) and Smuts et al. (2005) they found that vitamin B6 is one of the most important components in the formation of red blood cell (hemoglobin) and treatment of anemia respectively.

In previous studies it has been reported that the use of some formulations of COC increases the hemoglobin level to its users (Larsson et al., 1992). Thus the results of these studies go antagonistically with the present study results. Further studies are needed to be done on this to investigate the effect of COC on hematological parameters (Toryila et al., 2014).

The effects of vitamin B6 obtained in current study results have added the knowledge on therapeutic effect of various vitamins on treatment of various diseases and metabolic disorders. For example the administration of some vitamins have been reported to treat and prevent some diseases like night blindness by vitamin A , scurvy, hypochromic anemia by vitamin C and preventing against bacterial and viral infections by vitamin K (Nieman et al., 2004; Yu.Vysotsky et al., 2015).

Results obtained from this study indicate that administration of vitamin B 6 to mice receiving COC has a significant positive effect in reversing weight reduction caused by COC Fig 3. The COC use has been shown to cause PLP deprivation which affects the carbohydrate metabolism and uptake of glucose by tissues that result in insulin resistance and glucose intolerance, which affect negatively the utilization of glucose (Wynn et al., 1979). Impairment of glucose utilization in the body affects the normal growth of animal. Similar result was also obtained by Anderson et al. (1971) they found that the deprivation of PLP in two human infants resulted in an arrest of weight gain. Thus the use of hormonal contraceptives has been linked to the decrease in lean body mass.
On previous studies it has been reported that other hormonal drugs have affecting body weight of users by increasing fat deposition and increase glycogen synthesis which affect immunity of users against non communicable diseases (Baptista et al., 1987; Permery et al., 2013).

Vitamin B6 administrations have shown to prevent the effect of COC on body weight of mice in G3. The body weight of mice in G3 is similar to that in G1 indicating that concurrent use of vitamin B6 has regulated the carbohydrate and amino acids metabolism and thus stabilizes the body energy utilization. The administration of vitamin B6 to mice has shown to reduce the effect of COC by improving weight of G3. Not only that, other study has been reported recently (Ubbink et al., 1993) that the vitamin has therapeutic effects and improving metabolic problems. Previous studies show that the vitamin B6 administration up to 100 mg/day is likely to benefit in treating premenstrual symptoms and pre menstrual depression (Bender, 1999; Wyatt et al., 1999).

On the other hand there are studies done on effect of COC on body weight changes and they have found that COC have no significance effect on body weight (Coney et al., 2001; Ingela et al., 2011). Thus there is a need of other studies to be done on the effect of COC on body weight.

Observation from this study on survival rate of mice among groups, it has been shown that the number of mice died from G2, G1 and G3 was 4, 3 and 1 respectively. From vitamin B6 taking group the number of died mice was very small compared to non takers. This results show that perhaps the vitamin may have ability to improve life. The results shown by vitamin B6 agree with those reported by Hays et al. (2003) who found, the use of vitamin supplementation to people improves life and avoid infections. Therefore more studies are needed to be done to verify the therapeutic use of vitamin B6 to mice taking COC users.
CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The current study has clearly demonstrated negative effects of COC on glucose tolerance, hemoglobin level and body weight following its administration to female mice of reproductive age. It is further observed that concurrent administration of COC and vitamin B6 was able to significantly reduce the observed negative effects of COC on glucose tolerance, hemoglobin level and body weight.

6.2 Recommendation

Given the aforementioned results from this study, it is recommended that the use of COC by women of reproductive age should be accompanied by concurrent use of vitamin B6 to reduce the effects associated with oral contraceptive. Also further clinical trial should be carried to find out the benefits of vitamin B6 to COC users.

6.3 Area for Further Study

- More studies should be carried out to investigate the effect of COC on other hematological and physiological parameters in human subjects.

- Further investigation of the therapeutic effectiveness of vitamin B6 should be done using other dosage and formulations of COC.
REFERENCES


