

## **Abstract**

### **Background**

Drug abuse and its related complications are one of the most important public health problems in a world and it is becoming an increasing problem in Ethiopia. It can lead to social, health, economical and other problems. However, to the best of our knowledge data's regarding gonadal and cortisol hormone profile of drug abusers are limited, and there is no published data which is similar with our study in Ethiopia in general and the study area in particular.

**Objective:** to assess gonadal and cortisol hormone profile among drug abusers visited Zewditu memorial hospital for the purpose of HIV Behavioral and Biological Surveillance (IBBS) Survey, Addis Ababa, Ethiopia.

**Method:** A case control study was conducted to assess gonadal and cortisol hormone profile among drug abusers from stored serum sample from March 01-30, 2017. A total of 148 drug abusers and 23 controls using convenience sampling technique was included. Any information regarding the study population was obtained from questionnaire and interview filled previously. Serum level of LH, FSH, T, E2 and Cortisol was assayed. Data was entered and analyzed using SPSS version 20 statistical software and Mann-Whitney test and median was used to compare groups. Percentage and frequency was used to show distribution of descriptive data. In all cases, P-value less than 0.05 were considered as statistically significant.

**Result:** Serum testosterone and LH level were significantly decreased in khat, marijuana and heroin abusers compared to control group. But serum level of estradiol was significantly increased among the khat, marijuana and heroin abusers as compared to control groups. The other hormone parameters [cortisol and FSH] was not statistically significant among khat, marijuana and heroin users compared to control group.

**Conclusion:** In conclusion, our data suggest that gonadal [LH, testosterone and estradiol] hormones are affected as a result of drug use [khat, marijuana and heroin].

**Keywords:** Gonadal hormone, Cortisol hormone, Drug abuse

## **Acknowledgment**

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## **List of Abbreviations**

|       |                                             |
|-------|---------------------------------------------|
| ACTH  | Adrenocorticotrophic hormone                |
| CRH   | releasing hormone                           |
| CNS   | Central nervous system                      |
| DF    | Degree of freedom                           |
| DHT   | Di-hydro testosterone                       |
| E2    | Estradiol                                   |
| EPHI  | Ethiopian public health institute           |
| FSH   | Follicle stimulating hormone                |
| LHRH  | Luteinizing hormone-releasing hormone       |
| GnRH  | Gonadal releasing hormone                   |
| HPG   | Hypothalamus-Pituitary-Gonadal axis         |
| LH    | Luteinizing hormone                         |
| LJ    | Levey- jennings                             |
| QC    | Quality control                             |
| RDS   | Respondent Driven Sampling                  |
| THC   | Tetra hydro cannabinol                      |
| UNCND | United Nations Commission on Narcotic Drugs |
| WHO   | World health organization                   |

## **Operational definitions**

**Drug Abuse:** The use of the three common drugs: Heroin, marijuana /ganja, khat for purposes other than the reason for its prescribed recommendation.

**Drug abusers:** people who use any of three common drugs Heroin, marijuana /ganja , khat in the past 6 months.

**Hypogonadism:** failure of the testes to produce androgen, sperm, or both. A cut off value 410 ng/dl is used for testosterone hormone.

# **1. Introduction**

## **1.1. Background**

### **1.1.1. Gonadal Hormones**

The endocrine system is the collection of glands that produce hormones that regulate metabolism, growth and development, tissue function, sexual function, reproduction, sleep, and mood, among others. The endocrine system includes pituitary gland, thyroid gland, parathyroid glands, adrenal glands, pancreas, ovaries in females and testicles in males [1].

Hormones are synthesized in response to biochemical signals generated by various modulating systems. Many of these systems are specific to the effects of the hormone product; for example, parathyroid hormone synthesis is regulated by the concentration of ionized calcium, whereas gonadal, adrenal, and thyroid hormone synthesis is achieved by the homeostatic function of the hypothalamic pituitary axis. Cells in the hypothalamus and pituitary monitor the circulating hormone concentration and secrete trophic hormones that activate specific pathways for hormone synthesis and release. Typical examples are luteinizing [LH], follicle-stimulating [FSH], and adrenocorticotrophic [ACTH] hormones. These trophic hormones increase rates of hormone synthesis and secretion and also may induce target cell division. These various feedback signaling systems provide the hormonal homeostasis characteristic of virtually all endocrine systems [2, 3].

The hypothalamus is the coordinating center of the endocrine system. It consolidates signals derived from upper cortical inputs, autonomic function, environmental cues, and peripheral endocrine feedback. The hypothalamus receives input from virtually all other areas of the central nervous system and uses it to provide input to the pituitary. It controls output of the anterior pituitary by means of hormones, and it controls the output of the posterior pituitary or neurohypophysis by direct stimulation or inhibition [4]. The interaction between the hypothalamus, the pituitary gland, and the other endocrine glands is a feedback control system [4, 5].

The hypothalamic pituitary gonadal axis [HPG], which plays a critical role in development of body systems including immune and reproductive systems all of which need to be in balance for optimum functioning of the body, mind, and spirit. Several substances alter the delicately balanced HPG axis, causing either a decline in pituitary-secreted gonadotropins or an alteration in intratesticular testosterone concentrations which leads to hypogonadism [6].

According the endocrine society defines hypogonadism in men as a clinical syndrome that results from failure of the testes to produce adequate levels of testosterone and a normal number of sperm due to a disruption of the HPG axis. The follicle stimulating hormone [FSH] stimulates spermatogenesis by acting on the sertoli cells, while the Luteinizing hormone [LH] stimulates the leydig cells of the testes to secrete testosterone. Testosterone levels modulate the HPG axis, such that high testosterone levels inhibit the frequency and amplitude of the GnRH pulsatile release from the hypothalamus [7].

Hypogonadism may be congenital or acquired, and has many causes. The pathophysiologic mechanisms underlying hypogonadism involve either gonadal failure or failure of the hypothalamus or pituitary to release adequate stimulatory hormones for the gonadal production of sex hormones. Gonadal failure may be referred to as primary hypogonadism or hyper gonadotropic hypogonadism [e.g. low testosterone; high pituitary gonadotropins], while insufficiency of hypothalamic and/or pituitary gonadotropin release results in hypogonadotropic hypogonadism, also known as secondary or central hypogonadism [e.g. low testosterone, and low to low normal LH/FSH] [8]. Secondary hypogonadism may occur as a result of conditions such as hemochromatosis, pituitary tumors, and exposure to drugs such as corticosteroids or opioids [9].

### 1.1.2. Synthesis of gonadal and cortisol hormones

Steroid hormones are lipid molecules derived from common cholesterol precursor (Cholestane, C<sub>27</sub>). Three major classes of steroid hormones are included: androgens, estrogens, and corticoids, which contain 19, 18, and 21 carbons, respectively. Steroid hormones are synthesized by dehydrogenases and cytochrome P450 enzymes, which catalyze hydroxylation and dehydroxylation-oxidation reactions. Eukaryotic cytochromes P450 are membrane-bound enzymes expressed in either the inner mitochondrial or endoplasmic reticulum membranes of steroid-synthesizing tissues. A common and important rate-limiting step for the synthesis of all steroid hormones is cleavage of the side chain from cholesterol (C<sub>27</sub>) to yield pregnenolone (C<sub>21</sub>), the common branch point for synthesis of corticoids, androgens, and estrogens [10].

Expression of the side-chain cleavage enzyme cytochrome P450<sub>scc</sub> (cytP450<sub>scc</sub>), which converts cholesterol to pregnenolone, is one of the unique features of steroidogenic cells that participate in *de novo* steroid synthesis. In vertebrates, the synthesis and secretion of gonadal and adrenal steroid hormones are regulated by tropic hormones from the anterior pituitary such as folliclestimulating hormone (FSH), luteinizing hormone (LH), and adrenocorticotrophic hormone (ACTH) [10].

Common regulatory mechanisms for steroid synthesis and release are negative feedback loops in which elevated circulating levels of steroids suppress production of tropic hormones by acting at specific sites in the brain and the anterior pituitary [10].

### 1.1.3. Drug of abuse overview

Drug abuse has different definition but it is commonly defined as the intentional, non-therapeutic use of a drug product or substance, even once, to achieve a desired psychological or physiological effect [11]. Drugs of abuse induce pleasant feelings [euphoria in the initiation phase] or relieve distress. Regular use of drugs induces adaptive changes in the central nervous system that lead to a state of tolerance to the drug, lack of physical independence, sensitization, craving, and relapse. Drug abuse can lead to tolerance and dependence syndrome, cluster of behavioral, cognitive, and physiological phenomena that develop after repeated use and that typically include a strong desire to take the drug, difficulties in controlling its use. Drug abuse is a

significant public health issue in the world today [12]. drug abuse , particularly in developing countries like Ethiopia, has dramatically increased [13].In addition to being a serious, health-threatening behavior, it is often associated with detrimental consequences and creates certain difficulties for not only the individuals who misuse the drug, but also their parents, families, peers, and society as a whole [12].

The most common abused drugs includes: Alcohol , Cocaine ,Heroin ,Khat ,Marijuana [Cannabis] ,Methamphetamine ,Prescription Opioids , Prescription Sedatives [Tranquilizers, Depressants) ,Prescription Stimulants, Steroids [Anabolic] ,Synthetic Cannabinoids ,Synthetic Cathinone ["Bath Salts"] and Tobacco [14].Marijuana remains the most commonly used drug at the global level, with an estimated 183 million people having used the drug in 2014, while amphetamines remain the second most commonly used drug [15]. In Africa and countries of the horn of Africa Khat use is the most commonly used drug. Khat is a legal drug in Ethiopia, openly sold at markets and chewed in streets. It has different legal status in Africa [13].

#### **1.1.4. Drug of abuse and Endocrine**

Drugs of abuse often disrupt the hypothalamic-pituitary-endocrine axis, causing the endocrine glands to either over- or under produce. Disruptions of the endocrine system occur in various ways. Some chemicals mimic a natural hormone, fooling the body into over responding to the stimulus or responding at inappropriate times. Others will directly affect various endocrine glands leading to overproduction or underproduction of hormones. The effects of different drugs of abuse on the endocrine system are multiple and complex [4]. The type, length, and pattern of exposure; level of intoxication and withdrawal; and coexisting medical problems often predict the degree of endocrine disruption [4]. For example the effects of opioids are described below [figure 1] and the hypothalamic-pituitary-gonadal [HPG] axis is regulated by a negative feedback mechanism [Fig. 1]. Testosterone inhibits the frequency and amplitude of gonadotropin-releasing hormone [GnRH] release from the hypothalamus and also the secretion of luteinizing hormone [LH] from the pituitary. The Sertoli cells of the testes, in addition to stimulating spermatogenesis, also secrete the glycoprotein hormone inhibin, which

provides negative feedback to the pituitary, inhibiting the secretion of follicle stimulating hormone [FSH] [16] [Fig. 1].

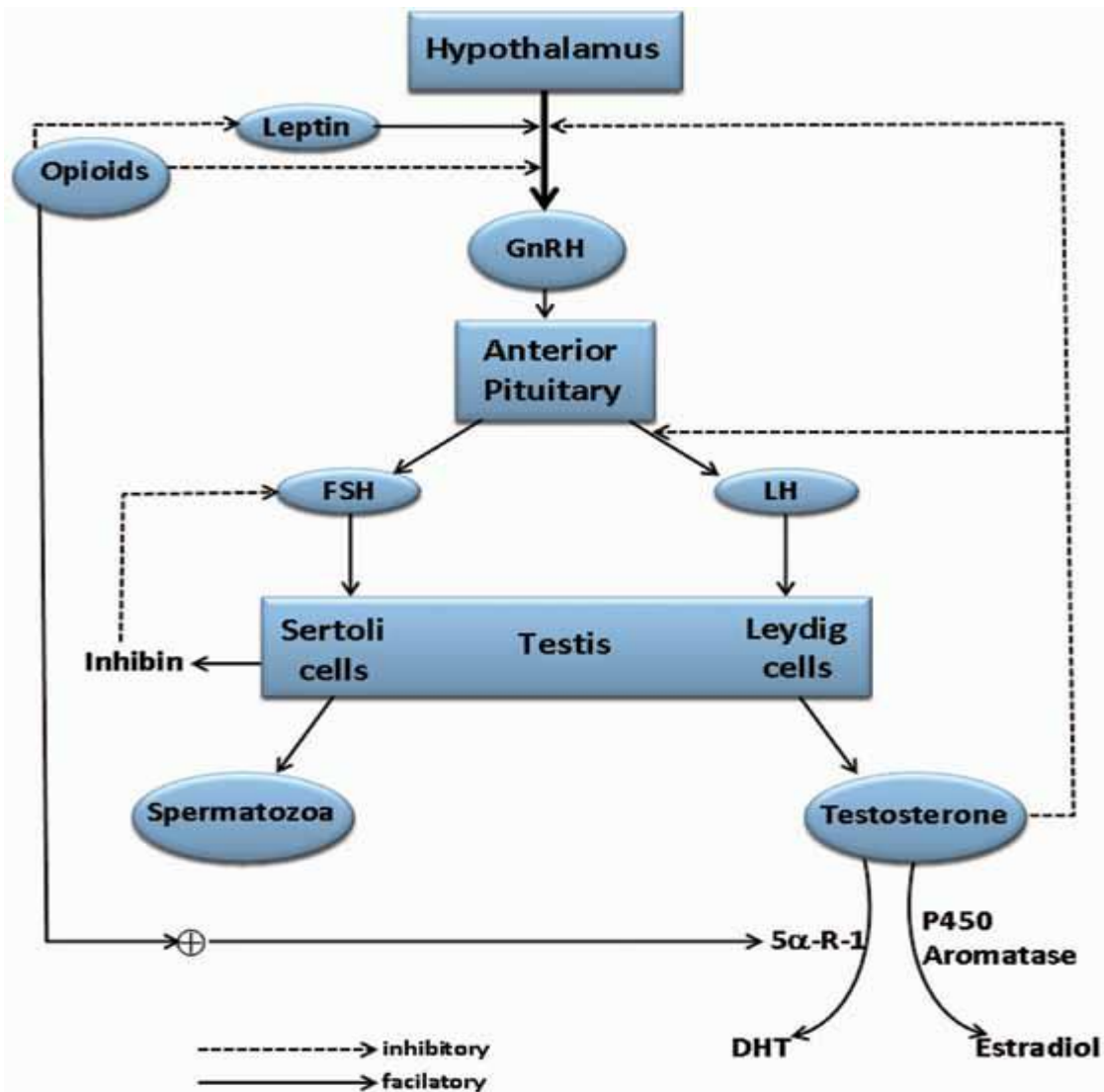


Figure 1: Schematic of opioid effects on the hypothalamus pituitary gonadal Axis in Men

Epidemiological studies provide basic information about the burden of the drug abuse and enhance prevention and control interventions. To this regard, there are limited studies conducted regarding gonadal hormone and cortisol profiles of drug abusers in Ethiopia in general and the study area in particular. Therefore, this study aims to assess the gonadal and cortisol hormone profile in khat, cannabis and heroin drug abusers in Addis Ababa.

## **1.2. Statement of the problem**

According to the 2014, WHO estimate shows a burden of 185 million drug abusers worldwide [18]. It is estimated that 1 in 20 adults, or a quarter of a billion people between the ages of 15 and 64 years, used at least one drug in 2014. Over 29 million people who use drugs are estimated to suffer from drug use disorders [15]. With an estimated 207,400 drug-related deaths in 2014, corresponding to 43.5 deaths per million people aged 15-64, the number of drug-related deaths worldwide has also remained stable.

In Ethiopia drug abuse is increasing at an alarming rate. According to the 2009 report of the 52th session of United Nations Commission on Narcotic Drugs [UNCND], Ethiopia is classified among the main illicit drugs trafficking routes destined to Europe and some Asian countries [17].

Drugs of abuse can be disrupting the hypothalamic-pituitary- gonadal axis, causing the endocrine glands to either over- or under produce. The effects of different drugs of abuse on the gonadal hormones are multiple and complex. These substances impair both the functions of the glands that release hormones and the tissues to which they are being sent [4]. Most drug of abuse like heroin and marijuana block dopamine in the CNS, leading to suppression of the HPG axis and decreased libido [19].

The use of drugs like opioids induces widespread endocrine dysfunction which leads to hypogonadism, infertility, reduced interest in sex, fatigue, depression, anxiety, menstrual irregularities, loss of muscle strength, osteoporosis, and compression fractures. The dynamic functioning of the HPA axis can be modulated by a complex series of exogenous and endogenous influence. Opioids bind to specific receptors in the hypothalamus and pituitary gland, disrupt the pulsatile release of corticotrophin releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH), and interfere with the production of cortisol and androgen precursors [20,21] .

Fertility is considered as a life conservative phenomenon among married couples which can be obliterated by various conditions affecting both males and females. For example, khat has been implicated as a causal factor for family instability divorce encouragement of prostitution and criminal behavior. The medical problems that arise from khat

consumption are partly due to its effect on mental health, and partly as a result of the sympathomimetic effects of the drug [22].

Anecdotal information from different sources reveals that drug use among youth in urban areas is expanding in unprecedented speed. The traditional Khat chewing practice is being diverted to a complex scheme that leads to a multi-drug [khat, Marijuana, Alcohol] use. The drug abuse had influence on serious societal problem like social maladaptation, decreased work productivity and job loss. Beside loss of control, drug abuse leads to the development of tolerance and demand to consume high doses in order to attain the desired stimulating effect and has serious negative consequences.

The illegality of using substance/drug abuse is one of the main reasons why addicts are not willing to participate in studies and hence, to the best of our knowledge ,data's regarding gonadal and cortisol hormone profiles of drug abusers are limited and there is no published data which is similar with our study in Ethiopia in general in the study area in particular .Therefore, this study was aimed to assess the effect of Khat, Heroin and Marijuana on gonadal [ $E_2$ , LH, FSH, and Testosterone] and cortisol hormones in adult male.

### **1.3. Significance of the study**

The abuse of drugs has an adverse impact, not only on the individual abuser, but also on the economy and society of a country, realizing the fact that drugs impose huge health, economic and social problems to individual users, families, the community and the nation as a whole. Sexual dysfunctions resulted from using drug of abuses include reduced libido and sexual performance , erectile dysfunction and delayed ejaculation [23].

Now a day, the consumption of drug abuse in Ethiopia is alarming from time to time [8]. Therefore, investigating the effect of drug abuse on the gonadal hormone is crucial to avert the health related and other complications.

As far as the current knowledge, there is no or little research has been conducted on effect of drug abuse in the gonadal and cortisol hormone profile in Addis Ababa, Ethiopia. Thus, findings of the present study would have a great importance, since it can assist in the clinical management of gonadal hormone disorders in drug abusers and may give convenient ways for solving it.

Furthermore, the associated factors identified could have implications and immediate benefits for the design, targeting and implementation of drugs education and prevention.

This study will also be useful to planners, policy makers and community at large for planning interventions targeting prevention and control of drug abuse in Ethiopia in general and specifically in the study area.

Moreover the findings of this study will serve as baseline information for the further studies.

## **2. Literature review**

### **2.1. Hormonal profile of drug abusers**

A study conducted in Iran showed that a declined serum levels of testosterone, LH and FSH hormones in test groups as compared with control groups. In this study, there were 46 opioid addicted 23-48 years old men who had a history of 1-15 years of opium consumption. Considering the duration of addiction to opium indicates that the most frequent duration [32.6%] was 10-12 years among this population. The minimum value for serum testosterone was measured in subjects who had a history of 13-15 years of addiction [ $9.8 \pm 3.3$  mmol/L] which was significantly [ $p < 0.01$ ] less than subjects who had the least duration of addiction [ $23.2 \pm 3.8$  mmol/L]. Similarly, along with the increase in duration of addiction serum level of LH was significantly reduced [4.9 m IU/ml for 13-15 years addicted subjects vs. 5.9 m IU/ml for one year addicted subjects,  $p < 0.01$ ]. There was a positive correlation between duration of addiction and the decline in the serum levels of testosterone and LH but there was no correlation between the reduction in FSH level and the duration of addiction [24].

A study conducted in Iran showed a significant [ $P < 0.005$ ] decrease in Testosterone levels in young [18-25 years] and mature [36-50 years] and a decreased FSH levels in only mature heroin addicts when compared with controls. Heroin show no effect on LH levels. Sixty eight subjects were included in this study. Thirty three were untreated heroin addicts visiting drug abuse treatment centers. The remaining 35 males of the same age as addicts were studied as controls. The study also showed that that the amount and duration of heroin intake had no effect on the hormone levels and the differences observed in three groups were statistically insignificant [25].

A case control study conducted in Makkah have been established the effect of khat on the hormonal levels in men. The study showed significantly higher serum levels of testosterone in khat chewers than the control group [ $P < 0.03$ ]. However, the mean level of serum cortisol was significantly [ $P < 0.001$ ] lower in khat chewer's serums than that in normal controls [26].

According the study conducted in USA, the level of serum testosterone, luteinizing hormone, follicle stimulating hormone, and cortisol measured in men and women. Chronic marijuana use showed no significant effect on hormone concentrations in either men or women [27].

## 2.2. Overview of khat

*Catha edulis*, commonly known as Khat or qat, chat or miraa [*Catha edulis*] is an evergreen shrub that belongs to the family celastraceae [28], which is cultivated in the Republic of Yemen and most of the countries of East Africa [29]. There is a controversy about the origin of khat. Some oral traditions claim that it is originated from Yemen; however literatures indicate that it is originated from Ethiopia, specifically in hararghe with a gradual expansion to different parts of Ethiopia, Yemen [30] and other parts of the world such as Somalia, Sudan, South Africa and Madagascar, Afghanistan and Turkestan [31]. The leaves of khat shrub have a stimulating effect, and the chewing of this material has been practiced for many centuries in certain areas of East Africa and Arabian Peninsula.



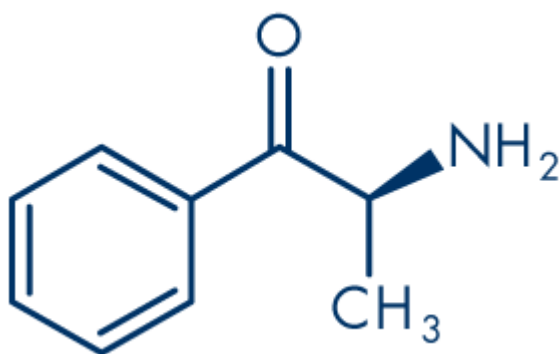
Figure 2: Young Khat shrub retrieved from [32]

## 2.3. Chemistry of khat

### 2.3.1. Chemical composition of khat

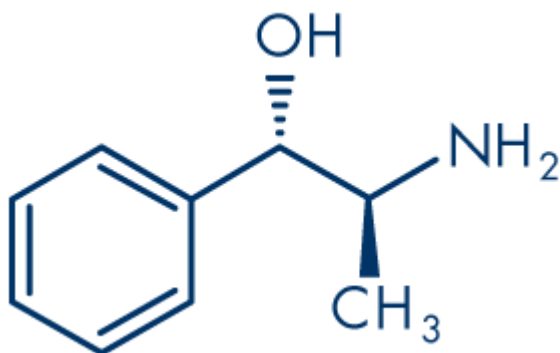
Khat contains a lot of chemical components that may have different effect on the body system. The major active ingredient of khat responsible for its psycho stimulant effect is an alkaloid chemical known as cathinone, which is structurally and chemically similar to amphetamine, and cathine, a milder form of cathinone. Cathinone is a highly potent stimulant, which produces central nervous system stimulation analogous to the effect of amphetamine [32]. Cathinone is found mainly in the young leaves and shoots. During maturation, cathinone is metabolized to cathine, and norephedrine. The leaves contain these two substances in a ratio of approximately 4:1 [33].

Molecular structure: Cathinone



Molecular formula: C<sub>9</sub>H<sub>11</sub>NO  
Molecular weight: 149.19 g/mol

Molecular structure: Cathine



Molecular formula: C<sub>9</sub>H<sub>13</sub>NO  
Molecular weight: 151.21 g/mol

Figure 3: Molecular structure of cathine and cathinone retrieved from [34]

## **2.4. General Mechanism of khat action**

The main characteristic property of khat is stimulation of the CNS. The cathinone chemical similarity to amphetamine and the amphetamine like effect, lead cathinone to be called a natural amphetamine. Experimental studies conducted to investigate khat's central and peripheral effects have determined that cathinone and amphetamine share a common pharmacological activity. Khat contains more than forty compounds, however only three phenylalkylamine alkaloids are responsible for its psychoactive effects [35]. These are cathinone  $\alpha$ -aminopropiophenone [cathinone], nor pseudo ephedrine [cathine] and nor ephedrine which are phenyl propyl amines structurally related to amphetamine and nor adrenaline [36]. Due to its potency and high liposolubility capacity, cathinone facilitates access in to the CNS. So it can be assumed that khat induced symptoms are mainly due to cathinone. Cathinone mainly increases levels of dopamine and norepinephrine in the brain by acting on catecholaminergic synapses, delaying the uptake and or enhancing the release of those neurotransmitters. Cathinone is unstable so it enzymatically degraded to cathine and nor ephedrine during maturation within a few days after harvesting. These molecules are less lipophilic and act at peripheral level, provoking the sympathomimetic effects. In view of its peripheral action, cathinone is accompanied by sympathomimetic syndrome [37, 38].

## **2.5. Effect of khat (cathinone) on HPG axis**

Detailed studies on the possible mechanism effects of khat on human reproduction are lacking. However, there are few available data suggesting its effect on human reproduction. Contradictory findings have been reported about Khat and its active components on reproduction effect these reports lead to dilemma as to whether khat is a boon or a bane to human [39]. Studies reported about khat as an aphrodisiac with its cathine and norephedrine alkaloids being shown to stimulate the final stages of sperm maturation and inhibition of acrosomal loss, another finding showed that rabbits fed on freeze-dried leaves of khat for three months had an increased rate of spermatogenesis [40, 41]. Similarly, studies showed enhanced plasma testosterone and decreased prolactin and cortisol levels following khat treatment [42]. Reports from World Health Organization

indicate effect of khat on impairment of sexuality, inability to sustain erection, loss of libido and spermatorrhoea [43].

Other studies reported a significant increase in number of abnormal sperm cells following (-)-cathinone exposure in mice ,rats and accompanying degenerative changes of testicular tissue with decrease in plasma testosterone levels in rats [44,45] . Other earlier findings have shown that cathinone acts at the catecholaminergic synapses to increase levels of dopamine in a dose dependant manner, higher dose having higher effect .Thus, it is plausible to assume that mild dose of khat extract might have enhanced LHRH release but moderate and high dose decreased hormonal release, resulting in variation of testosterone level with doses of khat [46]. Even if contradiction exists on the khat effect, a greater percentage of the studies show khat impairs sexuality than boosts sexuality.

## **2.6. Overview of marijuana**

Marijuana/cannabis is a natural psychoactive products obtained from the plant *Cannabis sativa* (Indian hemp) and some of its subspecies. It comes from leaves, stems, and dried flower buds of the cannabis plant. It is also known under numerous street names, including weed, pot, grass, 420, hashish, joint, dope, and many more [47]. Hashish is a resin obtained from flowering buds of the hemp plant .Cannabis has been used for both recreational and medicinal purposes since several centuries and it is undoubtedly the most widely cultivated, trafficked, and abused illicit drug in the world. Approximately 147 million people, or 2.5 % of the world population, consume cannabis [48].

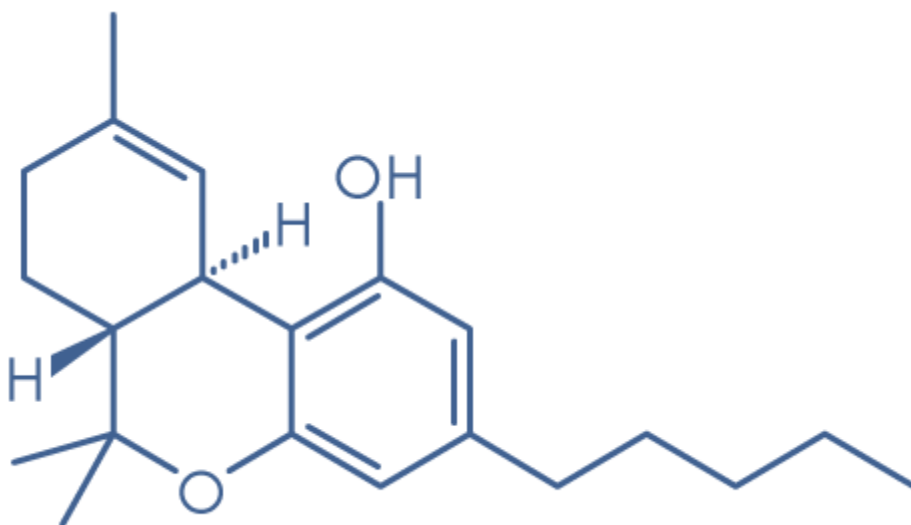


Figure 4: leaf of marijuana retrieved from [49]

## 2.7. Chemistry of marijuana/Cannabis

### 2.7.1. Chemical Composition of Marijuana

The cannabis plant contains more than 421 chemicals of which 61 are cannabinoids. More than 2000 compounds are produced by pyrolysis during smoking of cannabis and they are represented by different classes of chemicals including nitrogenous compounds, amino acids, hydrocarbons, sugar, terpenes and simple fatty acids [50]. These compounds altogether contribute to the unique pharmacological and toxicological properties of cannabis. Delta 9-tetrahydrocannabinol [ $\Delta^9$ \_THC] is considered as the most psychoactive component contributing to the behavioral toxicity of cannabis among the major compounds [51].



Molecular formula:  $C_{21}H_{30}O_2$

Molecular weight: 314.4 g/mol

Figure 5: Molecular structure of marijuana [52]

## **2.8. General mechanism of Actions of Marijuana**

$\Delta^9$ \_THC has a tri-cyclic 21- carbon structure without nitrogen and with two chiral centers in trans-configuration.  $\Delta^9$ \_THC is volatile viscous oil with high lipid solubility and low aqueous solubility.  $\Delta^9$ \_THC is present in cannabis as a mixture of mono-carboxylic acids, which gets readily and efficiently de-carboxylated upon heating. It decomposes when exposed to air, heat or light and readily binds to glass and plastic, due to this  $\Delta^9$ \_THC is usually stored in basic or organic solvents in amber silicate glassware to avoid loss during analytical procedures [53].

There are two mechanisms of  $\Delta^9$ \_THC action in vivo. The first mechanism of  $\Delta^9$ \_THC which is secreted as a glucuronide acts via non-specific interactions with cellular and organelle membranes in the brain supporting a membrane perturbation mechanism [54]. The second mechanism acts interaction of  $\Delta^9$ \_THC with specific cannabinoid receptors [55]. Molecular analysis has demonstrated  $\Delta^9$ \_THC to act on several intracellular targets including opioid and benzodiazepine receptors, prostaglandin synthetic pathway, protein and nucleic acid metabolism, so delineating a single mechanism of action  $\Delta^9$ \_THC of is very difficult. Further, cannabinoids inhibit macromolecular metabolism in a dose dependent manner and have a wide range of effects on enzyme systems, hormone secretion and neurotransmitters [56].

Cannabinoids exert various physiological effects by interacting with specific cannabinoid receptors [CB receptors] present in the brain and periphery [57]. CB1 receptors in the brain [58] are particularly concentrated in anatomical regions associated with cognition, memory, reward, anxiety, pain sensory perception, motor co-ordination and endocrine function. CB2 receptors are localized to the spleen and other peripheral tissues [59].

## **2.9. Effect of Marijuana on HPG axis**

The secretion of sex hormones is directly controlled by the pituitary and indirectly influenced by the hypothalamus in both males and females. From cells in the medial basal hypothalamus, gonadotropin releasing hormone [GnRH] is secreted in a pulsatile fashion under the influence of a variety of other factors. GnRH stimulates the production of follicle stimulating hormone [FSH] and luteinizing hormone [LH] in the anterior pituitary gonadotrophs. In both males and females, FSH and LH act on the gonads,

leading to the secretion of testosterone in males and estradiol and progesterone in females. These hormones feed back to the hypothalamus and anterior pituitary to modulate GnRH and gonadotropin release [59].

Marijuana,  $\Delta^9$ -THC, and other cannabinoids acutely alter hypothalamic-pituitary-gonadal [HPG] integrity and affect reproductive function by acting at the hypothalamus either directly through GnRH or indirectly through other modulators [Figure 1] [59]. These effects are mediated by central cannabinoid [CB1] receptors in the hypothalamus, which is an integral part of the endo cannabinoid system that modulates several functions in the CNS and periphery. CB1 receptors are GPCRs and also found in the ovaries, uterine endometrium, testes and vas deferens among others, suggesting a possible direct effect of cannabinoids on the gonads [60, 61].

In addition,  $\Delta^9$ -THC inhibits binding of dihydro testosterone [DHT] to the androgen receptor. The anti-androgenic effects associated with marijuana use result, at least in part, from inhibition of androgen action at the receptor level. But not to the estrogen receptor, it is the non-cannabinoid components of marijuana extract that bind to the estrogen receptor. How these non-cannabinoid components contribute to marijuana's effects on the HPG axis has not been clarified [62, 63, and 64].

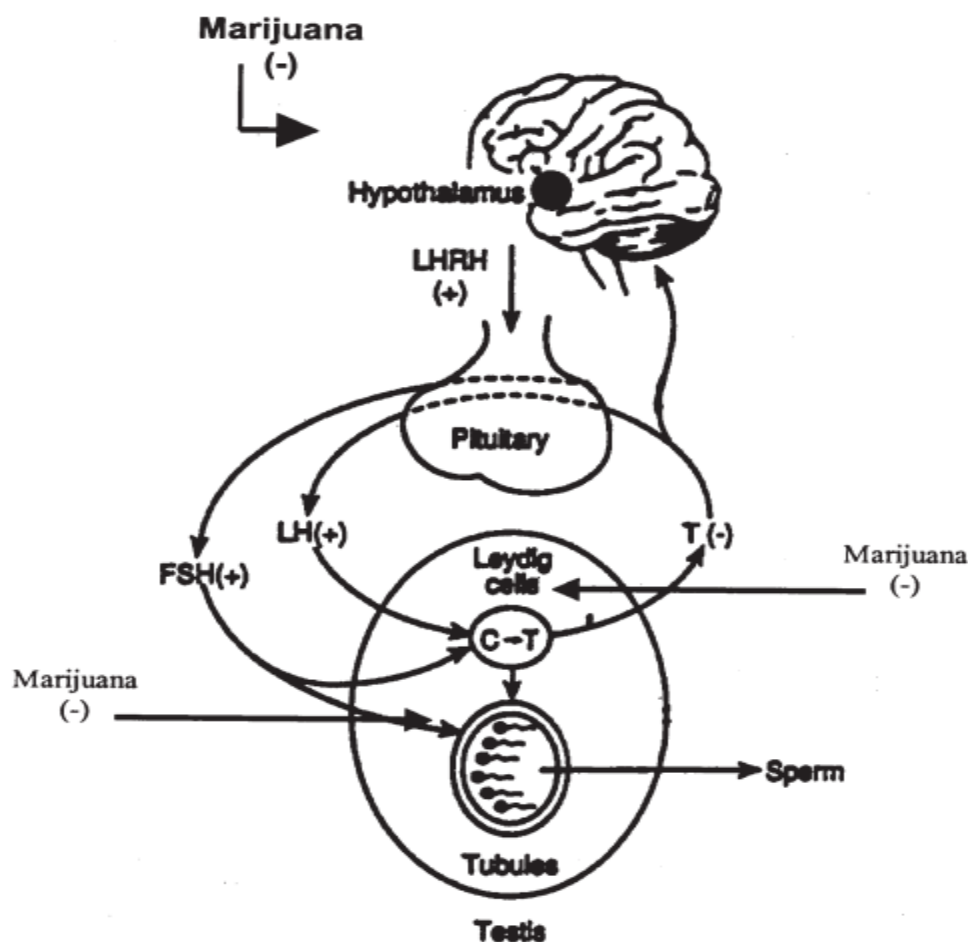


Figure 6. Effects of marijuana and  $\Delta^9$ -THC on male hypothalamic- pituitary-gonadal [HPG] function. Animal models demonstrate inhibition of the HPG axis by indirect suppression of LHRH [GHRH] secretion. In addition, direct effects on Leydig and Sertoli cells have been observed [59].

## 2.10. Overview of Heroin/Diacetylmorphine/

Heroin is a narcotic derivative of the opium poppy plant. It is a semisynthetic product obtained by acetylation of morphine, which occurs as a natural product in opium of certain poppy plant *Papaver somniferum*. Pure heroin is a white crystalline powder and is referred to as 'white sugar' by abusers. When the quality of heroin is poor, its color is no longer white, but brown; this inferior quality is called 'brown sugar'. Narcotic analgesics, being alkaline in nature, are not absorbed in the acidic medium of the stomach. It is estimated that about 90% of the effect is lost when taken orally [65]

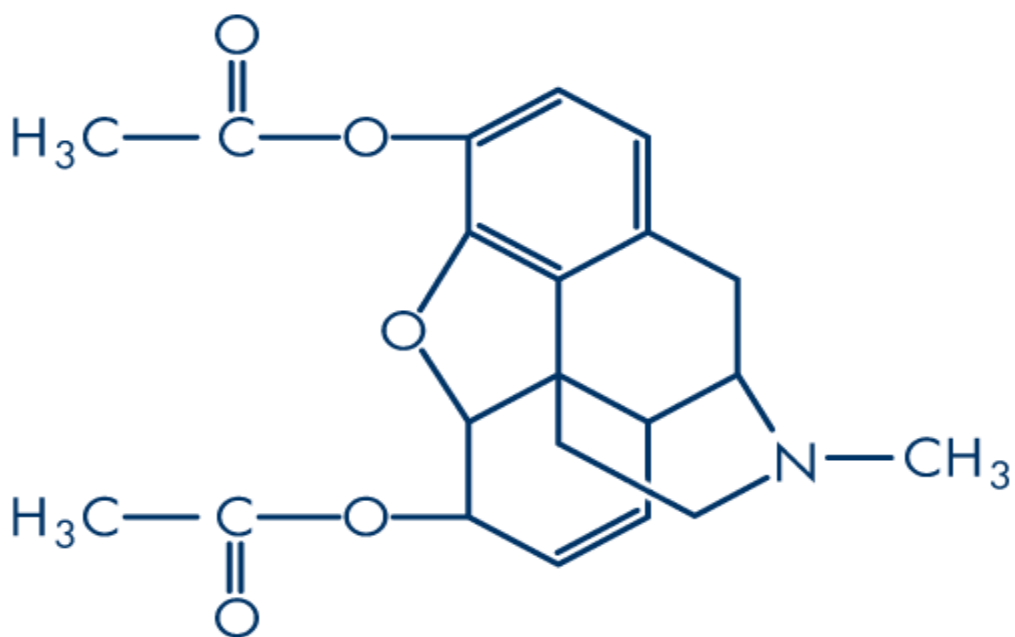


Figure 7: Heroin powder retrieved from [66]

## 2.11. Chemistry of Heroin/Diacetylmorphine

### 2.11.1. Chemical composition of Heroin

Heroin [diacetylmorphine] is produced by the acetylation of crude morphine. Diacetylmorphine is the principal psychoactive constituent of heroin. The systematic name (**Iupac**) is [5 $\alpha$ ,6 $\alpha$ ]-7,8-didehydro-4,5-epoxy-17 methylmorphinan-3,6-diol acetate [67]



Molecular formula:  $C_{21}H_{23}NO_5$

Molecular weight: 369.4 g/mol

Figure 8: Molecular structure of heroin retrieve from [68]

### **2.12. Effect of heroin on HPG**

Several studies in humans and in animal subjects have found evidence that opiates, such as heroin induce hypogonadism by suppressing the hypothalamic-pituitary-gonadal axis. Opioids induce hypogonadism by suppressing the secretion of the gonadotropin-releasing hormones, luteinizing hormone [LH] and follicle-stimulating hormone [FSH] by the hypothalamus [69]. Heroin, an exogenous opioids exert an effect on the endogenous opioid receptors and interfere with the release [including its pulsatile nature] of GnRH. Heroin also decrease the negative feedback of sex steroids on the anterior pituitary, as well as its response to GnRH. Generally exogenous opioids lead to decreased secretion of GnRH, which in turn leads to reduced levels of LH. This results in decreased testosterone and estradiol secretion, which leads to hypogonadism disorder or dysfunction [70].

### **3. Objective**

#### **3.1. General Objective**

- To assess the effect of Khat, Heroin and Marijuana on gonadal [E<sub>2</sub>, LH, FSH, and Testosterone] and cortisol hormones in adult males visited Zewditu memorial hospital for the purpose of HIV Behavioral and Biological Surveillance (IBBS) Survey on 2015, Addis Ababa, Ethiopia

#### **3.2. Specific objective**

- To compare the median difference gonadal [E<sub>2</sub>, LH, FSH, Testosterone] and cortisol hormone among khat, marijuana and heroin abuses versus control group.
- To determine correlation of different gonadal hormones [E<sub>2</sub>, LH, FSH, and Testosterone] and cortisol hormone among khat, marijuana, heroin and control groups.
- To assess associated factors within the use of different drugs.

#### **4. Hypothesis**

There is no change in gonadal [ E2, LH, FSH, and Testosterone ] and cortisol hormone profile among drug abusers and control group.

## **5. Materials and methods**

### **5.1. Study area**

The study area was Zewditu Memorial Hospital in Addis Ababa, Ethiopia which is stationed as a collection site for the data and sample via respondent driven sampling technique [RDS] sampling techniques which designated for HIV Behavioral and Biological Surveillance [IBBS] Survey among drug Abusers from all hot spot areas from March to May 2015. Zewditu Memorial hospital have rehabilitation center for drug abusers.

### **5.2. Study design and period**

Case-Control study was conducted from March 01 to 30, 2017.

### **5.3. Sample description**

Samples were collected for the purpose of HIV Behavioral and Biological Surveillance (IBBS) Survey among drug Abusers in Addis Ababa, Ethiopia and the sample was stored at  $-70^{\circ}\text{C}$  in National HIV Reference Laboratory, Ethiopian Public Health Institute (EPHI).

### **5.4. Population**

#### **5.4.1. Source population**

All adult men drug abusers from hot spot area of Addis Ababa based on mapping of drug use.

#### **5.4.2. Study population**

The study population was adult men visited Zewditu memorial hospital for the study of HIV Behavioral and Biological Surveillance [IBBS] Survey.

### **5.5. Eligibility**

#### **5.5.1. Inclusion criteria**

##### **Drug abuser groups**

- Males which has an experience of using drug of abuse in the past six months, and living in Addis Ababa.
- Drug abusers who have clinical data in the database and labeled serum sample .

- Samples with greater than 1 ml serum sample.

#### **Control groups**

- Apparently healthy males who didn't use drug of abuse

#### **5.5.2. Exclusion criteria**

##### **Drug abusers group**

- Drug abusers with incomplete clinical data in the database and unlabeled stored serum sample
- Samples with less than 1 ml serum sample
- Female drug abusers are excluded due to small study participants and absence of records related to menstrual cycle phases and co-factors.

##### **Control group**

- Males who uses drug of abuse

### **5.6. Study variables**

#### **5.6.1. Dependent variables**

- Serum level of luteinizing hormone [LH], testosterone [T], follicle stimulating hormone [FSH], Estradiol [E2] and Cortisol.

#### **5.6.2. Independent variables**

- Age
- Education status
- Occupation
- Marital status
- Type of drug
- Over dose

### **5.8. Quality Assurance and Sample storage stability**

Daily Maintenance was performed according the Manufacturer's instructions. Visual inspection of the reagent bottles and expiration date was checked. Two levels of quality control samples [Peci Control Universal 1 and 2] was run to assess the functionality of the instrument and reagents, and results was evaluated using Levey-jenning [LJ] chart.

All phases of quality assurance during laboratory analysis was performed in the EPHI national clinical chemistry reference laboratory which is accredited by the national accreditation office [ENAO] to perform tests in accordance with the requirement of ISO 15189: 2012, Medical laboratory requirements for quality and competence [Accreditation No.: M0025] by well trained and experienced laboratory professionals and Standard operating procedures [SOPs] was strictly followed of respective parameters .

The serum or plasma concentrations of several hormones after 33 freeze-thaw cycles and more than 5 years storage at -70°C, has no meaningful effects on the plasma and serum concentration of hormones [71, 72]. In this study serum sample stored for one and half year at -70 °C at National HIV Reference laboratory was used. According to the above evidence, no effect on the stability of the stored serum sample for measurement of concentration of hormones

### **5.9. Data analysis and interpretation**

The data obtained from the automated Cobas e 411 analyzer was entered and analyzed by using Statistical Package for Social Science SPSS version 20 [SPSS INC, Chicago, IL, USA]. Before the beginning of statistical analysis, the existence of outliers and normal distribution was checked, hence non - parametric statistical tests were done for all data's which were not normally distributed. Statistical analysis like median and frequency were performed for demographic variables and hormone parameters, displayed in numbers and percentage by using tables and graph. A non-parametric Mann-Whitney test was done to compare the gonadal and cortisol hormone parameters difference across the four groups [khat, marijuana, heroin and non-user group] by mean rank.

Similarly, a non-parametric Spearman correlation was done to assess relationship between the variables of FSH,LH, E2, testosterone and cortisol. A non-parametric chi-square and fisher exact test was also done to assess association between the variables of

marriage, occupation, school level, educational status, drug type with testosterone. In all cases p value less than 0.05 was considered as statistically significant.

#### **5.10. Ethical considerations**

The study was conducted after it is ethically reviewed and approved by the Department of Medical Laboratory Science research and ethical review committee (DRERC), College of Health Science, Addis Ababa. Permission was obtained from EPHI by providing a support letter from department of laboratory science. Samples were coded and confidentiality of patient data maintained throughout the study by locking and limiting accessibility of the study information

#### **5.11. Dissemination of the result**

The finding of this study will be presented and submitted to Addis Ababa University, College of Health Science Medical Laboratory Science Department and Ethiopian public health institute. It will also be presented for scientific community elsewhere and manuscript will be submitted to peer reviewed national or international journal and it will be presented in relevant workshops, seminars and scientific conferences.

## 6. Result

### 6.1. Socio-demographic characteristics of study subjects

The median age for the drug abusers and control groups were 27 and there was no statistically significantly difference in age among the drug abusers and control group. Most of the participants were within the age group of 20-24 years old (figure 9).

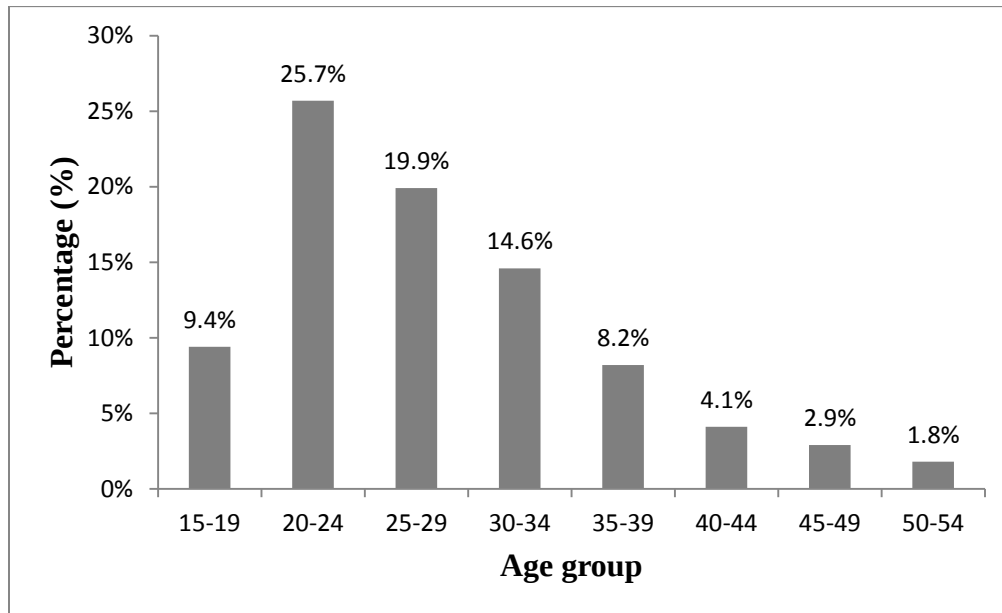


Figure 9: Age distribution of study participants visited Zewditu Memorial hospital, Addis Ababa, Ethiopia, 2017.

Among the 148 drug abusers, 77.7% (115) and 22.3% (33) were unmarried and married, respectively. Concerning educational background, 44.6% (66) and 27 % (40) of drug users were completed primary and high school, respectively. On the occupational status, most of the attendants 59.5(88) % were Hawker, street vendor, casual labor. [Table 1]

Table1: Socio-Demographic characteristics of study population visited Zewditu Memorial hospital, Addis Ababa, Ethiopia, 2017. [N=148]

| <b>Variables</b>                    | <b>Frequency</b> | <b>Percentage</b> |
|-------------------------------------|------------------|-------------------|
| <b>Marital Status</b>               |                  |                   |
| Married                             | 33               | 22.3              |
| Unmarried                           | 115              | 77.7              |
| Total                               | 148              | 100               |
| <b>School level</b>                 |                  |                   |
| Primary                             | 66               | 44.6              |
| high school                         | 40               | 27                |
| Preparatory                         | 23               | 15.5              |
| Vocational/Technical                | 8                | 5.4               |
| University                          | 11               | 7.4               |
| Total                               | 148              | 100               |
| <b>Occupational status</b>          |                  |                   |
| No Job                              | 28               | 18.9              |
| Waiter/bar manager/hotel            | 1                | 0.7               |
| Tourism/travel agent/tour guide     | 3                | 2                 |
| Mechanic, factory worker, labor     | 5                | 3.4               |
| Professional/teacher/banker         | 9                | 6.1               |
| Businessman                         | 1                | 0.7               |
| Hawker, street vendor, casual labor | 88               | 59.5              |
| Musician/dancer/performer           | 2                | 1.4               |
| Driver (private, taxi, truck)       | 7                | 4.7               |
| Drug dealer                         | 2                | 1.4               |
| Watchman/security guard             | 2                | 1.4               |
| Total                               | 148              | 100               |

## 6.2. Distribution of different drug of abuse used by study population

Three different drugs of abuse such as khat, marijuana, and heroin were included. Majority 44.6% (66) of study participants used heroin (Figure 2).

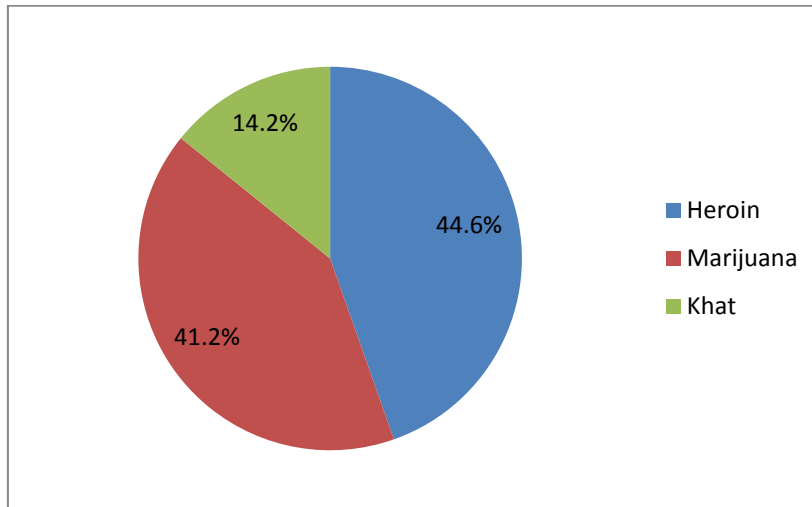


Figure 10: distribution of drug of abuse of study population visited Zewditu Memorial hospital, Addis Ababa, Ethiopia, 2017.

### 6.3. Comparison of hormone parameters among khat, marijuana and heroin users with control group

Serum testosterone level were significantly decreased in khat, marijuana and heroin abusers [ $p=0.03$ ,  $0.011$ , and  $0.004$ ], respectively, as compared to control groups. Similarly serum level of LH showed a significant decrease among three drug abuse groups compared to control. But serum level of estradiol was significantly increased among the khat , marijuana and heroin users [ $p=0.003$ ] for khat users,[ $p<0.001$ ] for marijuana and heroin users , respectively. The other hormone parameters [cortisol and [FSH] was not statistically significant among khat, marijuana and heroin users compared to control group [Table 2, 3, 4]

Table 2: Comparison of gonadal [E2,FSH,LH,T ]and cortisol hormone parameters among khat user and control groups visited Zewditu hospital, Addis Ababa, Ethiopia, 2017.

| <b>Hormone parameters</b> | <b>Control Median</b> | <b>Khat user Median</b> | <b>*P-value</b> |
|---------------------------|-----------------------|-------------------------|-----------------|
| Cortisol [ug/dl]          | 15.02                 | 16.23                   | 0.934           |
| E2 [pg/ml]                | 22.59                 | 39.42                   | 0.003           |
| FSH [mlu/ml]              | 3.22                  | 3.24                    | 0.869           |
| LH [mlu/ml]               | 6.16                  | 4.97                    | 0.003           |
| Testosterone [ng/dl]      | 7.95                  | 6.07                    | 0.03            |

\*Mann-Whitney test       $P\text{-value} : < 0.05$

Table 3: Comparison of gonadal [E2, FSH, LH, T] and cortisol hormone parameters among marijuana user and control groups visited Zewditu hospital, Addis Ababa, Ethiopia, 2017.

| <b>Hormone parameters</b> | <b>Control Median</b> | <b>Marijuana user Median</b> | <b>*P-value</b> |
|---------------------------|-----------------------|------------------------------|-----------------|
| Cortisol [ug/dl]          | 15.02                 | 14.01                        | 0.120           |
| E2 [pg/ml]                | 22.59                 | <b>43.93</b>                 | <0.001          |
| FSH [mlu/ml]              | 3.22                  | 3.01                         | 0.055           |
| LH [mlu/ml]               | 6.16                  | 5.01                         | 0.021           |
| Testosterone [ng/dl]      | 7.95                  | 6.31                         | 0.01            |

*\*Mann-Whitney test P-value: <0.05*

Table 4: Comparison of gonadal [E2, FSH, LH, T] and cortisol hormone parameters among heroin user and control groups visited Zewditu hospital, Addis Ababa, Ethiopia ,2017.

| <b>Hormone parameters</b> | <b>Control Median</b> | <b>Heroin user Median</b> | <b>*P-value</b> |
|---------------------------|-----------------------|---------------------------|-----------------|
| Cortisol [ug/dl]          | 15.02                 | 17.2                      | 0.120           |
| E2 [pg/ml]                | 22.59                 | <b>40.15</b>              | <0.001          |
| FSH [mlu/ml]              | 3.22                  | 2.37                      | 0.055           |
| LH [mlu/ml]               | 6.16                  | 5.65                      | 0.021           |
| Testosterone [ng/ml]      | 7.95                  | 6.62                      | 0.004           |

*\*Mann-Whitney test P-value: <0.05*

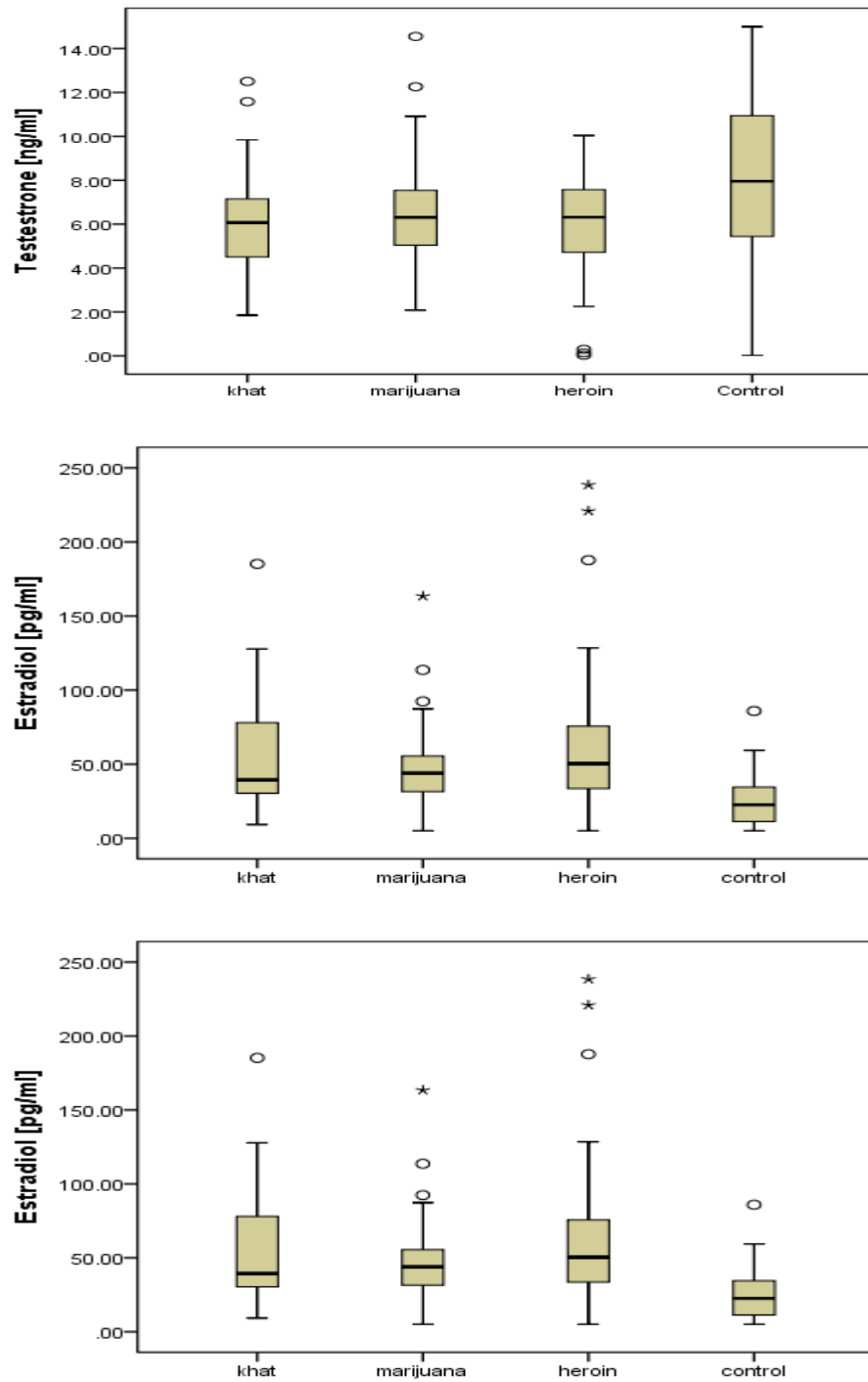


Figure 11: Box and whisker plot data comparison graph of median Testosterone, Estradiol and Luteinizing hormone levels among khat, marijuana, heroin and control groups.

#### 6.4. Correlation between the hormone parameters among heroin, marijuana and khat users and control group

There was a positive correlation between FSH and LH [ $p=0.001$ ], [ $p= <0.001$ ] among heroin and marijuana users respectively ,which lacks such correlation among control groups and khat users [table 5]. Moreover, FSH was negatively correlated with estradiol in heroin, marijuana and khat users [ $p= 0.033, 0.029, 0.018$ ] respectively. There was also a positive correlation between cortisol [ $p=0.005$ ] and estradiol [ $p=0.012$ ] in the heroin and marijuana groups. Estradiol was positively correlated with testosterone [ $p=0.002$ ] in the heroin group and negatively correlated with LH [ $p=0.039$ ] in the khat groups. None of the hormone parameters show correlation in the control group.

Table 5: Correlation between hormone parameters among heroin, marijuana, khat and control groups visited Zewditu Memorial hospital, Addis Ababa, Ethiopia, 2017.

| Hormone parameters         | Heroin(rho)     | Marijuana (rho)<br>Khat(rho) | Control (rho) |        |
|----------------------------|-----------------|------------------------------|---------------|--------|
| <b>FSH vs. LH</b>          | <b>0 .406**</b> | 0.519**                      | 0.292         | -0.274 |
| <b>FSH vs. E2</b>          | <b>-0.262*</b>  | -0.280*                      | -0.356*       | -0.356 |
| <b>Cortisol vs.E2</b>      | <b>0.343 **</b> | 0.320*                       |               |        |
| <b>E2 vs. Testosterone</b> | <b>0 .367**</b> | 0.210                        | 0.151         | 0.266  |
| <b>LH vs. E2</b>           | <b>-0.106</b>   | -0.119                       | -0.156*       | 0.003  |

*Rho: spearman correlation*

*p-value <0.05 \*: significant correlation*

## 6.5. Testosterone and associated factors in drug abusers visited Zewditu memorial hospital

The study result indicated that no significant association of socio demographic characteristics with testosterone.

Table 6: Testosterone and associated factors of khat, marijuana and heroin abusers visited Zewditu Memorial hospital, Addis Ababa, Ethiopia, 2017.

| Variables            | Testosterone  |           | Chi-square | p-value | df | Fisher exact |
|----------------------|---------------|-----------|------------|---------|----|--------------|
|                      | Normal (>4.1) | Low(<4.1) |            |         |    |              |
| Age                  |               |           |            |         |    |              |
| 15-19                | 15(93.8% )    | 1 (6.2%)  | 7.505      | 0.378   | 7  | 7.396        |
| 20-24                | 39(88.6%)     | 5(11.4%)  |            |         |    |              |
| 25-29                | 28(82.4%)     | 6(17.6%)  |            |         |    |              |
| 30-34                | 21(84%)       | 4(16%)    |            |         |    |              |
| 35-39                | 13(92.9%)     | 1(7.1%)   |            |         |    |              |
| 40-44                | 4(57.1%)      | 3(42.9%)  |            |         |    |              |
| 45-49                | 4(80%)        | 1(20%)    |            |         |    |              |
| 50-54                | 2 (66.7%)     | 1(33.3%)  |            |         |    |              |
| Marital status       |               |           |            |         |    |              |
| Married              | 26(78%)       | 7(21.1%)  | 1.352      | 0.245   | 1  | 1.542        |
| Unmarried            | 100(87%)      | 15(13%    |            |         |    |              |
| School level         |               |           |            |         |    |              |
| Primary              | 56(84.8%)     | 10(15.2%) | 4.156      | 0.385   | 4  | 3.288        |
| High school          | 35(87.5%)     | 5(12.5%)  |            |         |    |              |
| Preparatory          | 17(73.9%)     | 6(26.1%)  |            |         |    |              |
| Technical/vocational | 8 (100%)      | 0         |            |         |    |              |
| University           | 10(90.9)      | 1(9.1%)   |            |         |    |              |
|                      |               |           |            |         |    |              |

| Variables                | Testosterone  |           | Chi-square | pvalue | df | Fisher exact |
|--------------------------|---------------|-----------|------------|--------|----|--------------|
|                          | Normal (>4.1) | Low(<4.1) |            |        |    |              |
| Occupation               |               |           |            |        |    |              |
| No job                   | 24(85.7%)     | 4 (14.3%) |            |        |    |              |
| Waiter/bar manager       | 1 (100%)      | 0 (0%)    |            |        |    |              |
| Tourism/tour guide       | 2(66.7)       | 1(33.3%)  |            |        |    |              |
| Mechanic/factory worker  | 5(100%)       | 0(0%)     |            |        |    |              |
| Teacher/banker           | 8(88.9)       | 1(1.3%)   |            |        |    |              |
| Businessman              | 1             | 0         |            |        |    |              |
| Hawker, street vendor,   | 77(87.5%)     | 11(12.5%) |            |        |    |              |
| Casual labor             |               |           | 17.32      | 0.068  | 10 | 14.08        |
| Musician/dance performer | 0             | 2(100%)   |            |        |    |              |
| Driver                   | 5(71.4%)      | 2(28.6%)  |            |        |    |              |
| Drug dealer              | 2(100%)       | 0         |            |        |    |              |
| Watch man/security guard | 1(50%)        | 1(50%)    |            |        |    |              |
| Over dose                |               |           |            |        |    |              |
| Yes                      | 93(86.9%)     | 14(13.1%) |            |        |    |              |
| No                       | 32(82.1%)     | 7(17.9%)  | 2.512      | 0.285  | 2  | 2.913        |
| Don't know               | 1(50%)        | 1(50%)    |            |        |    |              |
| Types of drug abuses     |               |           |            |        |    |              |
| Khat                     | 18(85.7%)     | 3(14.3%)  |            |        |    |              |
| Marijuana                | 51(83.6%)     | 10(16.4%) | 0.197      | 0.906  | 2  | 0.256        |
| Heroin                   | 57(86.4%)     | 9(13.6%)  |            |        |    |              |

## 7. Discussion

Fertility is considered as a life conservative phenomenon among married couples which can be obliterated by various conditions affecting both males and females. In the other hand addiction to the use of drug abuse is a problem which increasingly developed among the various populations throughout the world, and there are evidences that drug of abuse affect the hypothalamus-pituitary-gonadal axis and sexual functions [73].

In the present study, the effect of marijuana , heroin and khat on the level of (LH, FSH ,testosterone, estradiol and cortisol),was tested in 61 marijuana ,66 heroin and 21 khat abuses male and the values were compared to the level of 23 non-users control.

Compared to the controls the present finding showed significant reduction in LH [ $p=0.021$ ] and testosterone [ $p=0.01$ ] among marijuana abuses .This findings are in agreement with the findings of previous study [74]. The latter study [74] reported that cannabinoids transiently impair pituitary function due to drug metabolic stress as reflected by decrement in LH and FSH hormones and hence reduced testosterone level, in which FSH was non-significant in our study.

The finding is also in agreement with a study done on Sudan [75], which reported highly significant decrease on LH and testosterone in marijuana abuse. It is again supported by an animal model study done in India[76] showed intraperitoneal injection of cannabis extract at low doses induced adverse effect on testes ,histology finding revealed significant shrinkage of tubular diameter and detrimental change in seminiferous epithelium of testes with resulting lowered serum testosterone and pituitary gonadotropins [LH ,FSH] levels.

A decrease of testosterone among marijuana users in the current study is in agreement with a study done on Nigeria [77,78], contradicts with a study done on USA on chronic marijuana users [79] which reported non-significant difference.

The decrease of testosterone might be attributed to inhibition of the gonadotrophin Releasing Hormone (GnRH) pulse generator in the hypothalamus by  $\Delta^9$ -THC [80].

A decrease in a testosterone observed in khat users in our study contradicts with previous study done in KSA [81] ,in which khat consumption resulted an increase in testosterone. On the other hand the finding which showed increased in estradiol is in agreement with the previous finding in KSA[81].It was previously reported that cathinone, which is one of the active ingredients in khat, is responsible for the decrease in testosterone concentration [82]

Effects of khat on testosterone was shown to be dose dependent [83] reported that, low concentrations of khat extract significantly increased whereas high concentrations suppressed testosterone production. This might imply that the decrease in testosterone observed in our study could be due high dose consumption of khat [in our study 72.3% were high dose users].

The increased concentration of estradiol observed in our study might be due to the conversion of testosterone to estradiol by aromatase or it might be due to estrogen saturates testosterone receptors in the hypothalamus, and so high estrogen may shut down the normal testicular production of testosterone [84,85].

In our findings heroin abuses had showed an increase in estradiol, whereas a decrease in LH and testosterone. This finding is in contrast with a study done on Pakistan and Iran [86, 87] which reported a non-significant difference between heroin addicts and control groups. Heroin use is thought to inhibit gonadotropin-releasing hormone production, which decreases the release of luteinizing hormone and subsequently reduces testosterone production [88].

A decreased serum level of Testosterone among heroin users in our finding is in accordance with a study done in Pakistan [89] which reported a significant decrease in testosterone values of young and mature addicts, and similar study on Hong kong [90] showed a significant decrease in testosterone and recovery of testosterone levels to normal occurred after about one month of heroin abstinence. This decrease of testosterone among heroin users might be due to the direct effect of heroin on the

hypothalamus and pituitary hormone secretions which can contribute to testosterone reduction [65].

The finding of decreased serum testosterone in our study is also in line with study done in Hong Kong [91] and USA [92] which reported decreased serum testosterone concentration in heroin abusers without consistent abnormalities in the other hormones.

## **8. Limitation of the study**

- ✓ This study was designed with small sample size
- ✓ The inclusion of only males and exclusion of females could potentially limit the validity and generalizability of these findings.
- ✓ Relevant background information related to the drug could not be accessed due to passive recruitment.
- ✓ Cutoff value for luteinizing hormone, follicle stimulating hormone, estradiol and cortisol was not used, only for testosterone is used.
- ✓ The failure to obtain objective measures of erectile function [impotence], such as measurement of SHBG and free hormones.

## 9. Conclusion

Our study indicated that estradiol was significantly higher in the three drug abuser groups than control groups. Similarly, Luteinizing hormone and testosterone were significantly lower in the drug abuser groups. However, follicle stimulating and cortisol hormone were not significantly different between drug abuse and control group.

Results showed a positive correlation between FSH and LH among heroin and marijuana users, which lacks such correlation among khat and control group. Similarly FSH was negatively correlated with estradiol among heroin, marijuana and khat users. There was a positive correlation between cortisol and estradiol in the heroin and marijuana groups ( $p=0.005$  and  $0.012$ ) respectively. Estradiol was positively correlated with testosterone in the heroin group and negatively correlated with LH in the khat groups. None of the hormone parameters show correlation in the control group.

No association between socio-demographic characteristics and testosterone was observed among the drug abusers.

## 10. Recommendation

Based on the above information:

- We recommend clinicians and health workers to primarily screen the level of gonadal hormones [LH, testosterone and estradiol] among drug abusers before further metabolic and physiatric support is initialized.
- Further experimental model research should be done on gonadal hormones (free hormones) in drug abusers to establish a clear mechanism and effect of these drugs which enhance intervention.
- Further prospective, randomized, controlled study with large sample size including female participants is recommended to investigate gonadal hormone profiles in those who abuse drug.
- The central mechanisms by which khat may affect reproductive functions is not yet clearly known although neurotransmitter such as dopamine is thought to play a role. Therefore, effect of khat extract and cathinone on brain neurotransmitter levels should be assessed in further works.
- Drug of abuse especially khat is commonly chewed together with other drugs such as coffee, tea and cigarette. In addition, alcohol is sometimes taken after the chewing session is over. The effect of concomitant use of such drugs on reproductive behaviors should be comprehensively studied.

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## **Annex**

### **Annex I: Information Sheet for the study**

**Addis Ababa University**

**College of Health Sciences**

**School of allied Health Sciences, Department of Medical  
Laboratory**

**Title:** Assessment of gonadal and cortisol hormone profile among drug abusers visited Zewditu memorial hospital for the purpose of HIV Behavioral and Biological Surveillance (IBBS) Survey ,Addis Ababa, Ethiopia

The study was conducted from stored serum sample and review of related information of the participants from previously collected data. There won't direct contact with study participants.

**Principal investigator:** Messeret Yibrah (BSc, MSc candidate)

**Advisors:**

Samuel kinde (MSc, PHD fellow)

Abebe Edao (MSc)

Atsbeha G/egziabxier (MSc)

**Sponsor:** **Mekelle University**

## **Annex II: SOP (Standard operating procedure)**

### **A. Test principle and procedure of total testosterone test**

Competitive immunoassay with analyte liberation was applied.

1st Incubation (9 minutes): 20  $\mu$ L of the sample is incubated with a biotinylated monoclonal testosterone specific antibody and 2-bromoestradiol to release testosterone, with the amount of antibody binding sites subsequently occupied depending on the concentration of testosterone in the sample.

2nd Incubation (9 minutes): Streptavidin-coated micro particles and a ruthenylated testosterone derivative are added to the reaction mixture and the complexes bind to the solid phase via biotin-streptavidin interactions.

Measurement method: Electro chemiluminescent

The reaction mixture is transferred to a measuring cell and the micro particles are magnetically captured onto the surface of an electrode; unbound sample is washed away before a chemiluminescent reaction is induced by applying a voltage to the electrode. Chemiluminescence is measured by a photomultiplier and the concentration of Testosterone within the sample is calculated using a calibration curve .[50].

### **Reagent:**

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL, preservative.

R1 Anti-testosterone-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-testosterone antibody (sheep) 40 ng/mL; releasing reagent 2-bromoestradiol; MES buffer 50 mmol/L, pH 6.0; preservative.

R2 Testosterone-peptide~Ru(bpy) (black cap), 1 bottle, 9 mL: Testosterone derivative, labeled with ruthenium complex 1.5 ng/mL; MES buffer 50 mmol/L, pH 6.0; preservative

## **B. Test principle and procedure of LH test: Sandwich principle**

1st Incubation (9 minutes): 20  $\mu$ L of the sample is incubated with both a biotinylated, monoclonal LH-specific antibody and a ruthenylated, monoclonal LH-specific antibody to form a sandwich complex.

2nd Incubation (9 minutes): Streptavidin-coated microparticles are added to the reaction mixture and the complex binds to the solid phase via biotin-streptavidin interactions.

Measurement: The reaction mixture is transferred to a measuring cell and the microparticles are magnetically captured onto the surface of an electrode; unbound sample is washed away before a chemiluminescent reaction is induced by applying a voltage to the electrode. Chemiluminescence is measured by a photomultiplier and the concentration of LH within the sample is calculated using a calibration curve [51].

### **Reagent:**

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-LH-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-LH antibody (mouse) 2.0 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.

R2 Anti-LH-Ab~Ru(bpy) (black cap), 1 bottle, 10 mL: Monoclonal anti-LH antibody (mouse) labeled with ruthenium complex 0.3 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.

Quality control: PreciControl Universal. PC U1 and PC U2 was used, in addition other suitable material can be used.

### **C. Test principle and procedure of FSH test**

Sandwich principle. Total duration of assay: 18 minutes.

1st incubation: 24  $\mu$ L of sample, a biotinylated monoclonal FSH-specific antibody, and a monoclonal FSH-specific antibody labeled with a ruthenium complex) form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induce chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the cobas link .

#### **Reagent:**

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-FSH-Ab biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti - FSH antibody (mouse) 0.5 mg/L, MES buffer 50 mmol/L, pH 6.0; preservative.

R2 Anti-FSH-Ab Ru(bpy) (black cap), 1 bottle, 10 mL: Monoclonal anti - FSH antibody (mouse) labeled with ruthenium complex 0.8 mg/L, MES buffer 50 mmol/L, pH 6.0; preservative.

#### **Quality control**

Preci Control Universal. PC U1 and PC U2 was used, in addition other suitable material can be used.

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## Declaration

### Assurance of principal investigator

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this application.

**Name of the student: Messeret Yibrah Yeebio**

Date \_\_\_\_\_

Signature \_\_\_\_\_

### Approval of Advisors:

**Samuel Kinde ,MSc, PhD candidate**

Date \_\_\_\_\_

Signature \_\_\_\_\_

**Abebe Edao ,MSc**

Date \_\_\_\_\_

Signature \_\_\_\_\_

**Atsbeha G/egziabxier , MSc**

Date \_\_\_\_\_

Signature \_\_\_\_\_

