THE ROLE OF KHAT IN EXACERBATING MANGANESE-INDUCED DELETERIOUS EFFECTS AND PUTATIVE RESCUE BY COENZYME-Q_{10} IN A MOUSE MODEL

KENNEDY CHEPUKOSI WEKESA
BSc (Periyar); MSc (Periyar)
(SSAY/06460P/2014)

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DECLARATION

This thesis is my original work and has not been presented in any other institution for a degree award or other qualification.

KENNEDY CHEPUKOSI WEKESA
REG. NO. SSAY/06460P/2017
Signature _____ Date _____

This thesis has been submitted with our approval as supervisors:

Signature _____ Date _____

PROF. ALFRED ORINA ISAAC
PROFESSOR OF PHARMACEUTICAL SCIENCES
THE TECHNICAL UNIVERSITY OF KENYA

Signature _____ Date _____

DR. PATRICK W. OKANYA
SENIOR LECTURER
DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY
THE TECHNICAL UNIVERSITY OF KENYA

Signature _____ Date _____

DR. NGALLA JILLANI
SENIOR RESEARCH SCIENTIST
INSTITUTE OF PRIMATE RESEARCH

DEDICATION

I dedicate this thesis to the Almighty God for the blessing of life and never-ending love. Secondly, I dedicate this thesis to my father Martin Chepkosi and my late mother Caroline Nasambu for their mentorship and upbringing. I also dedicate this thesis to my wife Caren Akoth and my siblings Phanice, Michael, Mary, Jackline, Faith, Purity, Janet and Chris. Lastly, this thesis is dedicated to my beloved children, Keysha and Jason Chepukosi.

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Co-Q_{10}	Coenzyme-Q_{10}
DAB	3.3, diaminobenzidine
ELISA	Enzyme linked immunosorbent assay
ETC	Electron transport chain
GSH	Reduced glutathione
GSSG	Oxidized glutathione
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA reductase
IL	Interleukin
INF-γ	Interferon gamma
MAPK	Mitogen-activated protein kinase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MPTP	1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
MPV	Mean platelet volume
NAC	N-Acetylcystein
NADPH	Nicotinamide adenine dinucleotide phosphate reduced
NF-κB	Nuclear factor kappa B
PDW	Platelet distribution width
RDW-CV	Red cell distribution width coefficient of variation
RDW-SD	Red cell distribution width standard deviation

ROS	Reactive oxygen species
sGOT	Serum glutamic oxaloacetate transaminase
sGPT	Serum glutamic pyruvic transaminase
SOD-1	Superoxide dismutase-1
TNF-α	Tumor necrosis alpha

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ABSTRACT

Khat, a plant containing phytochemicals that act as psychostimulants is widely grown and used in Kenya and East Africa. It contains amphetamine-like chemicals, cathine and cathinone, largely responsible for hematotoxicity and organ damage. There is growing use of this herb in rural and urban areas heavily polluted with environmental toxicants. Limited research has been done on khat toxicity, more so, on how to counter it. In addition, there is limited information on the biochemical, physiological and neurological changes due to co-exposure to khat and the other metal toxicants commonly present in polluted environments, such as manganese. This study was conducted to elucidate the toxicological profile of khat with or without manganese and the putative ability of coenzyme-Q_{10} (CoQ_{10}) to modulate negative physiological and biochemical processes due to khat and manganese-induced toxicities. This laboratory-based research involved the use of a well characterized mouse model in which the groups of Swiss albino mice were either treated with khat or manganese or CoQ_{10} or the three treatments combined. Group one was the control group that was not given any of the treatments; group two was supplemented with CoQ_{10} (200mg/kg b/w); group three was given khat extract (1500mg/kg b/w); group four had free access to water dissolved in manganese *ad libitum* (4g/L); group five was co-treated with CoQ_{10} (200mg/kg b/w) and khat (1500mg/kg b/w); group six was given CoQ_{10} (200mg/kg b/w) and had free access to manganese in water (4g/L); group seven was given khat (1500mg/kg b/w) and had free access to manganese dissolved in water (4g/L b/w), lastly, group eight was treated with CoQ_{10} (200mg/kg b/w) and khat (1500mg/kg b/w) and had free access to manganese in water *ad libitum* (4g/L). The study was conducted in two phases to simulate subchronic (90 days) and chronic (132 days) exposure to khat. A series of experiments were performed according to the study objectives. They included: monthly body weight measurements and rapid murine and behavioral score (RMCBS), biochemical assay for liver and kidney biomarkers, reduced glutathione (GSH) assay for oxidative damage, cytokine assay for changes in immune response and histology for organ pathology. Subchronic and chronic exposure to khat induced hematological perturbations leading to anemia that were nullified in the presence of CoQ_{10}. During the subchronic phase, khat caused neurological disturbance that was exacerbated on exposure to manganese (khat+Mn^{++}). However, CoQ_{10} appeared to restore the khat and manganese-induced neurological dysfunctions. Additionally, there was severe hepatotoxicity and nephrotoxicity following simultaneous exposure to khat and manganese as depicted by the elevated liver (AST, ALT, GGT, total bilirubin) and kidney (creatinine) biomarkers. Notably, supplementation with CoQ_{10} attenuated khat and/or manganese-induced hepatotoxicity and nephrotoxicity. Exposure to khat for 90 days and 132 days resulted in oxidative damage in the brain, liver and kidney as depicted by the significant GSH elevation. Nonetheless, supplementation with CoQ_{10} nullified the oxidative damage. Further findings revealed that, in the presence of manganese, the oxidative damage was enhanced; with CoQ_{10} suppressing the heightened oxidative damage. Lastly, subchronic and chronic exposure to khat caused inflammation which was enhanced in the presence of manganese as exhibited by the increased levels of the pro-inflammatory cytokines (TNF-α and IFN-γ). Notably, CoQ_{10} modulated khat and/or manganese driven proliferation of inflammatory cytokines. The results in the current study demonstrate that khat negatively affects vital physiological and biochemical processes and induces multiple organ damage and affects the immune system. Moreover, and for the first time, the results show that chronic exposure to manganese exacerbated multiple forms of toxicities following co-exposure with khat. Additionally, the outcomes demonstrate that CoQ_{10} may be considered as an appropriate pharmacological mitigation against khat-driven toxicity.

Keywords: Khat, Coenzyme-Q_{10}, Manganese, Oxidative stress, Inflammation, Neurotoxicity

CHAPTER ONE

INTRODUCTION

1.1 Background information

Khat (*Catha edulis*, Forsk), is a stimulant herb of socio-economic importance in East Africa (Getasetegn, 2016; Patel, 2015). It belongs to the *Celastraceae* family (Getasetegn, 2016) and is widely consumed due to its stimulant properties (Engidawork, 2017; Kimani *et al.,* 2016). Some communities in East Africa use it for medicinal purposes (Ketema, 2015). Khat contains alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals (Getasetegn, 2016). The alkaloid fraction of khat contains cathinone and cathine, constitute its psychoactive components (Getasetegn, 2016). Cathinone and cathine share structural and pharmacological similarities with amphetamines; hence, it has been postulated that long term khat consumption causes toxicities similar to those of long term amphetamine use (Lemieux *et al.,* 2015). Abuse of amphetamine and its derivatives has been linked to structural abnormalities in the brain with subsequent behavioural abnormalities (Geresu, 2015; Kalix, 1996).

Side effects associated with long term khat consumption include direct effects on the brain, liver and kidney (Kimani *et al.,* 2016; Al-mehdar *et al.,* 2012). The various chemicals and to a larger extent the alkaloids, cathinone and cathine, have been implicated in khat-induced structural and neurobehavioral deficits in the brain of khat-exposed subjects. Studies in Yemen have shown that long term khat consumption causes psychotic behaviour (Ayano *et al.,* 2019; Odenwald *et al.,* 2005).

Additionally, cognitive dysfunctions, memory deficits, psychotic and manic behaviours following exposure in humans and animal models have been documented (Leyrer-Jackson *et*

al., 2019; Bogale *et al.,* 2016; Kimani *et al.,* 2016; Castellani, 1982;). These abnormalities are attributed to the direct and indirect effect of the various khat components on the dopaminergic regions of the brain critical in initiating motor activity, memory and cognitive function (Mihretu *et al.,* 2017; Geresu, 2015; Al-habori, 2005). Even though there is still insufficient data on the exact mechanism of metabolism of cathinone in the central nervous system (CNS), there is enough data proving the involvement of the dopaminergic pathways (Kalix, 1996).

Like many other psycho-stimulants, chronic khat consumption has been associated with addiction. Despite the increasing debates on the topic, this assumption is based on the dependence-inducing potential of amphetamines and cathinone derivatives (Bedada *et al.,* 2010; Kalix, 1996; Tariq *et al.,* 1987). From this, an assumption can be made that the various chemicals in khat interact with the striato-cortico-limbic dopaminergic pathways. Additionally, the chemicals present in khat appear to cause structural damages to the dopaminergic and serotonergic systems.

Cases of khat-induced hepatotoxicity and nephrotoxicity in human and animal models have also been reported widely. For instance, human fatalities following severe khat-induced hepatitis cases have been recorded in Yemen and Somalia (Riyaz *et al*., 2014; Kassim *et al.,* 2010). The latter hospital reports and fatalities involved subjects with a history of chronic khat chewing, and/or confounding habits such as smoking and/or alcohol drinking. Such reports may perhaps implicate khat directly in exacerbation of disease condition. Findings in animal studies have implicated the chemical components in khat in the induction of various forms of hepatotoxicity (Abdul-Mughni *et al.,* 2018; Riyaz *et al.,* 2014; Alsalahi *et al.,* 2012). In addition, adverse khat-induced nephrotoxicity has also been reported in animal models, and especially rodents (Abdul-Mughni *et al.,* 2018; Alsalahi *et al.,* 2012). A patient of Somalia decent reported to a United Kingdom (UK) hospital with khat-induced mixed nodular cirrhosis in his liver and kidney congestion and died from the complications (Riyaz *et al.,* 2014). Khat-induced cardiovascular complications in human and animal models have also been recorded severally

(Ayano *et al.,* 2019; Cochrane *et al.,* 2016). It has been shown that exposure to cathinone induces increased diastolic pressure in mice rats. In addition, in a study involving human volunteers it was noted that khat chewing increases blood pressure following increased plasma levels of cathinone (Al-motarreb *et al.,* 2010). The above mentioned studies implicate khat use in various forms of organ pathology.

Blood is vital for mammalian system functioning; hence, a measure of the blood parameters is often used to assess the normal functioning of the subjects. Subchronic and chronic exposure to khat extracts in animal models resulted in anaemia as shown by the low levels of Red Blood Cells (RBCs) (Kennedy *et al.,* 2020; Ismaeel *et al.,* 2014). The potential of khat to induce anaemia has also been shown in human beings (Kedir *et al.,* 2013). Thrombocytosis and leucocytosis have additionally been reported as the side-effects associated with subchronic and chronic khat exposure (Ketema *et al.,* 2015).

The above-mentioned studies suggest that consumption of khat is accompanied by a myriad of adverse and life-threatening side effects. Despite the wide body of literature on the khat-induced deleterious effects, there has been an increase in the number of khat consumers locally and globally. Compounding the crisis further, is the fact that there has been limited focus on studies on intervention strategies and molecular events following simultaneous exposure to khat and manganese. An additional concern is the fact that a majority of the khat chewers reside in urban areas that are heavily polluted with heavy metals like manganese and other toxicants such as lead, mercury arsenic and cadmium; this influenced the choice of manganese to simulate co-exposure to khat and heavy metal in the current study.

Manganese is a trace element and a key component of the metalloenzymes (Santamaria, 2008). It is also a known neurotoxin with multiple routes of exposure (Isaac *et al.,* 2007; Perl *et al.,* 2007; Isaac *et al.*, 2006). People working in mining and welding have higher risks of occupational exposure (Guilarte *et al.,* 2008; Santamaria, 2008). The other routes of excess exposure include exposure to some hospital equipment, soil, contaminated air, food and water

(Frick *et al.,* 2011; Santamaria, 2008; Sánchez *et al.,* 1993). Reports of incidences of neurotoxicity, hepatotoxicity and nephrotoxicity following manganese exposure have been on the rise (Oladipo *et al.,* 2016; Milatovic *et al.,* 2009; Khan *et al.,* 1997).

Manganese neurotoxicity leads to *"manganism"*, a neurological disorder that presents with symptoms similar to Parkinson's disease (Milatovic *et al.,* 2009; Santamaria, 2008). The primary target for manganese toxicity in the brain is the globas pallidus, substantia nigra and other dopamine-rich brain regions because of their susceptibility to oxidative injury (Martinez-Finley *et al.,* 2013). Similarly, the dopamine rich regions of the brain are the primary targets of khat-induced neurotoxicity as earlier mentioned (Kimani *et al.,* 2016). Hence, it was hypothesized in this study that khat-induced injuries in the dopaminergic system may be exacerbated by acute exposure to environmental toxins such as manganese.

Etiologically, oxidative stress and inflammation play a key role in khat and manganese-induced pathology. In the brain, exposure to cathinone and cathine results in increased production of dopamine, which produces charged metabolites during its metabolism (Feyissa *et al.,* 2008; Kalix, 1996). In addition, charged metabolites during the metabolism of the non-alkaloid fraction of khat contributes to the increased oxidative burden in the cells; hence, cell damage via oxidative stress (Al-Zubairi *et al.,* 2003). On its part, manganese induces oxidative stress by sequestration of manganese ions into the mitochondria, hence, disrupting the normal cell respiration (Martinez-Finley *et al.,* 2013). It has also been shown that accumulation of manganese in the dopamine-rich region of the brain often induces auto-oxidation of dopamine, hence, generating reactive oxygen species (Martinez-Finley *et al.,* 2013). These findings are significant given that subchronic and chronic exposure to khat similarly result in increased cellular oxidative stress burden.

Cytokines play an immune-modulatory role in mammals, hence, perturbations in the levels of cytokines leads to disease conditions. There is sufficient data implicating khat in the proliferation of the pro-inflammatory cytokines (Ali *et al.,* 2015). Similarly, manganese exposure reportedly induces proliferation of inflammatory markers (Ozgur *et al.,* 2019; Mokgobu *et al.,* 2015). Proliferation of pro-inflammatory cytokines has been associated with diseases such as hepatitis (Connolly *et al.,* 2009). From the above studies, general assumptions can be made, that is co-exposure to khat and manganese may lead to heightened inflammation and oxidative stress conditions in the subject, and perhaps potent antioxidants and anti-inflammatory agents may be useful against khat and manganese-induced oxidative stress and inflammation. Therefore, in the current study, Co-enzymeQ_{10} (CoQ_{10}) was used in order to test effectiveness against khat and manganese induced oxidative stress condition and inflammation.

CoQ_{10} is a potent antioxidant and anti-inflammatory agent (Fuller *et al.,* 2006). The choice of CoQ_{10} in this study was informed by promising findings from previous studies in the treatment of neurodegenerative diseases following neurotoxin exposure (Mancuso *et al.,* 2009; Spindler *et al.,* 2009). In addition, choice was influenced by previous data about its potency as an anti-inflammatory and antioxidant (Monsef *et al.,* 2019; Zhai *et al.,* 2017; Schmelzer *et al.,* 2008). A wide body of literature has demonstrated CoQ_{10}'s usefulness in treating various diseases mediated by oxidative stress and inflammation (El-Laithy *et al.,* 2018; Fuller *et al.,* 2006; Shaeffer *et al.,* 1980). However, the impact of CoQ_{10} on oxidative stress and inflammation following exposure to khat and/or manganese is yet to be elucidated.

1.2 Statement of the problem

Consumption of khat is a major public health concern in Kenya. The debate on whether khat is harmful or not is increasing with no clear government policy direction. The shrub is chewed by

youths and adults during social events for stimulant properties and, in some communities for medicinal properties (Carrier, 2008; Carrier *et al.,* 2006). The alkaloid fractions (cathinone and cathine) have been associated with its stimulant properties, with cathinone being the most potent of the two (Kalix, 1996). In the USA, cathinone is grouped as schedule I drug with the highest potential for abuse, whereas cathine as a schedule IV drug (Kassim *et al.,* 2010). A wide body of literature implicates khat consumption in psychological and peripheral organ damages (Bedada *et al.,* 2010; Kassim *et al.,* 2010; Al-Akwa *et al.,* 2009).

Despite the widespread knowledge on the deleterious effects connected with khat use, the prevalence of consumption is still on the rise. Moreover, khat chewers range from young teenagers to aging adults with a khat chewing history that spans years (Carrier, 2008; Aden *et al.,* 2006). Khat-associated deleterious events have been the focus of several investigators, however, studies on co-exposure of chronic khat and environmental toxins such as manganese are still lacking. Importantly, both khat and manganese have been demonstrated to trigger their deleterious events via generation of reactive oxygen species (ROS), reactive nitrogen species (RNS), proliferation of inflammatory markers and hematological disturbances (Ali *et al.,* 2015; Mokgobu *et al.,* 2015; Frick *et al.,* 2011; Al-Zubairi *et al.,* 2003). Further investigations were done to determine the potential of CoQ_{10} in mitigating khat and manganese-driven toxicities. The justification for this approach is anchored on the fact that CoQ_{10} has demonstrated antioxidant and anti-inflammatory properties that have been well established in toxicities as a result of chemical toxins (Mwaeni *et al*., 2021;Kennedy *et al*., 2020; Nyariki *et al*., 2019). This approach endeavors to develop a potential nutritional and pharmacological intervention to assuage khat and manganese driven organ injury.

1.3 Justification

Cultivation and consumption of khat is a deep rooted culture in some communities in Kenya (Engidawork, 2017; Patel, 2015). Additionally, the psycho-stimulant herb is sold locally and internationally, this underscores its economic value to these communities. Despite the growing body of literature on the side effects accompanying khat consumption, there has been an upsurge in consumption rates locally and on a global scale (Kassim *et al.,* 2010; Aden *et al.,* 2006). Compounding this public health crisis further, is the growing population of youthful consumers, this presents a public health challenge given the high potential for addiction associated with cathinone (Patel, 2015; Kalix, 1996).

There is documented evidence of chronic khat use locally and globally (Ayano *et al.,* 2019; Carrier, 2008). This is worrying given the toxic nature of this herb even with subchronic consumption (Roelandt *et al.,* 2011). Moreover, there is lack of sufficient information on the scale of body organ damage associated with chronic exposure and co-exposure with environmental toxins such as manganese. Therefore, the current research findings are significant as they show that chronic exposure to this psycho-stimulant herb is associated with marked damages in the brain, liver and kidney. Furthermore, the findings also show exacerbated pathological conditions associated with co-exposure to the psycho-stimulant and the environmental toxin manganese.

There has been little focus by researchers in finding intervention strategies against subchronic and chronic khat-induced toxicities, this warrants urgent and further research into this grey area. As an intervention effort, this study sought to investigate the putative role of CoQ_{10} against subchronic and chronic khat and/or manganese.

1.4 Objectives

1.4.1 General Objective

To investigate the impact of chronic khat use and manganese on the induction of deleterious events, and potential ameliorative effects of CoQ_{10} in restoring resultant pathophysiology

1.4.2 Specific Objectives

i. To determine the effect of CoQ_{10} on host physiological and hematological changes following khat-induced toxicity
ii. To decipher the ameliorative effect of CoQ_{10} on khat and manganese driven oxidative stress and inflammation
iii. To elucidate the impact of khat and manganese driven pathological and immunological events

1.5 Research questions

1. What are the outcomes of CoQ_{10} supplementation on khat-driven physiological and hematological changes?
2. Does CoQ_{10} have the potential to ameliorate khat and manganese driven oxidative stress and inflammation?
3. What is the extent of chronic khat use and manganese on driving pathological and immunological events?

CHAPTER TWO

LITERATURE REVIEW

2.1.1 Khat

Khat (Figure 2.1) has been highlighted as a herb of significant public health concern in the East African and the Arabian regions, (Ayano *et al.,* 2019; Getasetegn, 2016; Carrier *et al.,* 2006). People chew khat to enhance their level of attention, for social interaction and increase their work output capacity (Ketema *et al.,* 2015; Carrier, 2008). It has been used in the treatment of asthma, depression, fatigue, stress and also for abating hunger (Ketema *et al.,* 2015; Kimani *et al.,* 2008).

The consumption of khat affects individuals' health and socioeconomic development of a nation (Carrier, 2008). For this reason some countries have categorized this psychostimulant drug as a schedule I drug given its higher potential for addition (Kassim *et al.,* 2010). In Europe, there have been debates on whether to ban its use in some European countries (Pantano *et al.,* 2016). In Kenya, khat holds a socio-economic value for many Kenyans and as such, it is frequently used during social functions by the youths, who also consider it fashionable (Carrier *et al.,* 2006). For the farmers, cultivation of this herb is the main source of income (Carrier, 2008). In Kenya, the national agency for the campaign against drug abuse (NACADA) has listed khat as one of the main drugs abused by Kenyans (Carrier, 2008). Despite this, the substance has not been banned from use leading to its growing popularity.

There are numerous forms of toxicities associated with khat use (Mega *et al.,* 2017). The range is from hematological disturbances, to multiple organ toxicities (Riley *et al.,* 2020; Berhe *et al.,* 2015; Alsalahi *et al.,* 2012). Importantly, the khat-induced toxicities are preceded

by cathinone and cathine's action leading to physiological, biochemical and multiple organ pathology. It has been shown that the khat variety found in Kenya contains the highest concentration of cathinone (Al-habori, 2005).

Figure 2.1: Khat leaves

2.1.2 Phytochemical composition of khat

The two main psycho-active constituents of khat, cathinone and cathine are alkaloids (Figure 2.2), (Kalix, 1996). Additionally, khat contains non-alkaloid fractions such as tannins in considerable quantities, sterols (a-sterol and b-sterol), terpenoids, carotene, calcium, thiamine, riboflavin, essential oils, niacin and iron.

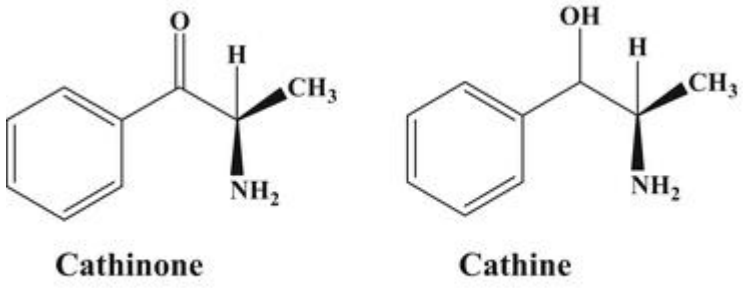


Figure 2.2: Psycho-active components of khat (Kalix, 1996).

2.1.3 Pharmacology of cathinone and cathine

The alkaloids cathine and cathinone are responsible for khat's pharmacological activity (Al-motarreb *et al.,* 2005). Optimal levels of an oral dose of khat are normally attained after 120 mins, while the half-life is approximately 4 hours (Widler *et al.,* 1994). The principal metabolite from the breakdown of cathinone, is norephedrine (Patel, 2015) and minute quantities of norpseudoephedrine are also produced (Getasetegn, 2016). Pharmacological studies have shown that less than 7% of norpseudoephedrine is excreted in its unchanged form in urine (Nichols *et al.,* 2015; Cox *et al.,* 2003).

Body temperature, and increased blood pressure are some of the effects that appear immediately after khat consumption (Feyissa *et al.,* 2008). The effects depend on the potency and amount absorbed into the system (Cox *et al.,* 2003; Kalix, 1996). On the central nervous system (CNS), immediate effects include euphoria, alertness, and feeling of well-being (Kimani *et al.,* 2016). The cathinone and cathine in khat exert their effects via the dopamine and noradrenaline pathways. Cathinone enhances dopamine release, inhibits dopamine reuptake and blocks the action of monoamine oxidase (Figure 2.3).

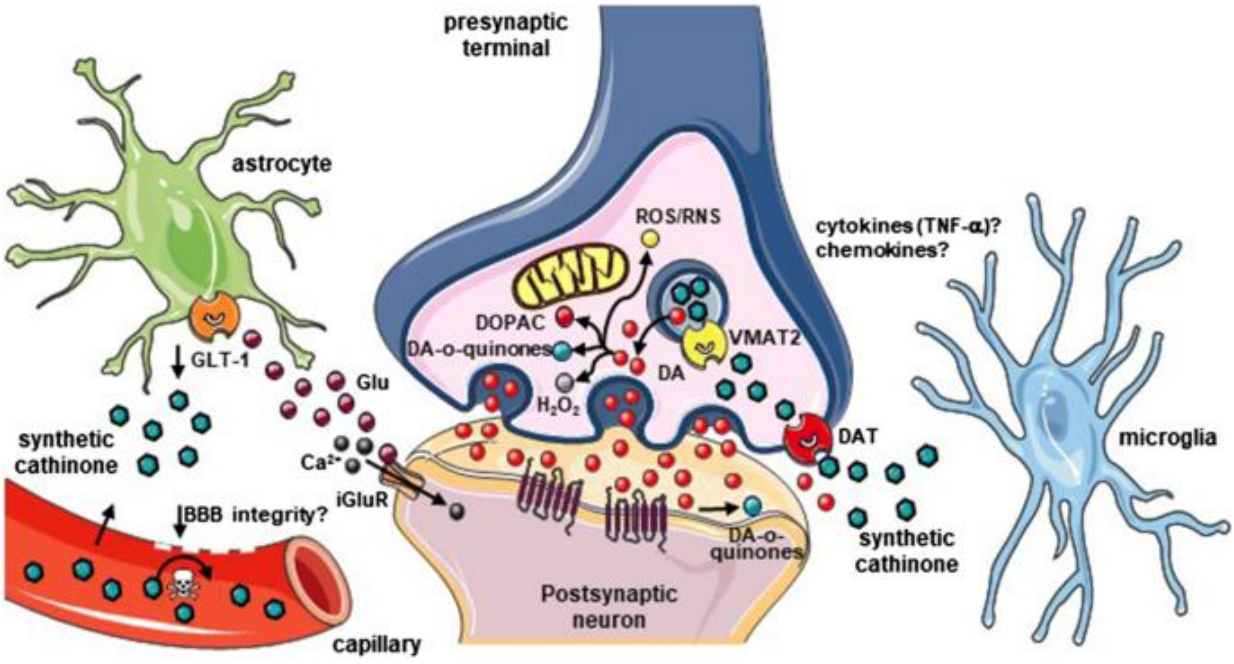


Figure 2.3: Mechanism of action by which cathinone and cathine induce their stimulatory effects (Leyrer-Jackson *et al.*, 2019).

2.1.4 Effects of khat on neurobehavioral function and memory

Kimani and Nyongesa (2008), utilizing the Morris water maze task performance, found out that khat extract selectively affects long term and short-term memory as well as learning, with a low dose having no effect on learning but impairing memory. In the same study, a high dose impaired learning but improved memory. These findings demonstrate that exposure to khat either at small or high doses with varied durations of exposure may have varied effects on neurophysiological systems of the subjects. In other studies, exposure to khat causes loss of both long term and short term memory as well as learning memory (Assefa *et al.,* 2018; Mohammed *et al.,* 2014). In another Morris water maze experiment, Mohammed *et al.* (2014), demonstrated that 200mg/kg Khat results in functional changes in mice without morphological effects.

Other neuropsychopharmacological studies have been carried out as well using animal models. In a previous study using lactating mice at postnatal day 28, effects of crude khat extract on motor coordination and emotional stability was evaluated (Bedada *et al.,* 2010). Interestingly, the study demonstrated increased latency in cliff avoidance and reduced forelimb strength following amphetamine and khat treatment. The observed change may be attributed to the development of tolerance to the performance-disrupting action of khat after repeated dosing (Adorjan *et al.,* 2017).

Studies to elucidate the underlying neurochemical mechanisms by which khat and amphetamine-like substances cause morphological and behavioral changes in humans and animals have provided sufficient evidence of khat-induced alteration of dopaminergic and serotonergic neurotransmitters (Riley *et al.,* 2020; Bedada *et al.,* 2010; Murray *et al.,* 2008).

2.1.5 Khat and addiction

Khat has a high potential for addiction demonstrated by an earlier study conducted in the North Eastern region of Kenya where a high percentage (62%) of khat chewers developed withdrawal symptoms and exhibited moodiness following attempts to stop khat consumption (Aden *et al.,* 2006). Nightmares, mild trembling and depression have been reported as some of the withdrawal symptoms following khat consumption (Kassim *et al.,* 2010). In addition, there's a compulsive need by the khat chewers to secure their daily portion of this psycho-stimulant herb (Feyissa *et al.,* 2008).

Even though there is still no known clear mechanism behind khat's addiction induction potential, it is believed that this could be due to the presence of cathinone in khat, which bears chemical similarities with amphetamines; hence, producing similar behavioral and

physiological impacts in users (Patel, 2015; Feyissa *et al.,* 2008; Kalix, 1996). Worthy of note, tolerance and withdrawal symptoms are associated with amphetamine use (Kassim *et al.,* 2010).

2.1.6 Khat-induced oxidative stress

Free radical scavenging systems and antioxidants protect the cell against oxidative damage (Dukhande *et al.,* 2009; Nyariki *et al.,* 2019). Free radicals and their reactions are involved in the etiology, development and progression of some diseases, including life threatening ones (Abdelwahab *et al.,* 2018). In an effort to evaluate the modulatory effects of khat on circulating free radicals, Al-Qirim *et al.*(2002) established that khat decreased the free radical metabolizing enzymes glutathione transferases (GST), superoxide dismutase (SOD) and catalase in rats while the concentration of uric acid was increased. Superoxide dismutase, catalase and GST play an important role in scavenging free radicals and their products (Al-qirim *et al.,* 2002). The reduced activities of catalase, SOD and GST may be responsible for the elevated free radical levels in stress (Al-Zubairi *et al.,* 2003). The observed increase in uric acid may be attributed to the body's natural response to scavenge excessive free radicals produced because uric acid is one of the quenchers of free radicals (Ketema *et al.,* 2015).

In a study by Al-Akwaa *et al*. (2009), humans consuming khat over a period of over 12 months, at weekly interval, had increased plasma free radicals when compared to control subjects (abstainers). Elevated free radicals observed in the study was linked directly to the effects of cathine and cathinone, or indirectly through the exposure of khat to pesticides during or after cultivation (Al-Akwa *et al.,* 2009). It has been suggested that the metabolism of the various khat components result in charged metabolites; hence the increase in reactive oxygen species (Abdelwahab *et al.,* 2018; Al-mehdar *et al.,* 2012; Al-Zubairi *et al.,* 2003)

2.1.7 Khat-induced nephrotoxicity

It is well established that exposure to khat induces nephrotoxicity in animal models (Ketema *et al.,* 2015; Alsalahi *et al.,* 2012). One study for instance demonstrated that a moderate dose of khat (400mg/Kg body weight) was able to induce mild nephrotoxicity in rats where the kidney biomarker creatinine was increased, and the Bowman's capsule damaged (Shewamene *et al.,* 2014). In other studies khat-induced nephrotoxicity was demonstrated by treatment of rabbits with moderate to high doses of khat, which induced subscapular hemorrhages with vacuolar degeneration of renal tubular epithelium (Abdul-Mughni *et al.,* 2018).

In another study, Gitonga et al. (2017) observed that mice exposed to khat extract for a subchronic period of time presented with enlarged capsular glomeruli, a finding that was accompanied by increased kidney damage biomarkers creatinine and blood urea nitrogen (BUN). Furthermore, high dose of khat induced kidney lesions and necrotic lesions upon subchronic administration of khat extract to mice (Alsalahi *et al.,* 2012). Human studies have demonstrated the potential of khat to induce nephrotoxicity. In line with this, a 35 year old patient with a history of repeated khat consumption presented with kidney congestion and high levels of cathinone in his blood (Corkery *et al.,* 2011).

While there is growing body of literature on khat-induced nephrotoxicity in animal models and in humans, there is still no clear information on the exact mechanism behind the reported khat-induced nephrotoxicity. However, from the histopathological findings from earlier studies, it is plausible to assume that the various chemicals present in khat trigger biochemical processes within the cells leading to kidney cell death and ultimately kidney damage.

2.1.8 Hepatotoxic effects of Khat

Herbal induced liver injury (HILI), is a rare event that occurs in a small number of susceptible individuals (Riyaz *et al.,* 2014). Characteristics of HILI are similar to those of drug induced liver injury (DILI). There has been an increasing number of documented severe khat-induced liver injury cases in several European nations and particularly European nations with relaxed laws against khat. Non-viral hepatitis is seemingly a common factor in all these cases (Pantano *et al.,* 2016).

Cases of khat-induced hepatitis in humans have been reported (Riyaz *et al.,* 2014). As an additional mechanism leading to khat-induced liver damage, inhibition of the xenobiotic metabolizing liver enzyme CYP2 by cathinone has been reported (Hu *et al.,* 2010). In animal models, hepatocellular necrosis, hepatic fibroblast proliferation, hepatic hyperplasia and hepatic vessel congestion have been highlighted as some of the effects following repeated exposure to khat (Alsanosy *et al.,* 2020; Abdul-Mughni *et al.,* 2018; Alsalahi *et al.,* 2012). While few studies have been done to simulate chronic exposure to khat in animal models, it appears that there is a close association between chronic exposure to the herb and hepatotoxic conditions such as hepatic fibroblast proliferation and hepatic vessel congestion (Abdul-Mughni *et al.,* 2018). To date there is no clear mechanism documented behind the histopathological changes induced by khat, however, the hepatic vessel congestion may be attributed to the capacity of the cathinone in khat to induce vasoconstriction (Berhe *et al.,* 2015).

2.1.9 Immuno-modulatory effects of khat

There has been a growing body of literature showing evidence of khat-induced proliferation of pro-inflammatory cytokines. Studies using rodent models have shown that oral administration of khat induces proliferation of pro-inflammatory cytokines tumor necrotic factor-alpha (TNF-α)

and interferon gamma (IFN-ϒ) in mice. A study by Ali et al. (2015) not only showed that khat induced TNF-α and interleukin 1 beta (IL-1β), but also inflammation that trigger apoptosis through activation of caspase-dependent pathways. Subchronic exposure to khat induces elevation of the inflammation biomarkers; c-reactive protein (CRP), uric acid and the monocyte: lymphocyte ratio (Ketema, 2015).

2.1.10 Khat-induced ischemia

Ischemia is the blockage or obstruction of blood flow which often results in irreversible tissue damage (Raichle, 1983). Khat-induced ischemia has been reported in various organs with the heart, being the most affected organ (Mega *et al.,* 2017; Ali *et al.,* 2011; Patanwala, 2011). Khat-induced ischemia has life-threatening effects such as heart failure (Mega *et al.,* 2017). A recent study conducted in Southern Ethiopia concluded that the mean values of systolic and diastolic pressure is high among khat chewers than in non-khat chewers (Geta *et al.,* 2019). Additionally, chronic users of khat were found to be prone to ST-segment–elevation myocardial infarction when compared to non-khat chewers (Ali *et al.,* 2011). These findings imply that khat chewers have a high risk of heart diseases and death when compared to non-khat chewers.

2.1.11 Khat-induced cardiovascular complications

There has been a growing number of studies done in humans in regard to khat-induced cardiovascular complications. In the United Kingdom (UK), a pregnant khat user was presented at a London hospital with chest pain, tachycardia hypertension (Kuczkowski, 2005). In a structured study involving women with a history of khat and tobacco use, findings showed that the women had blunted cardiovascular responses to stress (al'Absi *et al.,* 2014). Acute myocardial infarction (AMI) was observed in a patient with a history of khat use confirmed by high levels of blood cathinone (Al-motarreb *et al*., 2010).

Khat exposure in mice increases the levels of uric acid, which is associated with high blood pressure and consequently cardiovascular complications (Ali *et al.,* 2015). Furthermore, there is sufficient evidence demonstrating the potential of khat to induce different forms of cardiovascular complications both in animals and humans. There is paucity of data on the potential mechanism behind this phenomenon, however, there is an assumption that this could be due to the capacity of cathinone to cause vasoconstriction of blood vessels like amphetamines do (Alkadi *et al.,* 2002). Additionally, cathinone, a potent psychoactive constituent of khat increases heart rate and blood pressure by triggering the release of noradrenaline from neurons (Ismaeel *et al.,* 2014).

2.1.12 Khat-induced anemia

Khat induced hematological perturbations have been reported (Mohammed et al., 2014; Kedir *et al.,* 2013). Specifically, subchronic exposure to khat induces leucopenia and anemia denoted by the suppressed white blood cell count and red blood cell count accompanied by reduced hematocrit levels (Ketema *et al.,* 2015). A similar study involving pregnant women in Ethiopia, revealed that pregnant women with a history of khat consumption had a higher risk of developing anemia (Kedir *et al.,* 2013).

Even though there is still sparse data on the additional mechanisms leading to khat-induced anemia, the available literature points to the possibility of khat to cause RBC breakdown via RBC suppression in various studies (Ismaeel et al., 2014; Kedir et al., 2013). Additionally, another mechanism leading to khat-induced anemia could be that the various khat components have deleterious effects on the hematopoietic process in the bone marrow (Ketema, 2015; Ismaeel *et al.,* 2014).

2.1.13 Effect of khat on reproductive health

Toxicities on the reproductive system following repeated khat consumption have also been reported (El-Shoura *et al.,* 1995). Exposure to high doses of khat resulted in low sperm counts and decreases sperm quality with reduced testosterone levels in rats and baboons (Nyachieo *et al*., 2013; Mohammed *et al.*, 2011). Other studies have shown that khat use affects the sperm morphology demonstrated by deformed heads and flagella (El-Shoura *et al.,* 1995). In women, khat results in higher chances of fetal death (Masood et al., 2015).

2.1.14 Khat-induced cancer

There have been numerous reported cases of different forms of khat-induced cancers (Fasanmade *et al.,* 2007; Soufi *et al.,* 1991). Previous studies show that patients with a history of khat chewing presented with squamous cell carcinoma of the mouth (Soufi et al., 1991). Additionally, early cancer presentation among the khat users was associated a synergistic effect of khat and other known carcinogens such as 4-(Nmethyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. Different forms of oral cancers have been reported among patients with a history of Khat chewing. There have been ongoing efforts to unravel the mechanisms behind the khat-induced cancers (Lu *et al.,* 2017; Lukandu *et al*, 2010). Lu et al. (2017) demonstrated that 400µ/ml of khat was able to induce cell death in a breast cancer cell line. In another study, Lukandu and co-workers reported that khat alters the physiology of the oral mucosa by decreasing the epithelial thickness and reducing the expression of cytokeratin-13 expression (Lukandu *et al.,* 2010). The two highlighted studies show potential of khat to induce cancer at molecular level by regulating the expression of cancer-promoting genes.

2.1.15 Khat-induced genotoxicity

The genotoxic potential of khat has been determined in several studies. For instance, Alsanosy et al. (2020) reported khat chewers from Jazan province of Saudi Arabia had higher levels of 8-hydroxydeoxyguanosisne (8-OHdG) in their blood plasma, which correlates with the level of DNA damage. In another study, it was reported that khat induced chromosomal aberrations in the bone marrow cells of mice (Abderrahman *et al.,* 2008). In the latter study, the chromosomal aberrations included broken, sticky and ring chromosomes. In addition, khat induced disturbed metaphase and anaphase cycles of the bone marrow cells of mice.

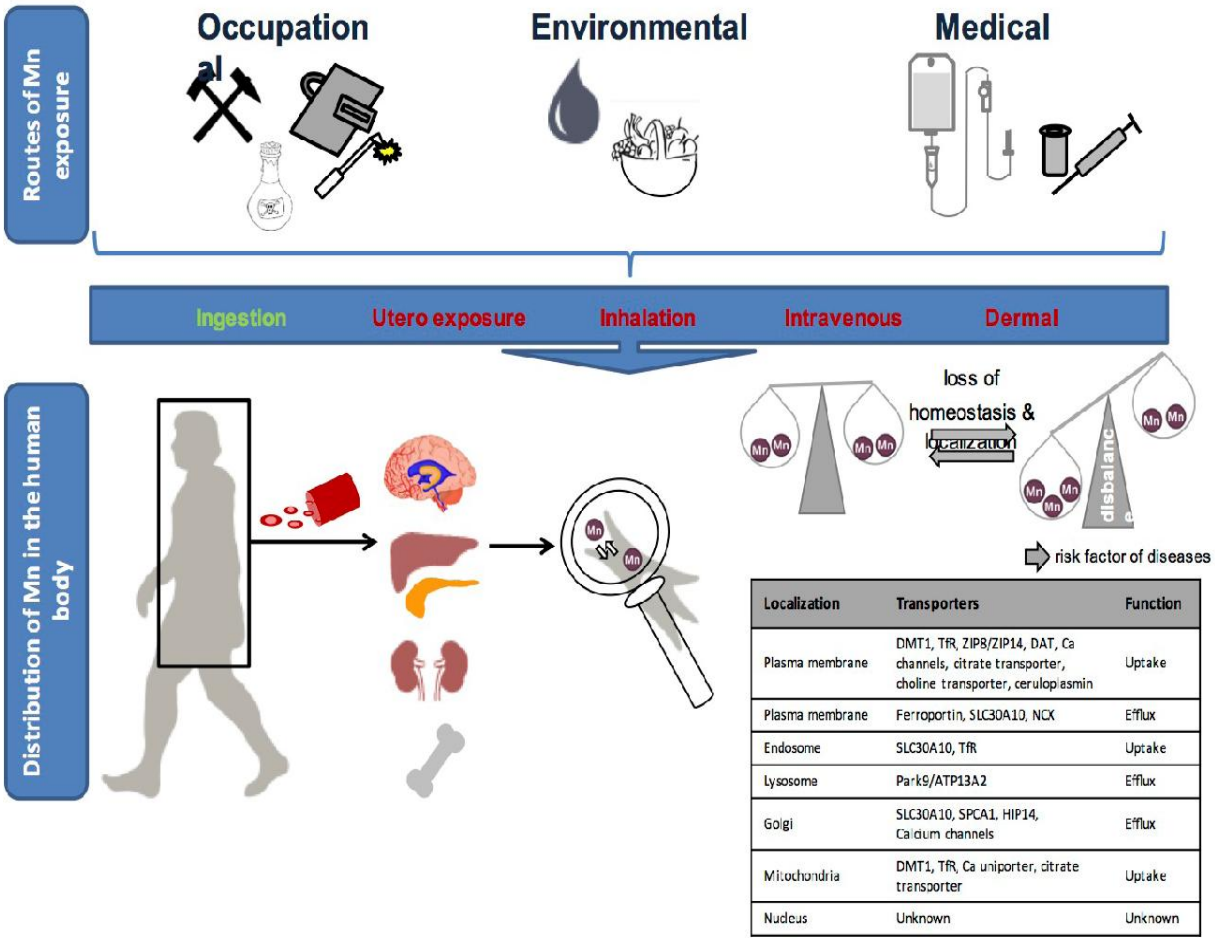
There is growing literature on multiple forms of toxicity following exposure to khat. However, there is paucity of information on the effect following co-exposure to khat and environmental metal toxins such as manganese. Therefore, in the current study, the impact of simultaneous exposure to khat and manganese was investigated. This is of significance since a majority of khat users reside in urban settings heavily polluted with metal toxins such as manganese.

2.2 Manganese

Manganese (Mn) is an essential trace element that is critical in various physiological processes like brain development, diverse metabolism and proper function of anti-oxidative enzymes like Acetylcholinesterase (AchE) (Isaac *et al.,* 2007). Overexposure can lead to the degeneration of dopaminergic neurons inducing Parkinson-like condition referred to as *"manganism"* (Fan *et al.,* 2020; Santamaria, 2008). Some clinical manifestation of *"manganism"* include psychiatric symptoms and cognitive deficits followed by movement abnormalities resembling idiopathic Parkinson's disease (IPD) (Perl *et al.,* 2007).

2.2.1 Routes of Manganese exposure

Mn is naturally present in food, with the highest concentration in nuts, cereals, legumes, fruits, vegetables, grains and tea. A small percentage is also present in drinking water. Typical daily intake range from 2-9mg/Kg for adults and approximately 3-5 per cent is absorbed from the gastro-intestinal tract (Santamaria, 2008). Exposure from soil erosion is the most common (Santamaria, 2008). Higher inhalation exposures may be experienced in occupational settings such as Mn Mines, foundries, smelters and battery manufacturing facilities (Figure 2.4), (Khan *et al.,* 1997).



Localization	Transporters	Function
Plasma membrane	DMT1, TfR, ZIP8/ZIP14, DAT, Ca channels, citrate transporter, choline transporter, ceruloplasmin	Uptake
Plasma membrane	Ferroportin, SLC30A10, NCX	Efflux
Endosome	SLC30A10, TfR	Uptake
Lysosome	Park9/ATP13A2	Efflux
Golgi	SLC30A10, SPCA1, HIP14, Calcium channels	Efflux
Mitochondria	DMT1, TfR, Ca uniporter, citrate transporter	Uptake
Nucleus	Unknown	Unknown

Figure 2.4: Routes of manganese exposure (Chen *et al.*, 2018).

2.2.2 Manganese neurotoxicity

Manganese is a constituent of metalloenzymes and an enzyme activator (Chen *et al*., 2018). Manganese Superoxide dismutase (MnSOD) is a catalytic enzyme that detoxifies superoxide free radicals (Martinez-Finley *et al.,* 2013). Mn activates it's metalloenzymes directly or indirectly interacting with ATP, to activate enzyme activity by initiating conformational change (Isaac *et al.,* 2007).

Manganese is required by some antioxidant enzymes such as manganese catalase and MnSOD (Isaac *et al.,* 2007). Mn is required as part of the diet but when in excess can lead to toxicity. Manganese-induced toxicity is due to autoxidation, production of free radicals and toxic metabolites, mitochondrial dysfunction and oxidative stress. Mn^{2+} can be converted to Mn^{3+} once in the body. A study utilizing rat model showed that Mn^{3+} is highly cytotoxic than Mn^{2+} (Ali *et al.,* 1995).

Evidence from earlier studies shows that Mn is able to pass through the blood brain barrier via the choline transporter (Martinez-Finley *et al.,* 2013). The other channels facilitating this entry include the voltage-gated and store-operated calcium channels, Mn citrate transporter and ionotropic glutamate receptor Ca2þ channels (Au *et al.,* 2008).

2.2.3 Manganese and reactive oxygen species

Reactive Oxygen Species (ROS) produced by cellular mechanisms include superoxide, hydroxyl radical and hydrogen peroxide (Martinez-Finley *et al.,* 2013). The main site for ROS production is the mitochondrial ETC while other sites include the NADPH oxidases, xanthine oxidases, enzymatic activation of cytochrome p 450 and cyclooxygenases 1 and 2 (Frick *et al.,* 2011; Rama Rao *et al.,* 2004).

Superoxide radical (O_2^-) is the first ROS formed but is converted by superoxide dismutase to hydrogen peroxide (H_2O_2). The MnSOD catalyzes this conversion within the mitochondrial matrix while copper/ zinc superoxide dismutase (Cu/Zn SOD) facilitates the conversion within the intermembrane space. Hydrogen peroxide can be converted into hydroxyl radicals (OHd) in the presence of iron and other transition metals such as Manganese (Melo *et al.,* 2011).

Production of superoxide ions in the mitochondria increases the electrochemical proton gradient (Hoffman *et al.,* 2010). Each of the three main ROS species, superoxide, hydrogen peroxide and hydroxyl ions can cause damage to nucleic acids, phospholipids and proteins within mitochondria (Melo *et al.,* 2011). Additionally, ROS generated in the mitochondria can contribute to the induction of Mitochondrial Permeability transition (MPT) inducing apoptosis. (Dirhem *et al.,* 2013; Takahashi *et al.,* 2010).

2.2.4 Manganese-induced hepatotoxicity

Studies to determine the effect of excess manganese on the liver have been done with outcomes showing that acute and chronic exposure to manganese results in hepatotoxicity (Chtourou *et al.,* 2012; Khan *et al.,* 1997). A study using a rat model showed that injection of 5mg/mL of Mn^{2+} to rats resulted in liver injury. Histopathological findings showed evidence of hepatocyte degeneration, having characteristic pink condensed nuclei (Fan *et al.,* 2020).

In another study using beagle dogs, Khan *et al.* (1997) found out that intravenous administration of manganese chloride ($MnCl_2$) to beagle dogs induced elevation of liver enzymes. The latter findings were accompanied with liver histopathological abnormalities showing regions of hepatocellular necrosis, mild epithelial hyperplasia and periportal

hemorrhages. Yet in another study, administration of 20mg/mL of manganese chloride to mice for thirty days resulted in elevated bilirubin levels (Chtourou *et al.,* 2012). This was accompanied by histopathological findings in the liver showing evidence of hepatic hemorrhage, cellular degeneration and necrosis.

The manganese-induced hepatotoxicity is not only limited to animals. The effect on the liver organ of human beings has also been reported. In one such study, Reynolds reported that long term parenteral administration of manganese to neonates results in cholestatic liver disease (Reynolds, 1994). Therefore, from literature, the hepatotoxic effects following excess manganese exposure are evident.

2.2.5 Manganese-induced inflammation

The potential of manganese to modulate the immune response by regulating cytokines has also been investigated in a number of studies (Fan *et al.,* 2020; Mokgobu *et al.,* 2015). A study using a rat model showed that when manganese was administered alone and in combination with ethanol, it induced an elevation of the pro-inflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor – alpha (TNF-α) (Nkpaa *et al.,* 2019).

A study by Fan *et al.* (2020) revealed that injection of manganese to rats induced an elevation of the pro-inflammatory cytokines IL-1β and TNF-α and interleukin-6 (Fan *et al.,* 2020). Another study demonstrated that when peripheral blood mononuclear cells were exposed to manganese chloride, they produced higher levels of IL-6, TNF-α, interferon gamma (IFN-γ) IL-1β, and IL-8 (Mokgobu *et al.,* 2015). Cumulatively, the above findings provide evidence that manganese has the potential to cause inflammation by up-regulating the pro-inflammatory cytokines.

The focus of many studies in regard to khat and manganese separately has been the toxicities following exposure to either of the two. This has resulted in information gap on the potential remedies against khat and/or manganese-induced toxicities. Therefore, as part of the objective of the current study, the potential role of CoQ_{10} in abrogating the khat and/or manganese-induced toxicities separately and in combination was investigated.

2.3 Co-enzyme-Q_{10}

Co-enzyme-Q_{10} (2,3-dimethoxy-5-methyl-6-decaprenil-1, 4-benzoquinone) (CoQ_{10}) is a lipo-soluble substance sometimes known as Ubidecarenone, CoQ_{10}, ubiquinone or Vitamin Q_{10}, (Figure 2.5) (Fuller *et al.,* 2006). External sources of this substance include diet and supplements, dietary sources include meat, poultry and fish which provide 2 to 20 milligrams. The non-animal sources include cereals, soya-beans, nuts and vegetables such as spinach and broccoli (Liu *et al.,* 2016). Its intestinal absorption is influenced by the presence of foods rich in lipids as well as beverages. CoQ_{10} has a key role in the mitochondria where it serves as an electron carrier between complex I and II (Figure 2.6) (Hargreaves *et al*., 2020).

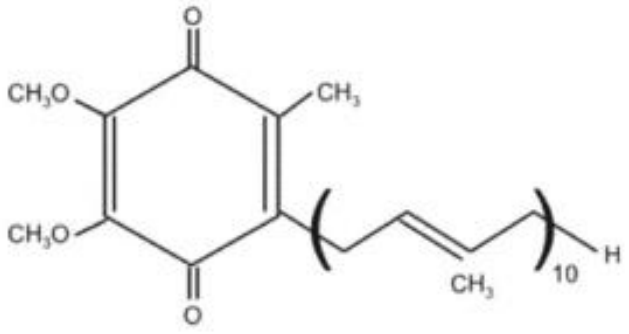


Figure 2.5: Structure of Coenzyme Q10 (Neela-Patil, 2012).

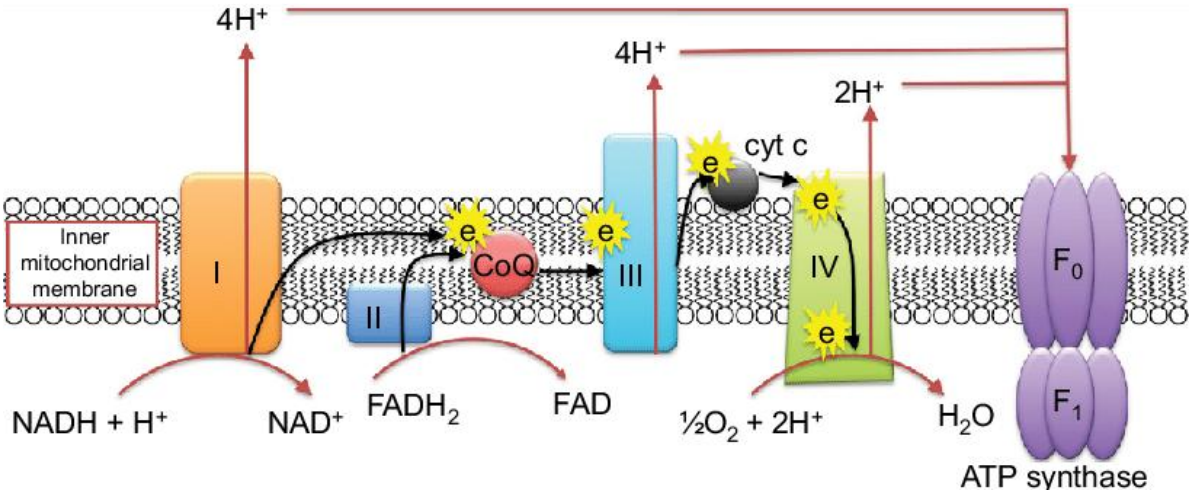


Figure 2.6: The electron transport chain in the mitochondrial matrix (Rodick et al., 2018).

2.3.1 Bio-synthesis of Co-enzyme-Q_{10}

CoQ_{10} is endogenously produced from the amino acid tyrosine in all cells and especially the kidney, pancreas, liver, and heart, and participates in intracellular energy production (Kamzalov *et al.,* 2003). CoQ_{10} is essential for maintaining the integrity of all organs and tissues (Liu *et al.,* 2016; Spindler *et al.,* 2009). Among the cofactors involved in synthesis include the niacin, vitamin B2, folic acid, vitamin B12, B6, C and pantothenic acid (Figure 2.7). At the age of 20 years concentration of CoQ_{10} is higher which then decreases progressively in the later years (Dos-Santos *et al.,* 2009).

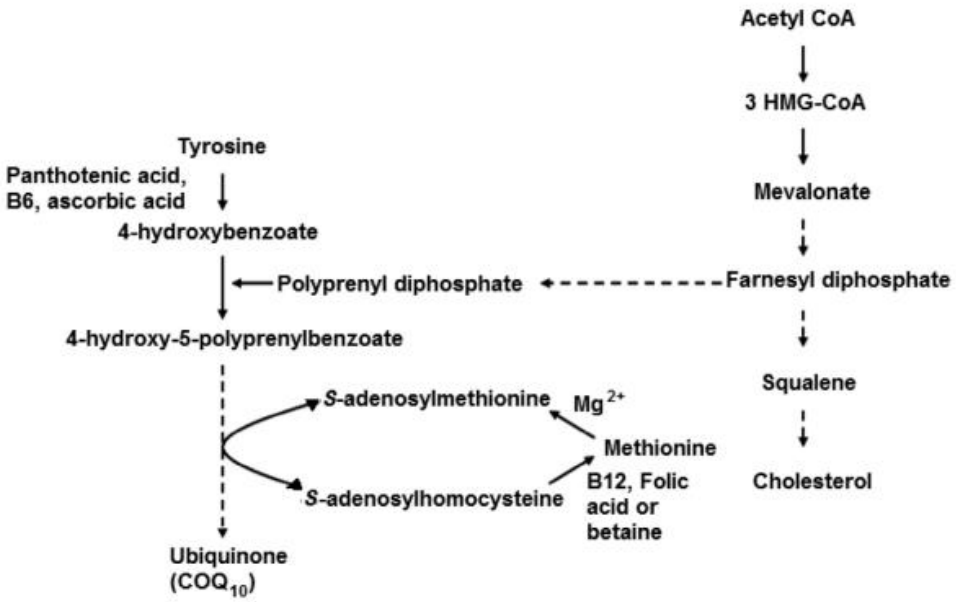


Figure 2.7: Coenzyme Q10 biosynthesis (Neela-Patil, 2012).

2.3.2 Clinical manifestations of co-enzyme Q_{10} deficiency

There is limited information on recommended daily doses but lack of CoQ_{10} is associated with symptoms such as congestive heart failure, cardiomyopathy, ischemic heart disease, hypertension, and breast cancer (Garrido-Maraver *et al.,* 2014; Folkers *et al.,* 1985). The deficiency can be due to low intake or insufficient endogenous production as a result of aging or deficiency of the precursors for its synthesis (Mancuso *et al.,* 2009).

2.3.3 Anti-oxidant function of Co-enzyme Q_{10}

Endogenous antioxidants are important in maintaining normal activities of cells, however, the cellular antioxidant capacity can be overwhelmed upon exposure to drugs, trauma, cold, infections, toxins, radiation, diet low in nutrients, or vigorous physical activity (Al-mehdar *et al.,* 2012; Novoselova *et al.,* 2009; Linda *et al.*, 2002). Earlier studies had shown a strong association between neuronal cell death and Reactive Oxygen Species (ROS) (Kim *et al*., 2002).

The main site for free radical production within the cell is the mitochondrion (Neyrinck *et al.,* 2015). A small amount this organelle's oxygen consumption leads to the production of hydrogen peroxide. The inner membrane of the mitochondrion contains the antioxidants CoQ_{10} and α-tocopherol, both known to possess antioxidant properties (Neyrinck *et al.,* 2015; Sohal *et al.,* 2006). CoQ_{10} has been shown to protect the mitochondrion from the effects of lipid peroxidation as a result of α-tocopherol depletion (Sohet *et al.,* 2009). There is documented evidence suggesting a synergistic free radical scavenging action involving both Coenzyme-Q_{10} and α-tocopherol (Kamzalov *et al.,* 2003).

2.3.4 CoQ_{10} as a treatment remedy for neurodegenerative diseases

The brain is one of the organs with the highest energy and oxygen consumption with high production of free radicals causing vulnerability to oxidative damage (Malthankar-Phatak *et al.,* 2008; Kamzalov *et al.,* 2003). Outcomes of previous studies have shown that oxidative damage may be an etiological factor in some neurodegenerative diseases (Ali *et al.,* 2015; Al-Zubairi *et al.,* 2003).

A common feature in neurodegenerative diseases is the progressive loss of neurons in some regions of the brain (Dos-Santos *et al.,* 2009). Parkinson's and Huntington's diseases (PD and HD, respectively) are the common neurodegenerative disorders, where the basal ganglia structures suffer neuron loss. Loss of neurons within the hippocampus and the cortex during Alzheimer's disease causes memory and cognitive capacity deficiency (Dos-Santos *et al.,* 2009). Studies using animal models for neurodegenerative diseases including Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease have demonstrated the beneficial role of CoQ_{10} in slowing down the respective degenerative disease progression (Dos-Santos *et al.,* 2009; Mancuso *et al.,* 2009; Spindler *et al.,* 2009).

Malonic acid is a known inducer of oxidative stress. In a study by Beat *et al.,* (1994) to determine the role of CoQ_{10} in malonic acid induced oxidative stress, it was found out that malonic acid depleted ATP with high levels of lactic acid. Coenzyme Q_{10} administration mitigated these effects. This clearly demonstrates that CoQ_{10} has the capacity to experimentally neutralize cellular ROS.

Findings from an earlier study demonstrated that oral administration of CoQ_{10} was beneficial in rats induced with Parkinson's disease, PD (Beal *et al.,* 1998). MPTP (a neurotoxin selectively toxic to dopaminergic neurons) was administered to a group of one-year old rats together with CoQ_{10}. Results indicated that the presence of CoQ_{10} and MPTP induced a high striatal dopamine levels when compared to the levels in rats receiving MPTP alone. This remarkable finding not only demonstrate the capacity of CoQ_{10} to neutralize ROS, but also clearly demonstrate that CoQ_{10} slows down PD's progression by targeting the dopaminergic system.

As mentioned earlier, one of the channels by which khat induces its cytotoxic effects is by induction of free radical species through metabolism of various phytochemical components. Additionally, manganese is known to induce neurotoxic effects via generation of free radicals in the cell which eventually leads to cell death. It is with this in mind that the current study employs the use of the potent antioxidant CoQ_{10}, to determine the impact in khat induced cytotoxicity as well as khat and manganese induced cytotoxicity.

2.3.5 Anti-inflammatory potential of CoQ_{10}

The potential anti-inflammatory ability of CoQ_{10} has been demonstrated in numerous studies (Monsef *et al*., 2019; Fuller *et al*., 2006; Xing *et al*., 2004). CoQ_{10} supplementation was shown

to be beneficial against aluminum-induced inflammation, exhibited by the aluminum-induced elevation of the pro-inflammatory cytokines: TNF-α and interleukin 1β, in a study done using a rat model (Ali *et al*., 2018). In another study using a mouse model, CoQ_{10} supplementation in mice having diet-induced inflammation resulted in reduced inflammation following reduced expression of the inflammation-linked genes: six trans-membrane protein of prostrate 2 (STAMP2) and C-reactive protein (CRP) genes (Sohet *et al*., 2009). Additionally, Lee and coworkers (2013) demonstrated that CoQ_{10} supplementation reduces osteoarthritis in a rat model by regulating the genes involved inflammation such as interleukin 1β, interleukin 15 and interleukin.

The potential anti-inflammatory capacity of CoQ_{10} has also been demonstrated in human beings. In this regard, supplementation with CoQ_{10} to patients with metabolic syndrome resulted in down-regulation of the markers of oxidative stress and inflammation: plasma glutathione and malondialdehyde (Raygan *et al*., 2016). Cumulatively, the above-mentioned data clearly demonstrated the putative role of CoQ_{10} against inflammation in both animal and human models of study. There is growing literature demonstrating the putative anti-inflammatory capacity of CoQ_{10}. Importantly, CoQ_{10} appears to regulate pathways and genes involved in inflammation. In line with this, Ali et al. (2018) demonstrated that supplementation of CoQ_{10} to mice down-regulates the expression of nuclear factor kappa B (NF*k*B) and caspase-3 genes that are involved in the induction of inflammation. In addition, it was established by Nyariki et al. (2019) that CoQ_{10} inhibits the pro-inflammatory cytokines TNF-α, IFN-γ, IL-18, IL-6 and IL2 (Figure 2.8) (Nyariki *et al*., 2019). The same study also established that CoQ_{10} stimulates in the production of the anti-inflammatory cytokines IL-10, IL-13 and IL-22.

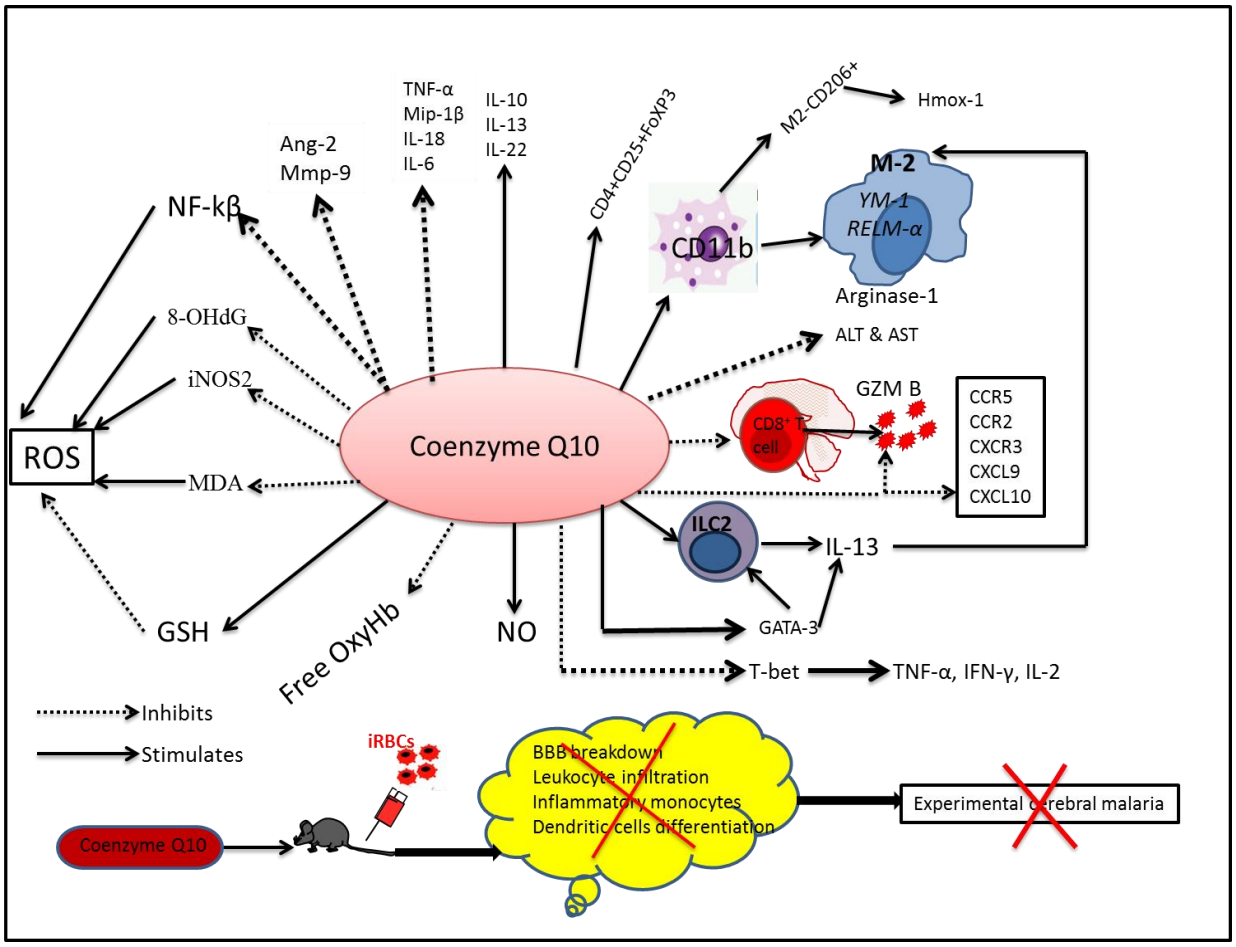


Figure 2.8: Schematic diagram of biochemical pathways modulated by CoQ_{10} (Nyariki *et al.*, 2019).

2.3.6 Ameliorating effect of CoQ_{10} against toxicity

CoQ_{10} has been shown to have ameliorating effects against other forms of toxicity (Gutierrez-mariscal *et al*., 2021; Garrido-Maraver *et al*., 2014;Lee *et al*., 2014). In efforts to unravel the role of CoQ_{10} in the modulation of lipopolysaccharide-induced toxicity in a rat model, El-laithy and co-workers found out that CoQ_{10} supplementation decreases lipopolysaccharide-induced brain malondialdehyde and beta amyloid (El-Laithy *et al*., 2018). Worthy of note is that both malondialdehyde and beta-amyloid are associated with toxicity with accumulation of the latter

accounting for toxicity in the brain leading to dementia (Barton *et al*., 2003). Additional findings in the same study showed that CoQ_{10} supplementation in rats increased the levels of reduced glutathione and paraoxonase-1 (PON1). Importantly, PON1 is a key enzyme involved in ameliorating toxicity resulting from lipid peroxidation; hence, the enzyme offers cytoprotection (Shunmoogam *et al*., 2018).

In another study to assess the putative capacity of CoQ_{10} against oxytetracycline-induced reproductive toxicity in mice, it was revealed that CoQ_{10} restores the oxytetracycline-induced suppression of testosterone levels in male mice (Oda *et al*., 2018).

CHAPTER THREE

THE EFFECT OF CoQ_{10} ON HOST PHYSIOLOGICAL AND BIOCHEMICAL EVENTS IN KHAT-INDUCED TOXICITY

3.1 INTRODUCTION

There is growing evidence associating khat with deleterious physiological and biochemical events (Bedada *et al.,* 2010; Al-Dubai *et al.,* 2006). However, studies aimed at investigating the effects following chronic exposure to khat are still limited. Compounding this crisis further is the fact that there is little effort by the scientific community to find potential remedies against the khat-induced toxicities.

Findings from previous experimental studies have shown that khat induces organ toxicity to the brain, kidney, liver and the reproductive organs; with the brain being among the most affected organ (Kimani *et al.,* 2016; Alsalahi *et al.,* 2012). It has been postulated that since cathine and cathinone bear structural similarities with amphetamines, then the mechanism by which the two alkaloids induce their mode of action and toxicity is similar to those of amphetamines (Patel, 2015; Gashaw *et al.,* 2014).

Additionally, it has been shown that the serotonergic and dopaminergic regions of the brain such as the substantia nigra are the most affected owing to the fact that this is a center for khat-induced toxicity (Tariq *et al.,* 1987). Damages on the dopaminergic regions of the brain potentially results in movement disorders similar to Parkinson's disease in the subjects (Milatovic *et al.,* 2009). Administration of 300mg/kg body weight to mice resulted in impairment of working and learning memory in a recent study (Assefa *et al.,* 2018). Other related studies have shown the relationship between repeated exposures to khat and behavioral abnormalities such as agitation, aggression, anxiety and hallucinations (Geresu, 2015;

Mohammed *et al.,* 2014). In another study alterations in the serotonin levels induced by khat resulted in aggressive behavior in rodents (Banjaw *et al.,* 2006). Hence an assumption can be made that chronic exposure to khat will result in severe damages to the central nervous system.

Subchronic exposure to khat has also been associated with body weight loss in humans and mice models (Al-Sharafi *et al.,* 2015; Gashaw *et al.,* 2014). It has been suggested that this may be due the effect of khat on the endocrines system; leading to endocrine-induced suppression of appetite and subsequent anorexia (Murray *et al.,* 2008). The khat-induced weight loss has also been suggested to be due to the ability of khat to inhibit nutrient absorption in the small intestine (Geta *et al.,* 2019; Gashaw *et al.,* 2014). A general decrease in body weight could also be attributed to the khat-induced cell death and subsequent tissue damage; previous reports have demonstrated khat-induced apoptotic mechanisms and tissue damage (Lu *et al.,* 2017; Dirhem *et al.,* 2013).With this in mind, there is a likelihood that subjects chronically exposed to khat will suffer severe anorexigenic effect.

Assessment of the level of various blood constituents is frequently used to assess the level of toxicity induced by chemicals. Haematological abnormalities following subchronic exposure to khat have been explored with outcomes showing the potential of khat to affect the haematopoiesis process (Ismaeel *et al.,* 2014). For instance, khat-induced suppression of white blood cells (WBCs) and WBC subtypes has been reported in previous studies (Ketema *et al.,* 2015; Alsalahi *et al.,* 2012; Ismaeel *et al.,* 2014). In another study, khat-induced suppression of RBC's was noted in mice upon oral exposure to the extract (Ismaeel *et al*., 2014). In humans, experimental studies have similarly demonstrated the potential of khat to cause different forms of blood abnormalities (Kedir *et al.,* 2013). The findings implicate khat in hematotoxicity in both humans and animals.

There is a growing effort by the scientific community to increase knowledge on various forms of toxicities induced by exposure to khat and its constituents. However, there are few studies geared towards finding counter strategies to khat-induced toxicities. Therefore, in the present study, the role of CoQ_{10} was evaluated against physiological and biochemical changes following exposure to khat. The putative role of CoQ_{10} against injurious physiological and biochemical changes following simultaneous exposure to khat were also investigated.

CoQ_{10} is one of the most potent endogenous antioxidant in mammals (Belardinelli *et al.,* 2008; Eal, 1998). Importantly, the putative role of CoQ_{10} in management of physiological and haematological abnormalities upon exposure to different toxins and parasitic infestation has been demonstrated (Mermel *et al.,* 2009; Rashid *et al.,* 2014). However, the role of CoQ_{10} in modulation of khat-induced toxicities is yet to be established.

3.2 MATERIALS AND METHODS

3.2.1 Ethical statement

Approval of the protocols involving animal handling was obtained from the Institute of Primate Research (IPR) Ethics Committee (number IRC/03/16).

3. 2. 2 Experimental animals

Forty male Swiss albino mice aged between 3-4 weeks old were obtained from the Kenya Medical Research Institute (KEMRI) and transported to the School of Biological and Life Sciences Animal House (Technical University of Kenya). The animals were kept in clean cages under controlled room temperature of 23-25℃ and a 12-hour light/dark cycle and allowed to acclimatize for one week before the start of the experiments. Mice were fed on mice pellets (Unga feeds) and had access to clean water *ad libitum*.

3.2.3 Preparation and administration of coenzyme-Q10

CoQ_{10} solution (Now Foods, Bloomingdale, IL, USA) was freshly prepared fresh on each day of experiment by dissolving it in olive oil. A dose of 200mg/Kg was used for CoQ_{10} treatment. The choice of the dosage was based on a previous finding that 200mg/kg of this supplement is sufficient to cross the blood brain barrier and offer neuroprotection (Matthews *et al.,* 1998).

3.2.4 Preparation of khat

Thirty (30) grams of fresh khat leaves were chopped into small pieces and then homogenized in distilled water (30gms/10ml) after which filtration was done and the resulting supernatant was stored at -4°C. Two hundred microliters from the filtrate (200 µl/kg body weight) was used for intragastric administration into mice on khat treatment (Graziani *et al.,* 2008).

3.2.5 Mice grouping and treatment

Forty (40) Swiss albino mice randomly divided into four groups were used in this study. Group one was the control with free access to mice pellets and clean water ad-libitum only; group two was given CoQ_{10} at a dose of 200 mg/Kg body weight, group three was administered with 1500mg/Kg body weight of crude khat extract and group four received 1500mg/kg body weight of khat extract and 200mg/Kg body weight of CoQ_{10}. CoQ_{10} was administered daily for two weeks prior to the start of khat treatment to boost its systemic concentration as shown in previous studies (Rashid *et al.,* 2014). CoQ_{10} was administered one hour prior to the administration of khat for group four that received both khat and CoQ_{10}. For this study, two parallel experiments using the above experimental design were used to simulate subchronic and chronic exposure to khat. For subchronic exposure, the experiment was terminated at 90 days post treatment (90dpt). While the experiment for chronic exposure was terminated at 132dpt.

3.2.6 Sample collection

Twelve hours after the last day of experiment, prilumbal surgical sectioning of mice was performed to draw blood via cardiac puncture after the mice were partially anesthetized with ketamine injection (0.2ml/kg body weight). The blood samples for hematological assay were collected in heparinized tubes.

3.2.7 Determination of organ and body weights

The mice were weighed after every 15 days throughout the experiment. The weights of brain, liver, kidney and spleen samples were measured at the end of the experiment. Subsequently, the relative organ weights were determined. Individual organ weight divided by the respective body weight at 90dpt for subchronic exposure and 132dpt for chronic exposure multiplied by 100 provided the relative organ weight. All the weight measurements were done using an analytical electronic balance (Mettler PM34, DoltaRange®).

3.2.8 Hematological assays

The blood samples were collected into heparinized tubes for full hemogram analysis and later analyzed using automated Benchman Coulter counter (Benchman, Indianapolis, USA).

3.2.9 Determination of neurological integrity

Rapid murine coma and behavior scale (RMCBS) was utilized for the determination of neurological integrity of mice using the procedure by Carroll (Carroll *et al.,* 2010). The mice were checked for clinical symptoms then scored according to gait and balance for co-ordination; motor performance, body position and limb strength; and touch escape, pinna reflex, toe pinch, aggression and grooming for self-preservation.

3.2.12 Statistical analysis

Statistical analysis was done using Graphpad prism software package (Version 5.0). One way ANOVA was done to compare the treatment groups with controls. For internal comparisons, Turkey's post-hoc test was used. The results were given as a mean ± with significance set at $p<0.05$.

3.3 Results

3.3.1 Exposure to khat induced increase in the weight of the brain

A significant increase ($p<0.0001$) in the weight of the brain was noted following subchronic exposure to khat and co-exposure to khat and CoQ_{10} (Fig. 3.1A). The liver weight was unaffected by khat exposure (Fig. 3.1B). Subchronic exposure to khat resulted in a marginal rise in the weight of the spleen with a significant increase following co-treatment with CoQ_{10} and khat ($p<0.001$) (Fig 3.1C). Chronic exposure to khat had no effect the brain weight (Fig 3.2A), however, the liver weight was significantly decreased following treatment with CoQ_{10} (Fig 3.2B). Additionally, there was a significant increase in the weight of the spleen following chronic exposure to khat (Fig 3.2C). A comparative analysis of subchronic and chronic exposure to khat revealed that there was a significant drop in the relative weight of the brain following chronic exposure to khat as well as co-exposure to khat and CoQ_{10} ($p<0.001$) (Fig 3.3A). On the contrary, relative organ weights of the liver (Fig 3.3B) and the spleen (Fig 3.3B) registered no significant changes after this comparison.

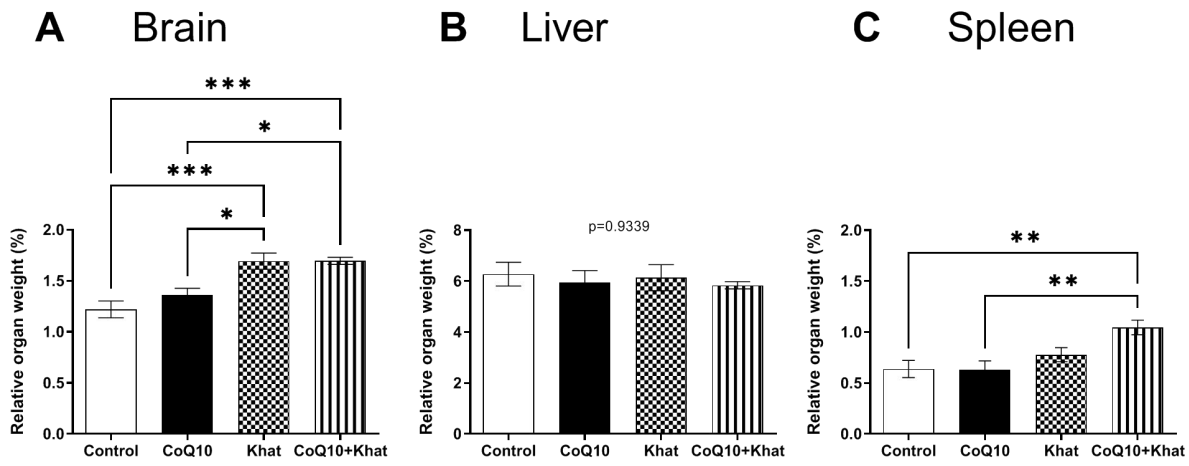


Figure 3.1: The figure shows the effect of subchronic exposure to khat and or CoQ_{10} on the weights of the brain (Fig. A), liver (Fig. B) and spleen (Fig. C). Administration of khat extract resulted in organ weight changes in spleen and brain. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

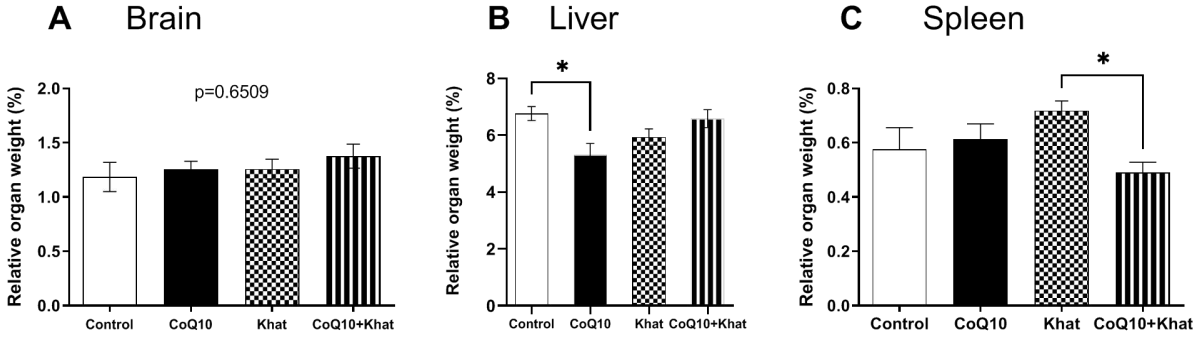


Figure 3.2: The figure shows the effect of chronic to khat and or CoQ_{10} on the weights of the brain (Fig. A), liver (Fig. B) and spleen (Fig. C). Administration of khat extract resulted in organ weight changes in spleen and brain. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

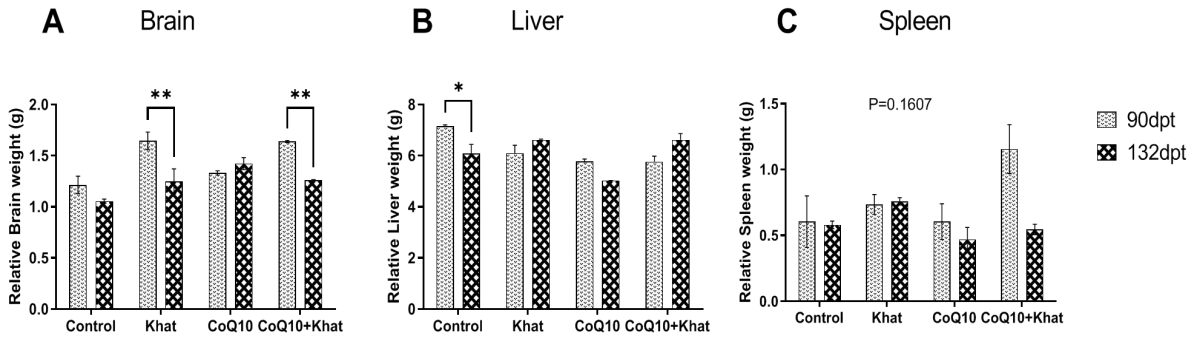


Figure 3.3: The figure shows the comparative effect of subchronic and chronic exposure to khat and or CoQ_{10} on the weights of the brain (Fig. A), liver (Fig. B) and spleen (Fig. C). Administration of khat extract resulted in organ weight changes in spleen and brain. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

3.3.2 Subchronic and chronic exposure to khat induced significant body weight decrease

Subchronic exposure to khat (90dpt) resulted in a significant body weight loss ($P<0.05$) (Figure 3.4 A and B). Similarly, chronic exposure to khat (132dpt) resulted in a significant body weight loss in mice ($P<0.001$) (Figure 3.4 C). Remarkably, mice co-treated with khat and CoQ_{10} appeared to resist the weight loss at 132dpt (Figure 3.4C). The was no significant change in body weight upon a comparative analysis of subchronic versus chronic exposure to khat (Figure 3.5).

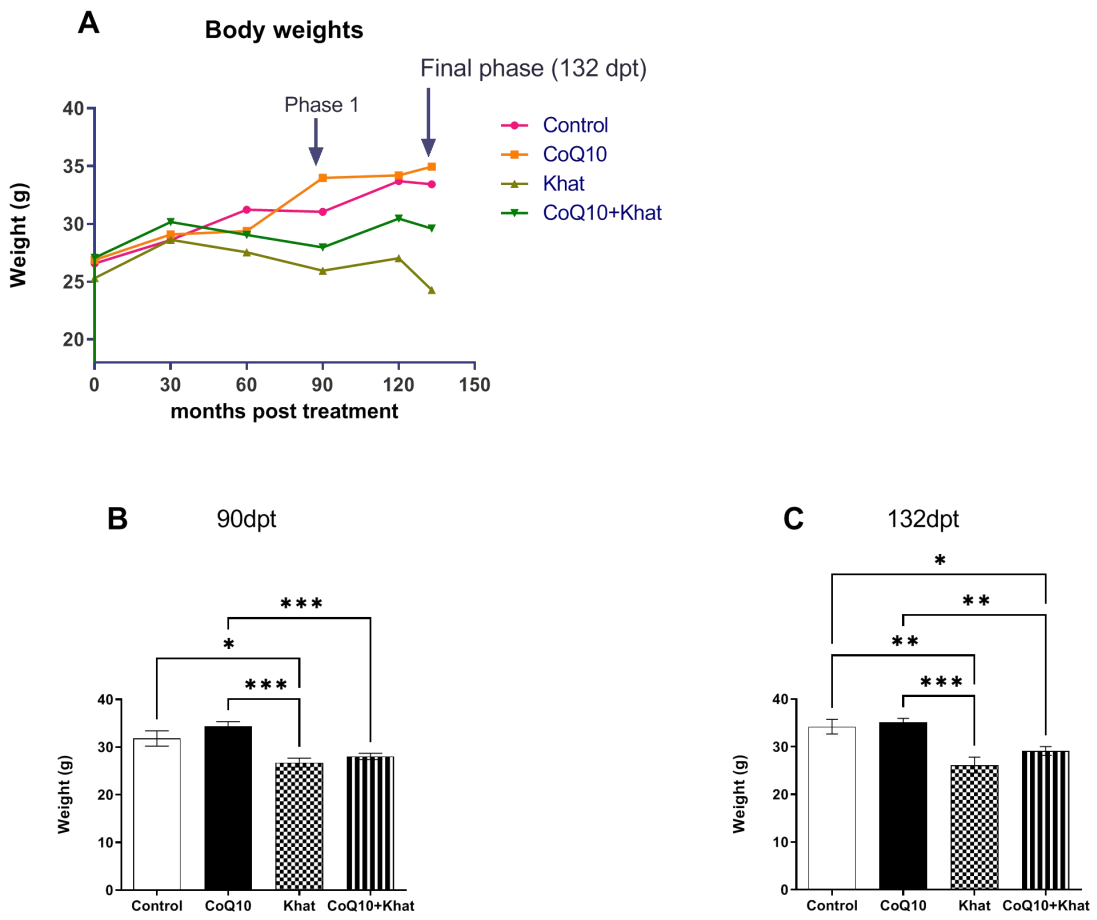


Figure 3.4: The figure shows the effect of subchronic and chronic exposure to khat and or CoQ_{10} on the body weights of mice: general changes in body weight presented as a trend (Fig. A), changes in body weight at 90 days post treatment (Fig. B) and changes in body weight at 132 days post treatment (Fig. C). Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

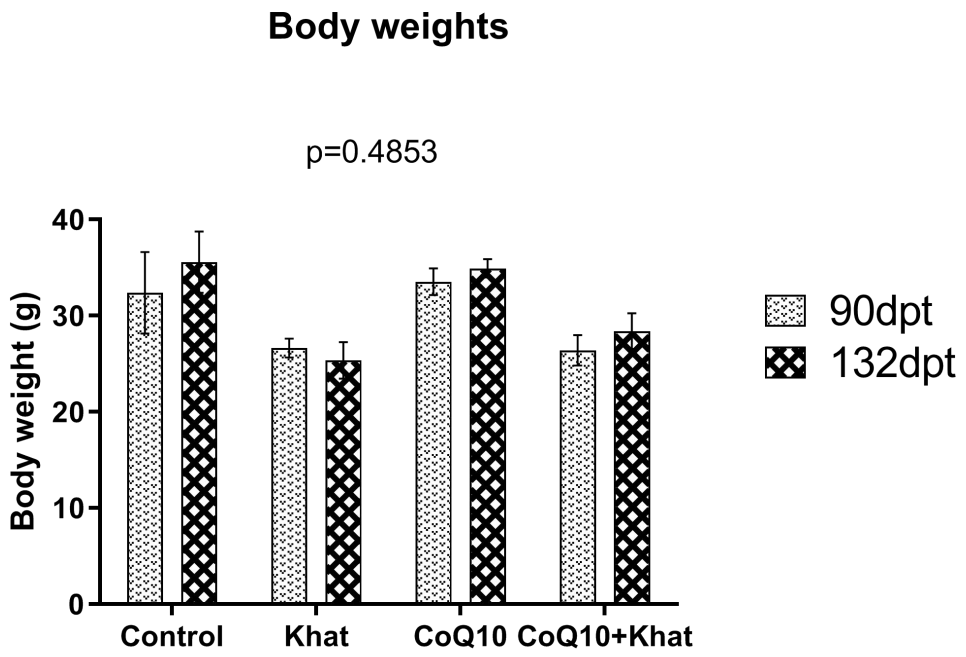


Figure 3.5: The figure shows the comparative effect of subchronic and chronic exposure to khat and/or CoQ_{10} on the body weights of mice. Administration of khat extract resulted in organ weight changes in spleen and brain. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

3.3.2 CoQ_{10} restored khat-induced RBC and hematocrit suppression

Subchronic exposure to khat marginally suppressed the levels of hematocrit (HCT) (Fig. 3.6A) with a significant decrease in the levels of red blood cells (RBC) (Fig. 3.6B). This was accompanied with no change in hemoglobin (HGB) (Fig. 3.6C); indicating khat-induced anemia. In addition, exposure to khat had no effect on the percentage hematocrit and the levels of RBCs (Fig 3.7A and B). On the contrary, exposure to khat for the same duration resulted in a marginal suppression of hemoglobin (Fig 3.7C). A comparative analysis of the effect of treatments upon subchronic and chronic exposure revealed that the hematocrit levels were unaffected (Fig 3.8A), while the levels of RBCs were significantly elevated (P<0.001) in the chronic phase following treatment with CoQ_{10} (Fig 3.8B). Finally, the HGB levels were unaffected (Fig 3.8C).

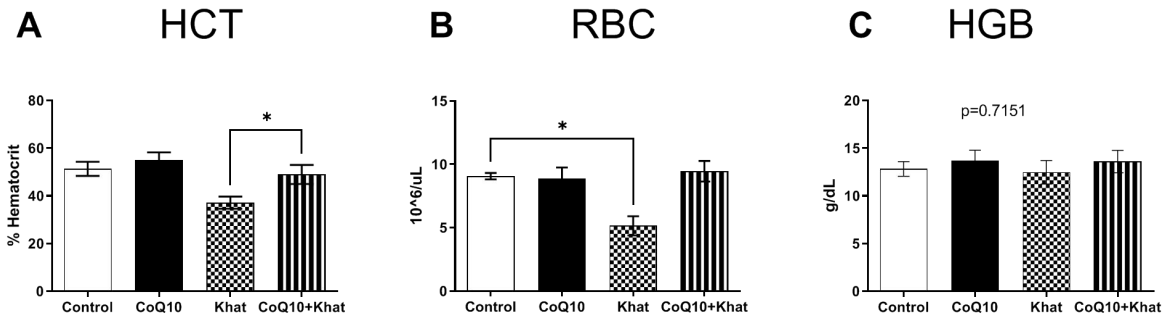


Figure 3.6: Effect of subchronic exposure to (90 days post treatment) khat and CoQ_{10} on Hematocrit (HCT), Red blood cells (RBC) and Hemoglobin (HGB).
The figure shows the effect of khat and or CoQ_{10} on HCT (Fig. A), RBC (Fig.C) and HGB (Fig. C). Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. u/L: microliters per Liter, g/dL: grams per deciliter.

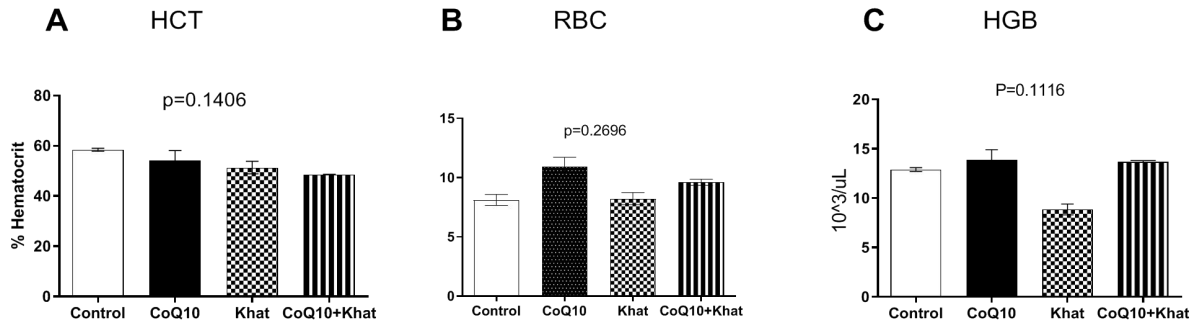


Figure 3.7: Effect of chronic exposure (132 days post treatment) to khat and CoQ_{10} on Hematocrit (HCT), Red blood cells (RBC) and Hemoglobin (HGB). The figure shows the effect of khat and or CoQ_{10} on HCT (Fig. A), RBC (Fig.C) and HGB (Fig. C). Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. u/L: microliters per Liter, g/dL: grams per deciliter.

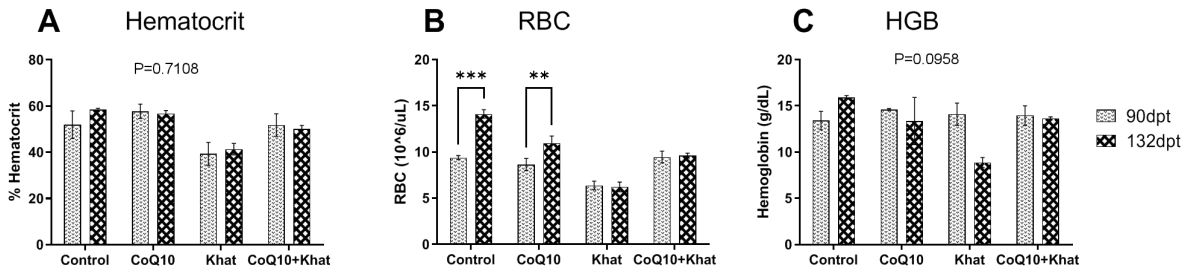


Figure 3.8: Comparative analysis of the effect of subchronic (90 days post treatment) and chronic exposure (132 days post treatment) to khat and CoQ_{10} on Hematocrit (HCT), Red blood cells (RBC) and Hemoglobin (HGB). The figure shows the effect of khat and or CoQ_{10} on HCT (Fig. A), RBC (Fig. C) and HGB (Fig. C). Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. u/L: microliters per Liter, g/dL: grams per deciliter.

3.3.3 CoQ_{10} abrogated khat-induced suppression of RBC indices.

Subchronic exposure to khat resulted in significant suppressions of mean corpuscular volume (MCV) ($P<0.05$), mean corpuscular hemoglobin (MCH) ($P<0.05$), red cell distribution width standard deviation (RDW-SD) and a reduction in the percentage red cell distribution width coefficient of variation (RDW-CV) ($P<0.001$) (Figs 3.9A-D). Importantly, the khat-induced suppressions of the RBC indices were abrogated in the presence of CoQ_{10}. No significant changes in the levels of RBC indices were noted upon chronic exposure to khat (Fig 3.10). A comparison of the subchronic versus chronic effects following exposure to khat revealed that there was a significant increase in MCV ($P<0.001$), MCH ($P<0.001$) and RDW-SD ($P<0.001$) (Figs 3.11A-C). However, the percentage RDW-CV was unaffected (Fig 3.11D).

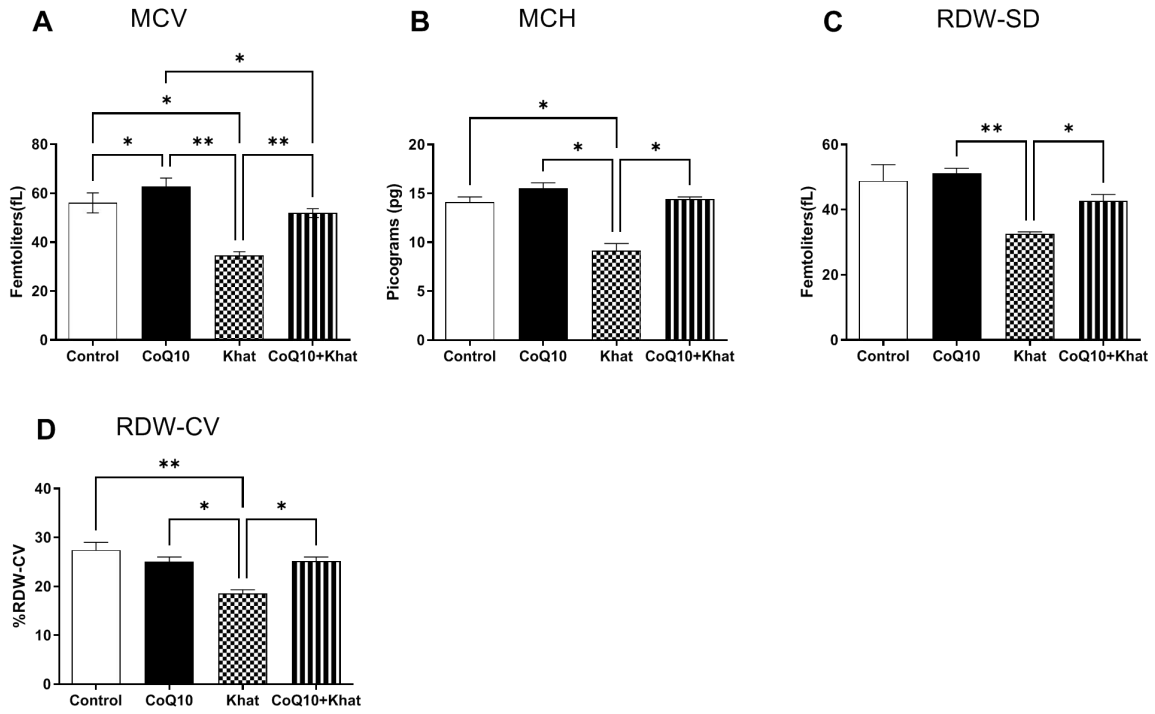


Figure 3.9: RBC indices in subchronic phase. CoQ_{10} restored khat induced reduction of MCV (mean corpuscular volume) (Fig. A), MCH (mean corpuscular hemoglobin) (Fig B), RDW-SD (red cell distribution width –standard deviation) (Fig. C) and RDW-CV (red cell distribution width –coefficient of variation) (Fig D). Red Blood Cell subtype levels from each group were compared by One way ANOVA with Tukey multiple comparisons post hoc test. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

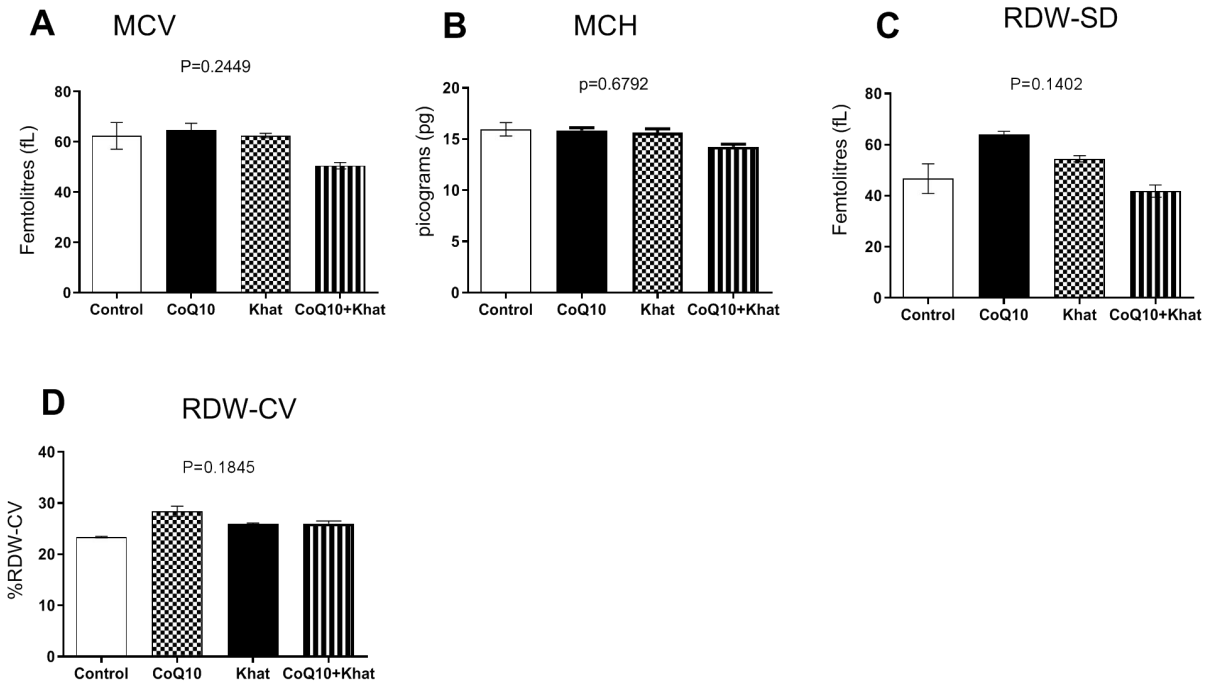


Figure 3.10: Chronic exposure to khat. Chronic exposure to khat and/or CoQ_{10} did not affect the levels of MCV (mean corpuscular volume) (Fig. A), MCH (mean corpuscular hemoglobin) (Fig B), RDW-SD (red cell distribution width –standard deviation) (Fig. C) and RDW-CV (red cell distribution width –coefficient of variation) (Fig D). Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. Bars represent mean ± SEM.

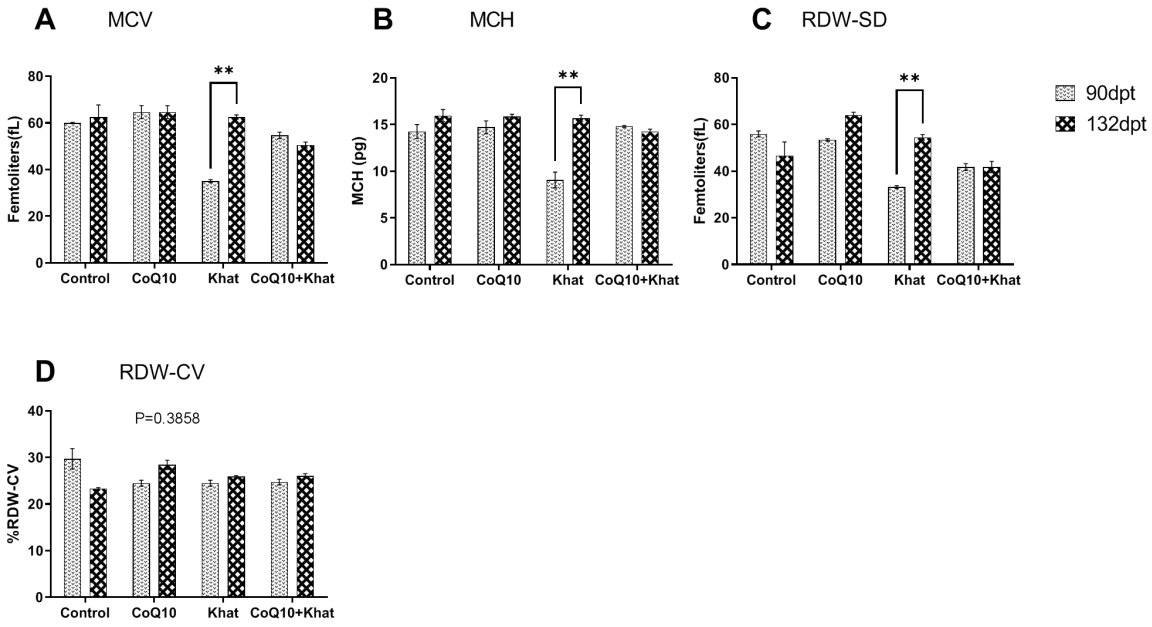


Figure 3.11: Changes in RBC indices following subchronic and chronic exposure to khat and/or CoQ_{10}, Red Blood Cell indices levels from each group were compared by One way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

3.3.3 CoQ_{10} supplementation modulated khat-induced changes in the levels of WBC subtypes

Subchronic exposure and chronic exposure to khat resulted in marginal suppressions WBC (Fig 3.12A) and (Fig. 3.13A), respectively. On the contrary, there was a significant elevation in the levels of monocytes, neutrophils, eosinophils and lymphocytes following subchronic exposure to khat (Figs 3.12 B, C, D and F, respectively). Importantly, the mentioned khat-induced elevations were down-regulated in the presence of CoQ_{10}. Lastly, the levels of basophils were suppressed by following exposure to khat (Fig 3.12E). Worthy of note, co-treatment with CoQ_{10} and khat up-regulated the khat-suppressed basophils.

In additional findings, chronic exposure to khat resulted in no significant change in the WBC levels (Fig 3.13A). However, a significant khat-induced elevation of monocytes, neutrophils and eosinophils was registered (Figs 3.13B-D). Remarkably, the elevations in the WBC subtypes were down-regulated in the presence of CoQ_{10}. Finally, the levels of basophils and lymphocytes were suppressed upon chronic exposure to khat (Figs 3.13 E and F). A comparative analysis of subchronic versus chronic exposure data revealed that there was no significant change in the levels WBC, monocytes, basophils and lymphocytes (Figs 3.14 A, B, E and F, respectively). However, marginal increases in the levels of monocytes following chronic exposure to khat were registered. In additional findings, there was a marginal elevation of lymphocytes following subchronic exposure to khat.

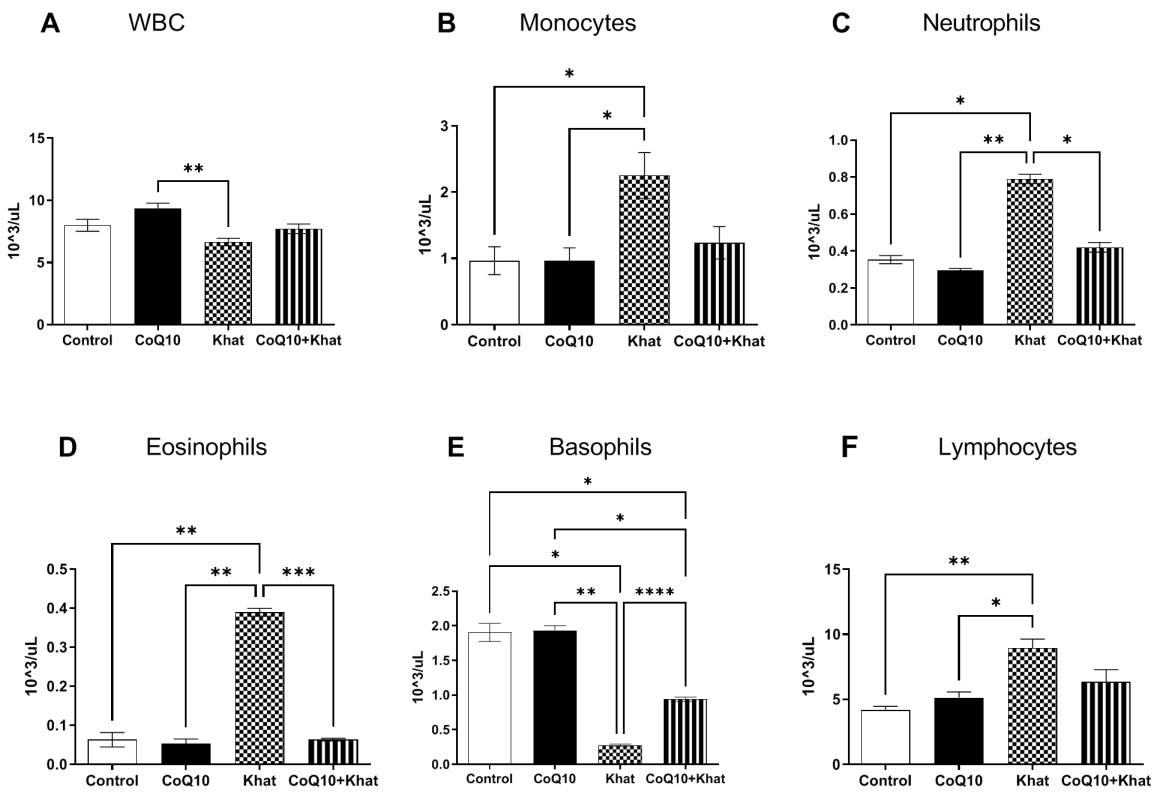


Figure 3.12: Effect of subchronic (90 days post treatment) exposure to CoQ_{10} and/or khat on WBCs. The figure shows the effect of khat and or CoQ_{10} supplementation on the WBC of Swiss albino mice. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

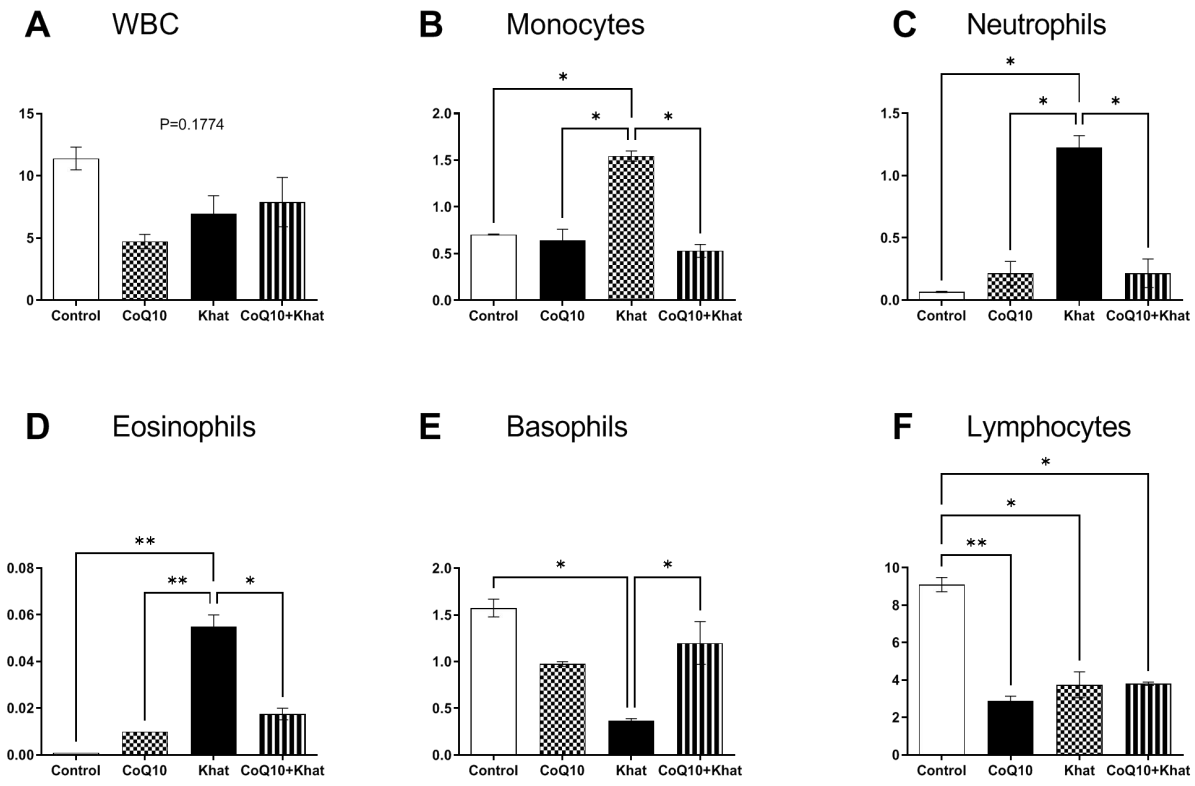


Figure 3.13: Effect of chronic exposure (132 days post treatment) to khat and/or CoQ_{10} on WBC subtypes. The figure shows the effect of khat and or CoQ_{10} supplementation on WBC subtypes. Fig. A: Effect on lymphocytes, B: Monocytes, C: Neutrophils, D: Eosinophils and E: Basophils. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

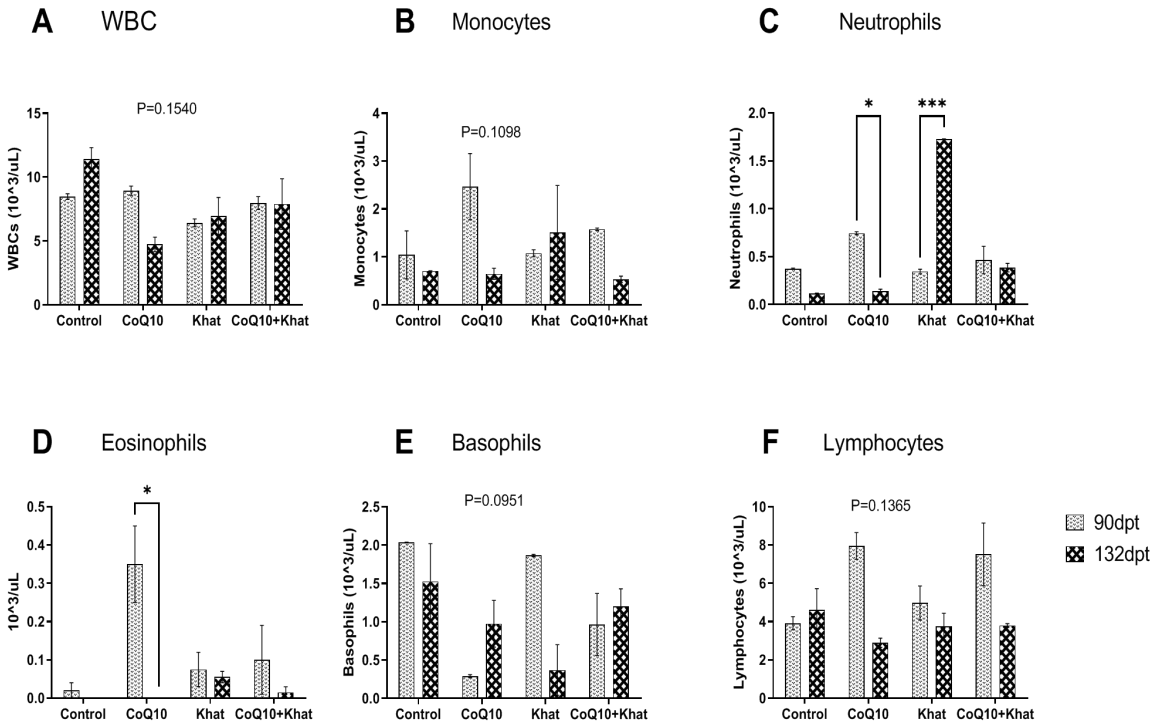


Figure 3.14: Comparative effect of subchronic (90 days post treatment) and chronic (132 days post treatment) exposure to khat and/or CoQ_{10} on WBC subtypes. The figure shows the effect of khat and/or CoQ_{10} supplementation on WBC subtypes. Fig. A: Effect on lymphocytes, B: Monocytes, C: Neutrophils, D: Eosinophils and E: Basophils. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.uL: microliters.

3.3.4 Subchronic exposure to khat induced suppression of platelets

Subchronic exposure to khat induced a significant suppression of platelets (P<0.05) (Fig. 3.15A). However, CoQ_{10} appeared to aid the recovery of platelet levels. An analysis of the platelet indices showed khat-induced down-regulation of the plateletcrit (PCT) that was not statistically significant (Fig. 3.15B). There was a significant reduction in the levels of platelet large cell ratio (P-LCR) (P<0.05) (Fig. 3.15C). Remarkably, CoQ_{10} showed the tendency to reverse the khat-driven reduction in PCT and P-LCR levels. The levels of mean platelet volume

(MPV) was unaffected by the treatments (Fig 3.15D). In additional results, subchronic exposure to khat significantly reduced the platelet distribution width (PDW) (Fig 3.15E). Chronic exposure to khat resulted in marginal suppression of platelets and plateletcrit (Figs 3.16A and B, respectively). However, CoQ_{10} showed signs of aiding the recovery of the platelets and plateletcrit. A khat-induced increase in the percentage P-LCR that was not statistically significant was noted (Fig 3.16C). The levels of MPV and PDW were unaffected by the treatments (Figs 3.16 D and E, respectively). A comparative analysis of the subchronic versus chronic effects of the treatments on platelets revealed that there was a significant khat-induced increase in the levels of platelets following chronic exposure to khat ($P<0.05$) (Fig 3.17A). Additionally, the platelet indices PCT, P-LCR, MPV and PDW were increased in a manner that is not significant during chronic exposure to khat (Figs 3.17 B-E).

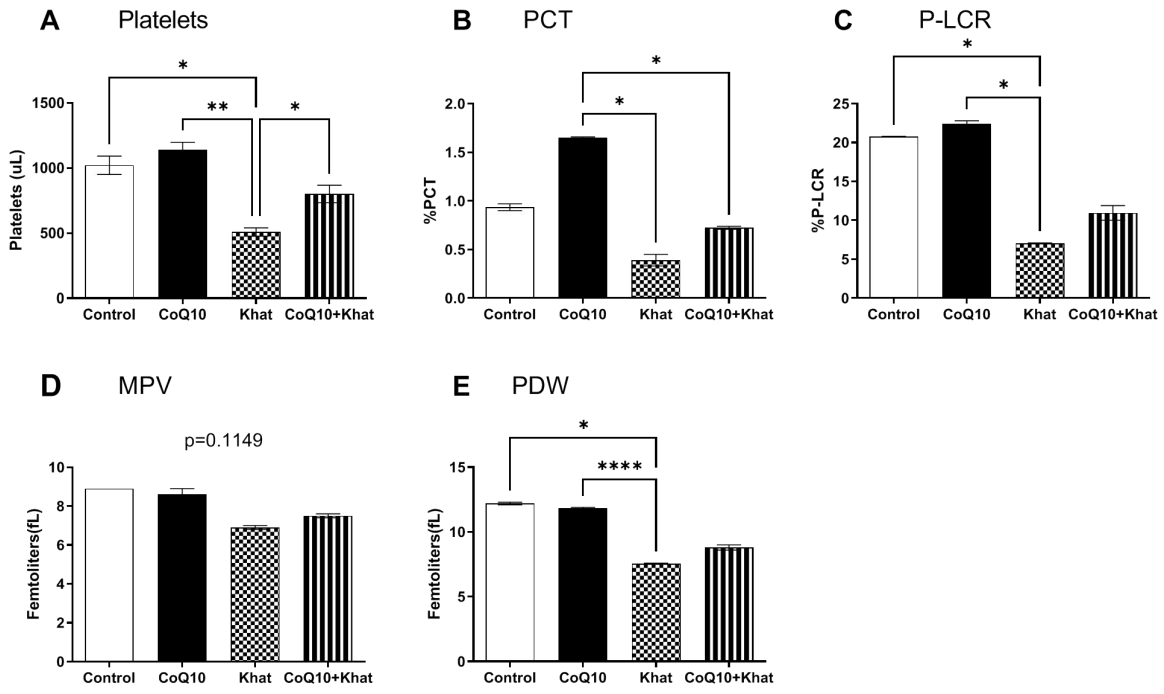


Figure 3.15: Coenzyme-Q10 supplementation restored suppression of platelets following subchronic exposure to khat. Fig. A: Effect on platelets, B: Plateletcrit (PCT), C: platelet large cell volume (P-LCR) and D: mean platelet volume (MPV) and E: platelet distribution width (PDW). Data was analyzed using

one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. uL: Microliters.

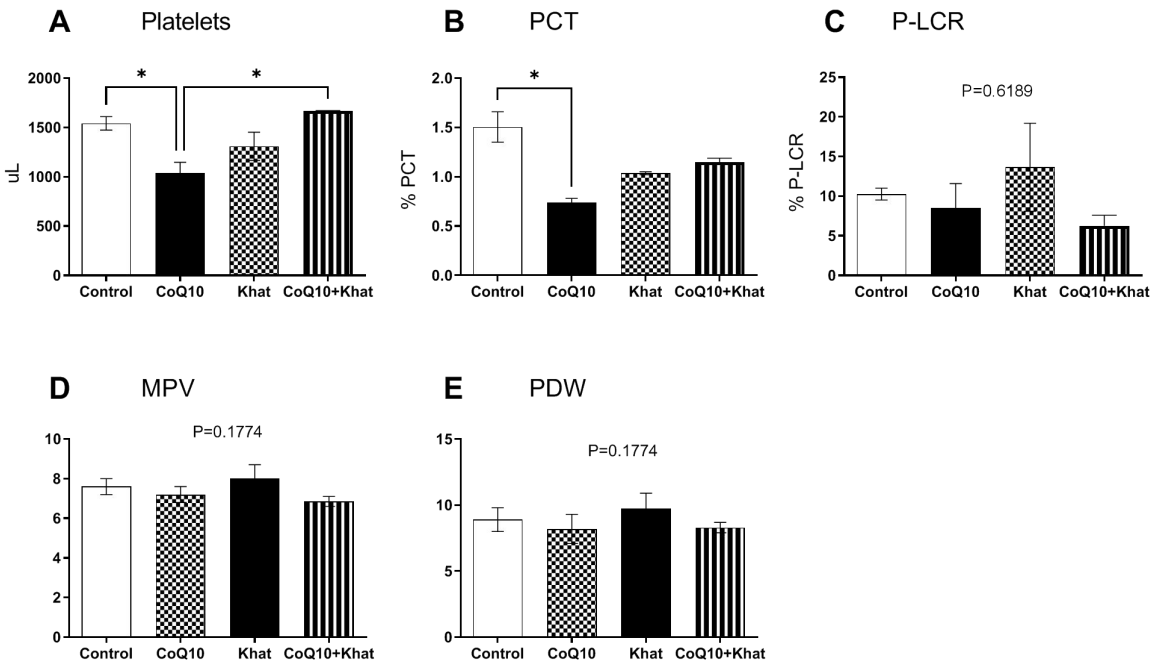


Figure 3.16: Coenzyme-Q10 supplementation restored suppression of platelets following chronic exposure to khat. Fig. A: Effect on platelets, B: Plateletcrit (PCT), C: platelet large cell volume (P-LCR) and D: mean platelet volume (MPV) and E: platelet distribution width (PDW). Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. uL: Microliters.

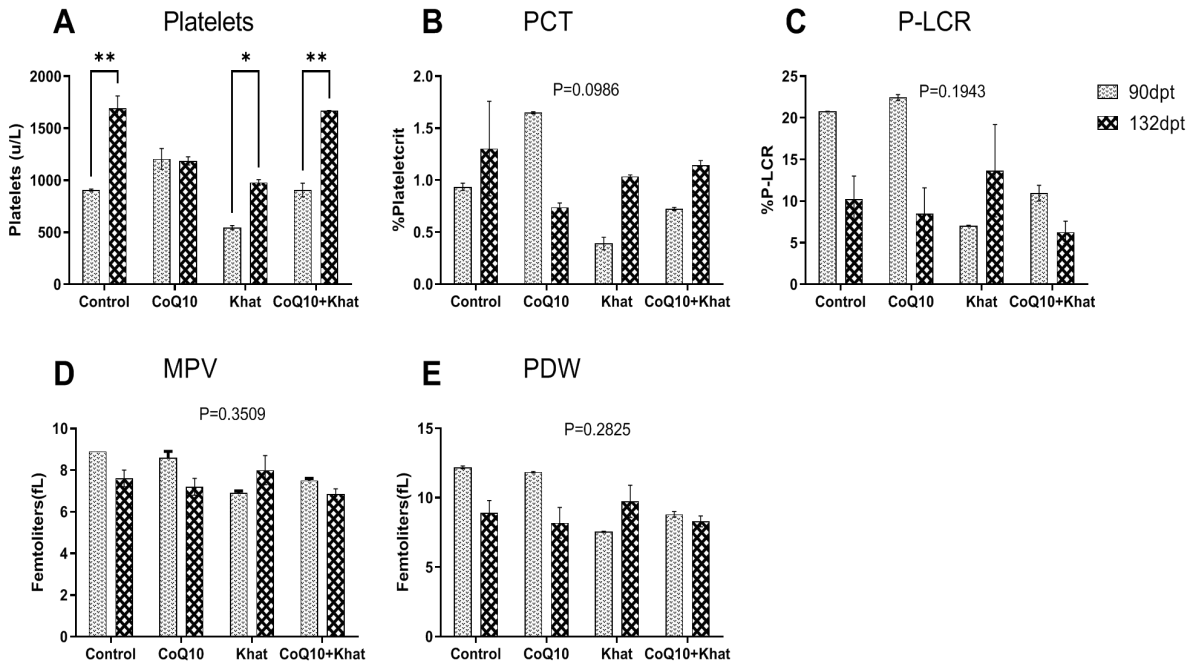


Figure 3.17: Comparative effects of subchronic (90 days post treatment) versus chronic (132 days post treatment) exposure to khat and/or CoQ_{10} supplementation on platelet subtypes. Fig. A: Effect on platelets, B: Plateletcrit (PCT), C: platelet large cell volume (P-LCR) and D: mean platelet volume (MPV) and E: platelet distribution width (PDW). Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

3.3.5 CoQ_{10} modulated khat-induced neurological injury

Rapid murine coma and behavioral scale (RMCBS) was used to gauge the neurological injury induced by subchronic and chronic exposure to khat, as well as ameliorative effects of CoQ_{10}. Subchronic exposure to khat induced neurological injury as depicted by the decreased RMCBS scores following subchronic exposure to khat (Fig 3.18A). A further analysis of the individual RMCBS parameters gait, motor performance, aggression and touch escape revealed significant khat-induced decrease in gait scores ($p<0.001$) (Fig 3.18B). Remarkably, in the presence of CoQ_{10}, the khat-induced decline in aggression was reversed. In addition, the motor performance

and aggression were unaffected upon subchronic exposure to the treatments (Fig 3.18C and D, respectively). However, a marginal decline in aggression was noted, importantly, CoQ_{10} showed a tendency to reverse the khat-induced decline of aggression. A khat-induced decline of touch escape was recorded ($p<0.05$) (Fig 3.18E). Similarly, CoQ_{10} showed a tendency to reverse the khat-induced decline of touch escape.

Chronic exposure to khat resulted in a general RMCBS decline; indicating compromised neurological integrity of mice following chronic exposure to khat (Fig 3.19A). Of significance, CoQ_{10} showed a tendency towards reversing this effect. Upon analysis of the parameters following chronic exposure to khat, a khat-induced decline of the parameters gait ($p<0.0001$) (Fig 3.19B) and touch escape ($p<0.05$) (Fig 3.19E) was noted. Remarkably, these outcomes were reversed in the presence of CoQ_{10}. Lastly, a comparative analysis of the subchronic versus chronic effects of khat on the RMCBS parameters revealed no statistically significant changes (Figs 3.20 A-D).

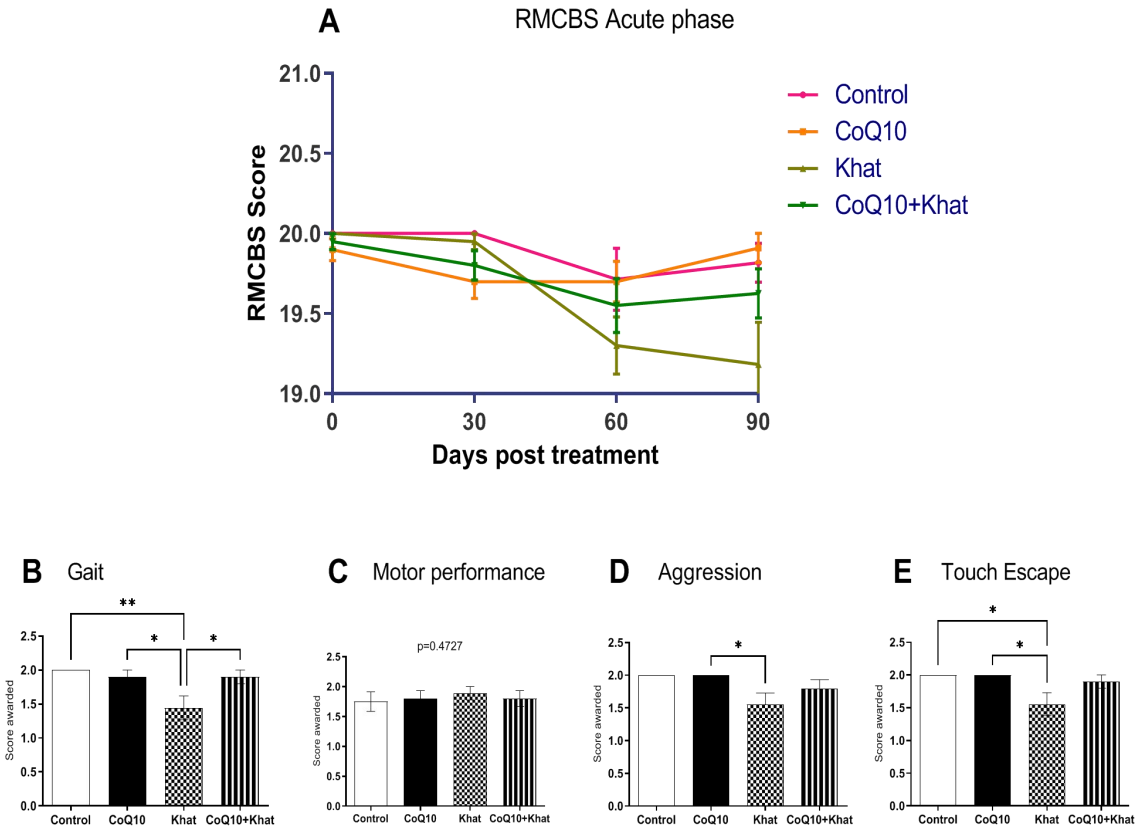


Figure 3.18: CoQ_{10} supplementation modulated khat-induced decline of rapid murine coma and behavioral scale (RMCBS) scores during subchronic exposure. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. U/L: Units per liter.

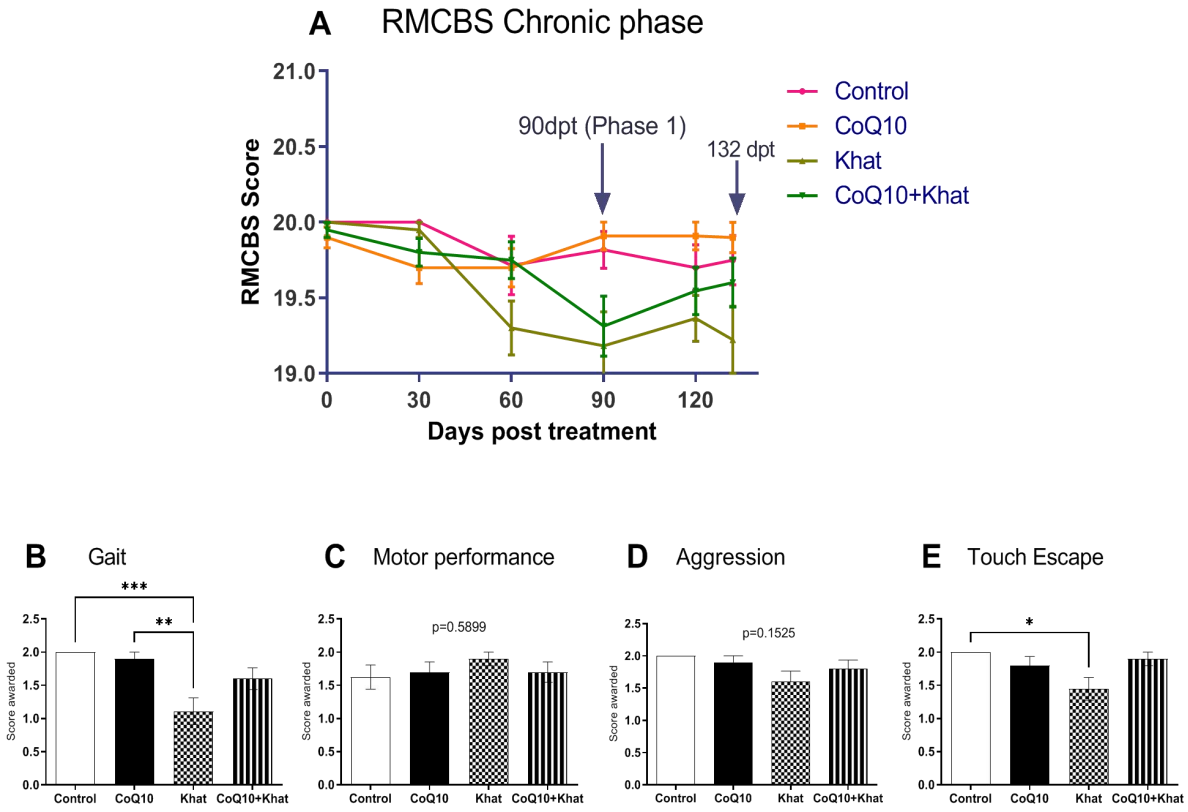


Figure 3.19: CoQ_{10} supplementation modulated khat-induced decline of rapid murine coma and behavioral scale (RMCBS) scores during chronic exposure. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

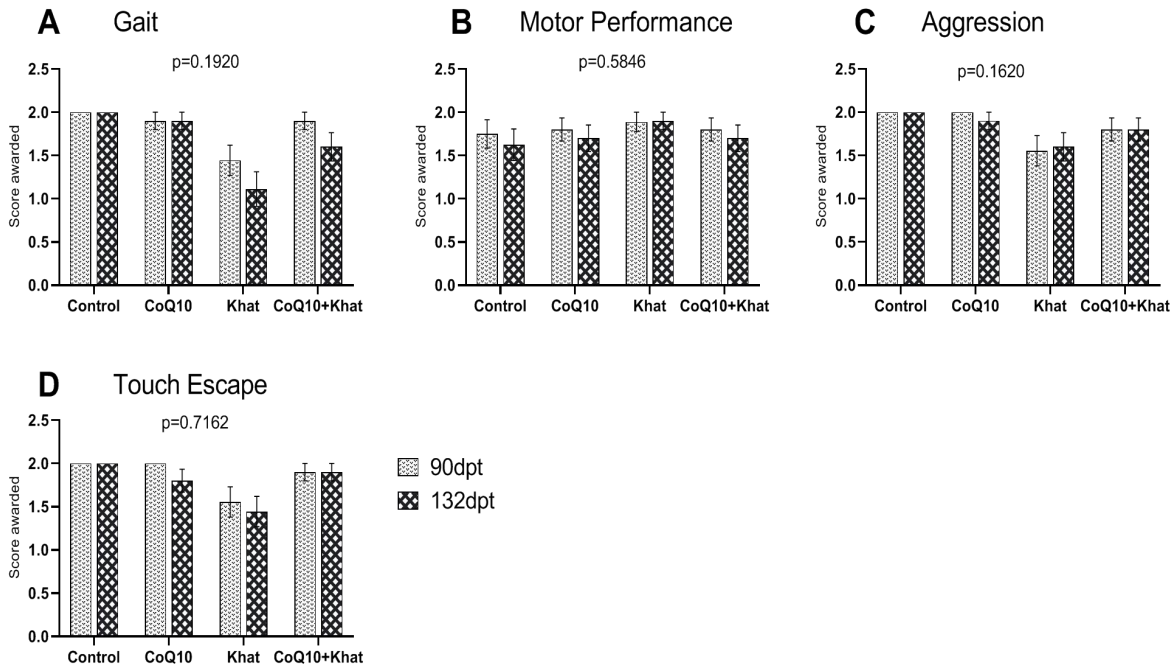


Figure 3.20: Comparative analysis of the effect of subchronic versus chronic exposure to khat and/or CoQ_{10} on rapid murine coma and behavioral scale (RMCBS) scores. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

3.4 DISCUSSION

Findings in this study demonstrate that exposure to khat extract results in deleterious effects in mice. Importantly, majority of the negative effects were abrogated by administration of CoQ_{10} during both the subchronic and chronic phase. In the current study, a marked increase in brain weight was noted during the subchronic phase; a phenomenon that may be attributed to the edema-inducing capacity of khat as shown by earlier studies (Abdul-Mughni *et al.,* 2018). A notable finding is that the khat-induced increase in brain weight was heightened during the subchronic phase when compared to the chronic phase. Even though there is no clear explanation for the noted finding, this may be a coping response to khat toxicity during the sub phase of exposure to khat.

Additionally, there was a general increase in the relative organ weight of the spleen that was not statistically significant following subchronic as well as chronic exposure to khat. The observed khat-induced splenomegaly during the subchronic phase may be attributed to the proliferation of B and T-lymphocytes. Notably, khat-induced T and B lymphocyte proliferation has been reported in a previous study (Ketema, 2015).

Both subchronic and chronic exposure to khat resulted in decreased body weights of mice in the present study. This outcome corroborates previous studies (Adel Al-Zubairi *et al*., 2008; Lemieux *et al*., 2015; Al-Dubai *et al*., 2006). There is evidence attributing this outcome to the effects of various khat constituents on the gastro-intestinal and endocrine systems; for instance khat's constituents inhibition of intestinal absorption of nutrients from food (Gashaw *et al.,* 2014; Murray *et al.,* 2008). A significant outcome in the present study is that, there is a persistent body weight loss following chronic khat exposure.

Khat-induced derangement of blood parameters has serious implications in blood related syndromes. Khat-induced anemia was notable in the current study as shown by the depletion of RBC and HCT following subchronic exposure to the extract. Notably, this outcome corroborates earlier studies which have demonstrated khat-induced anemia (Ismaeel *et al.,* 2014; Ketema *et al.,* 2015; Alsalahi *et al.,* 2012). A comparative analysis of subchronic versus chronic exposure to khat revealed that the khat-induced decrease in the level of hemoglobin was severe during the chronic phase. This finding may imply that continuous exposure to khat for longer period exacerbates hemoglobin suppression. Importantly, the suppression of RBC and HCT following subchronic exposure to khat was curtailed in the presence of $CoQ_{10;}$ this demonstrates the putative capacity of CoQ_{10} to ameliorate khat-induced hematotoxicity. In addition, the hemoglobin levels were restored in the presence of CoQ_{10} in the chronic phase. These findings are critical since it validates the common assumption that khat-induced RBC breakdown is mediated by increased cellular oxidative stress given that CoQ_{10} is a powerful antioxidant (Xing *et al.,* 2004). These outcome may not be surprising, as an earlier study has shown that RBCs enriched with exogenous CoQ_{10} are resistant to oxidative damage (Belardinelli *et al.,* 2008).

Further findings demonstrate that khat-induced microcytic anemia during the subchronic phase (90dpt) was evident as shown by the decreased levels of RBC indices: MCV, MCH, RDW-SD and RDW-CV. Of significance, the present study demonstrated for the first time that co-administration of khat with CoQ_{10} significantly restores the khat-induced effects on the levels of RBCs and its subtypes. Another significant outcome of the present study is that chronic exposure to khat resulted in no significant changes on hematocrit, RBCs and hemoglobin. In addition, the RBC indices: MCV, MCH, RDW-SD and RDW-CV were unaffected following chronic exposure to khat.

Subchronic exposure to khat resulted in a marginal suppression of WBCs and significant elevation of lymphocytes, monocytes, neutrophils and eosinophils. Notably, a similar pattern was observed following chronic exposure to khat with the exception of lymphocytes which were suppressed during this phase. The observed change in pattern of the derangement of WBC-subtypes following subchronic versus chronic exposure to khat warrants further investigation as this is a novel outcome. The khat-induced proliferation of neutrophils and eosinophils may account for the marginal increase in the relative organ weight of the spleen during both subchronic and chronic exposure to the extract. This may be as a result splenic sequestration and clonal expansion of these WBC subtypes. Importantly, studies have shown that elevated levels of monocytes and neutrophils trigger the release of the pro-inflammatory cytokines TNF-α, IL-8, IL-6 and IFN-γ leading to inflammation linked diseases condition (Das *et al.,* 2015; Hicks *et al.,* 2008), therefore, this may explain the role of khat in aggravating inflammation and disease conditions.

The observed impact of khat on some WBC subtypes might present diagnostic challenges for infections and some cancers that rely on WBC subtypes for diagnosis. This implies that repeated exposure to khat may lead to misdiagnosis for diseases that rely on WBC subtypes. This is a significant and profound phenomenon that may warrant further scrutiny. A significant finding in the present study is that in the presence of CoQ_{10}, the khat-induced increases in the WBC subtypes were nullified. Hence, this outcome indicates that CoQ_{10} protects from khat-induced increases in lymphocytes, monocytes, neutrophils and eosinophils.

The low platelet count exhibited by the khat group during subchronic and chronic exposure could be ascribed to the hemolytic anemia as earlier shown by the khat-induced RBC decrease. The khat-induced platelet depletion could predispose khat chewers to blood clotting

abnormalities and serious implications on people on blood thinning medications. Given that the repeated pattern of platelet suppression during both subchronic and chronic exposure to the stimulant herb, this could imply that chronic users could have severe blood clotting abnormalities. Decreases in platelet subtypes (PCT, P-LCR) were also noted during both and chronic exposure to khat. As much as there is not clear mechanism documented about this finding, there is a possibility of khat-induced effect on the hemopoietic system. Prolonged use of this stimulant herb could have far reaching implications on the hematopoietic system. Similar khat-induced platelet abnormalities have also been reported in previous studies (Ketema *et al.*, 2015; Ismaeel *et al.,* 2014). Remarkably, CoQ_{10} significantly restored the khat-induced depletion of platelet and its subtypes following subchronic and chronic exposure.

The central nervous system (CNS) constitutes a critical part of the mammalian system. A large volume of literature has associated khat with deleterious effects on the CNS (Kimani *et al.,* 2016; Geresu, 2015; Bedada *et al.,* 2010). In the present study, the subchronic and chronic effects of khat on the central nervous system of mice were gauged using the rapid murine coma and behavioral scale (RMCBS). Subsequently, the findings demonstrated khat-induced decline in neuronal integrity of mice; a pattern replicated in both subchronic and chronic exposure. A deeper analysis of the individual RMCBS parameters showed that during subchronic exposure to khat there was a decline in gait, aggression and touch escape. At this point, it is important to note that a decline in the RMCBS parameters aggression and touch escape highly suggests a compromised reflex and preservation (Carroll *et al.,* 2010).

In conclusion, chronic exposure to khat, affects the organ weights and a myriad of biochemical, physiological and pathological effects in Swiss albino mice. Subchronic and chronic exposure to khat resulted in anemia and derangement of RBC, WBC and platelets.

Importantly, there were heightened effects following chronic exposure to khat. These injurious effects are however abrogated upon supplementation with CoQ_{10}.

CHAPTER FOUR

THE ROLE OF CoQ_{10} SUPPLEMENTATION ON KHAT AND MANGANESE-DRIVEN OXIDATIVE STRESS AND INFLAMMATION

4.1 INTRODUCTION

Khat is a psycho-stimulant herb with high potential for abuse (Dasgupta, 2016). There is growing evidence suggesting the potential for khat to induce multiple forms of toxicities (Albaser *et al.*, 2021). However, there is little effort geared towards elucidating key mechanisms leading to khat-induced toxicities. In addition, there is paucity of data about toxicities following co-exposure to khat and other toxicants such as manganese and the exact mechanisms involved. Worthy of note is that manganese is a vital component of metalloenzymes, however, excess exposure is lethal (Neal *et al.,* 2015). Since there has been little effort aimed at finding potential remedies against khat-induced pathologies, the current study additionally sought to elucidate the role of coenzyme-Q_{10} (CoQ_{10}) in modulating the khat and/or manganese-induced oxidative stress and inflammation.

Urban areas are densely populated and have higher levels of metal toxicants such as manganese, cadmium, mercury and Arsenic (Eftekhari *et al*., 2018; Petejova *et al.*, 2019). Notably, most khat chewers reside in urban areas; therefore, they are exposed to higher levels of pollutants such as manganese. Contaminated air is among the routes for manganese and other metal toxin exposure (Santamaria, 2008). Both khat and manganese have been reported to separately induce systemic damages (Fan *et al.,* 2020; Assefa *et al.,* 2018; Santamaria, 2008; Al-Zubairi *et al.,* 2003). Therefore, there could be a possibility that khat users living in areas are vulnerable to heightened toxicities following khat and manganese co-exposure.

Oxidative stress has been highlighted as one of the major mechanism by which khat and manganese induce systemic damages (Adel Al-Zubairi *et al*., 2008; Milatovic *et al*., 2009). Cathinone, a psychoactive component of khat, has been reported as the most potent in induction of euphoria (Engidawork, 2017). It has been suggested that metabolism of cathine and cathinone produces charge metabolites in the cells, leading to increased oxidative stress and subsequent cell death and tissue damage (Bogale *et al.,* 2016; Al-Bekairi *et al.,* 1991). In addition, previous findings have shown that the presence of organophosphates used during cultivation of khat contribute to the khat-driven oxidative damage (Engidawork, 2017; Al-Akwa *et al.,* 2009). On its part, manganese causes oxidative damage when it accumulates in the mitochondria disrupting the respiratory processes (Rama Rao *et al.,* 2004). It is important to note that the manganese-induced damage follows excess exposure.

In addition to oxidative stress, inflammation has been highlighted as another key mechanism by which khat and manganese induce systemic injuries (Abdelwahab *et al.,* 2018; Ketema *et al.,* 2015a). Khat-induced elevation of the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) has been reported in previous studies (Abdelwahab *et al.,* 2018). On the other hand, there have been similar reports of manganese-induced elevation of inflammatory cytokines IFN-γ, TNF-α and IL-6 (Nkpaa *et al.,* 2019; Mokgobu *et al.,* 2015). Notably, there is evidence suggesting that elevations of the pro-inflammatory cytokines may lead to inflammation linked organ pathologies such as hepatitis (Riyaz *et al.,* 2014). Therefore, khat users in urban areas have a likelihood of severe organ pathologies when exposed to manganese present in polluted air.

Given that there have been limited efforts towards finding appropriate remedies against-induced toxicities, the present study sought to determine the role of coenzyme-Q_{10} (CoQ_{10}) on markers of oxidative stress and inflammation following chronic exposure to khat and acute exposure to manganese. Additionally, the role of CoQ_{10} in the modulation of simultaneous exposure to khat and manganese was evaluated.

Worthy of note is that, CoQ_{10} is an abundant endogenous antioxidant and anti-inflammatory in mammals that declines with age (Belardinelli *et al.,* 2008). The anti-inflammatory potential of CoQ_{10} has been demonstrated in studies utilizing animal and human models (Fuller *et al.*, 2006; Monsef *et al.*, 2019; Raygan *et al.*, 2016). The role of CoQ_{10} in modulating khat and manganese-induced oxidative stress and inflammation is yet to be established. Therefore, the choice of CoQ_{10} in the present study was informed by its promising findings that show its potency against neurological conditions, oxidative damage and inflammation-linked pathologies (Fuller *et al.,* 2006; Xing *et al.,* 2004; Matthews *et al.,* 1998).

4.2 MATERIALS AND METHODS

4.2.0 Experimental design and dosage for subchronic exposure study

Forty (40) Swiss albino mice randomly divided into four groups were used in this study. Group one was the control with free access to mice pellets and clean water *ad-libitum* only; group two was given CoQ_{10} at a dose of 200 mg/Kg body weight, group three was administered with 1500mg/Kg body weight of crude khat extract and group four received 1500mg/kg body weight of khat extract and 200mg/Kg body weight of CoQ_{10}. CoQ_{10} was administered daily for two weeks prior to the start of khat treatment to boost its systemic concentration as shown in

previous studies (Rashid *et al.,* 2014). CoQ_{10} was administered one hour prior to the administration of khat for group four that received both khat and CoQ_{10}.

4.2.1 Experimental design and dosage for chronic exposure study

Seventy (70) Swiss Albino mice aged 3-4 weeks were divided into seven groups. The first group was the control group that had access to clean water ad-libitum; the second group was given was given 200µl of (3g/mL) of khat extract per kg body weight; the third group was given free access to manganese (Mn^{++}dissolved in water to make a concentration of 4g/liter); the fourth group was given 200µl of 3g/mL of khat extract per kg of body weight and was given free access to manganese; the fifth group was co-administered with 200µl of CoQ_{10} (200mg/kg b/w) and 200µl of (3g/mL) of khat extract per kg body; the sixth group was given 200µl of CoQ_{10} (200mg/kg b/w) and free access to manganese; finally, the seventh group was co-administered with 200µl of CoQ_{10} (200mg/kg b/w) and 200µl of (3g/mL) of khat extract per kg body and had free access to manganese water. The mice were housed in clean plastic cages at room temperature and a 12 hour light/dark cycle; with free access to mice pellets.

4.2.2 Preparation of khat extract and manganese

Thirty (30) grams of fresh khat leaves were chopped into small pieces and then homogenized in distilled water (30gms/10ml) after which filtration was done and the resulting supernatant was stored at -4°C. Two hundred microliters from the filtrate (200 µl/kg body weight) was used for intragastric administration into mice on khat treatment (Graziani *et al.,* 2008). Manganese (Mn) was prepared by dissolving 4 grams/liter of manganese (II) tetrahydrate (Loba Chemie, Mumbai, India) in one liter (4g/L) of deionized water. This preparation was available to the mice ad-libitum following the protocol by Elbetieha et al (2002). Notably, administration of

manganese was done in the last twelve days to the termination of the experiment for group three and four.

4.2.3 Preparation of coenzyme-Q_{10}

CoQ_{10} solution (Now Foods, Bloomingdale, IL, USA) was prepared fresh on each day of experiment by dissolving it in olive oil. A dose of 200mg/Kg was selected for CoQ_{10} treatment in the current study. The choice of the dosage was based on a previous finding that 200mg/kg of this supplement is sufficient to cross the blood brain barrier and offer neuroprotection (Matthews *et al.,* 1998).

4.2.4 Sample collection

After 12 hours from the last day of treatment (132 days post treatment), mice were euthanized with ketamine and blood was drawn via cardiac puncture. The blood samples was left at room temperature for 30 minutes then centrifuged for 5 minutes at 10,000 rpm and 4℃ for immunological and serological assays. The organs (kidney, liver, brain and spleen) were extracted for biochemical and histopathological analysis. For reduced glutathione assay (GSH), the samples were homogenized in ice-cold conditions in a homogenizing buffer (0.5 ml of 0.25M sucrose, 5mM Hepes-Tris pH 7.4 with protease inhibitor) and aliquoted into 0.5 cryovial tubes. The homogenates were kept at -80℃ until further analyses.

4.2.5 Reduced glutathione assay

Fifty microliters (50µl) of the organ homogenates (brain, liver, spleen, kidney) were mixed with 50µl of solution A (Sulphosalicyclic acid (5%w/v), 0.25mM ethylene diamine tetra acetic acid (EDTA), followed by centrifugation of the mixture at 8000 x g for 10 minutes at 4℃). 200µmol of GSH standard solution was prepared in 0.5% sulphosalicylic acid (SSA) and serial dilutions

made using the same solution (0.5% SSA) to final concentrations of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56µmol of 5.5' Dithiobis (2-nitrobenzoic acid (DTNB). Ellman's reagent was prepared by dissolving 0.1M potassium phosphate buffer with 5mM EDTA disodium salt, pH 7.5 (KPE buffer) to a final concentration of 0.6mg/ml. 25µl of each standard was loaded on a 96-well microtitre plate wells (first two rows), followed by 25µl of the sample to the remaining wells in triplicate. To each well, 100µl of freshly prepared DTNB was added and the absorbance measured at 405nm at intervals of 30 seconds using a multi-detection microtitre plate reader (R and D Systems, Minneapolis, MN).

4.2.6 Cytokine assays

The levels of the cytokines TNF-α, IL-10 and IFN-γ were measured by sand-witch enzyme linked immune sorbent assay (sand-witch ELISA) using cytokine specific (Invitrogen Thermofischer Scientific, California, USA) following the manufacturer's instructions. The spectrophotometric reading was done using ELISA optical reader (Multiskan ex-355, Thermo electron corporation, Waltham, Massachusetts, USA) with absorbance set at 450nm.

4.2.7 Statistical analysis

Statistical analysis was done using Graphpad prism software package (Version 5.0). One way ANOVA was done to compare the treatment groups with controls. For internal comparisons, Turkey's post-hoc test was used. The results were given as a mean ± with significance set at $p<0.05$.

4.3 RESULTS

4.3.1 CoQ_{10} supplementation modulated khat-induced oxidative stress in the brain, liver and spleen at 90 dpt

Subchronic exposure to khat resulted in significant elevations of reduced glutathione (GSH) in the brain ($p<0.001$) (Fig 4.1A) and the spleen ($p<0.0001$) (Fig 4.1C), denoting khat-induced oxidative stress. In the presence of CoQ_{10}, the khat-induced GSH elevations were down-regulated. In the liver, subchronic exposure to khat induced a significant suppression of GSH levels ($p<0.001$). Another remarkable finding in this subchronic phase is that CoQ_{10} significantly restored the GSH levels in the liver (Fig 4.1B). There was a marginal elevation of the GSH level in the lungs (Fig 4.1D), while the GSH level in the heart were unaffected during this subchronic phase (Fig 4.1E).

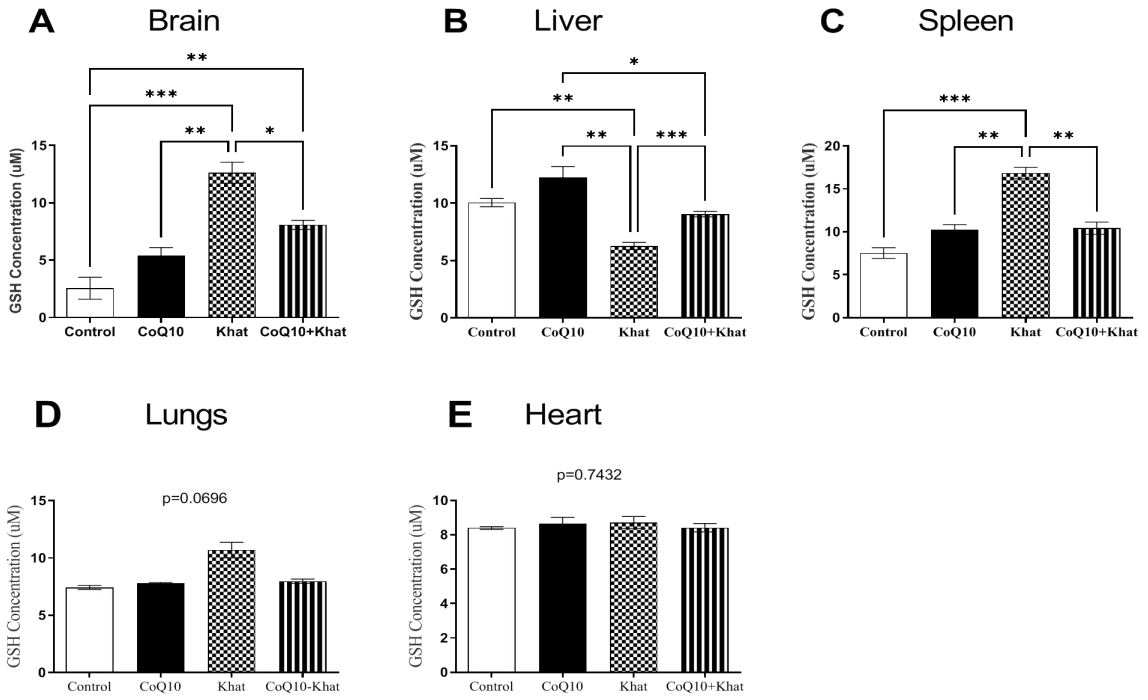


Figure 4.1: Effect of Subchronic khat administration on cellular GSH concentration in mice. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. GSH units expressed in micromoles/g (µmol/g)

4.3.2 CoQ_{10} restored khat and/or manganese-induced reduced glutathione levels in the brain, liver, spleen, lungs and heart

Chronic exposure (132 days post treatment) to khat resulted in significant suppression of cellular GSH in the brain (P<0.05) (Fig. 4.2A), liver (P<0.05) (Fig. 4.2B), spleen (P<0.00001) (Fig. 4.2C) and lungs (P<0.05) (Fig. 4.2D). In the heart, there was a marginal suppression of GSH that was not statistically significant (Fig. 4.2E). Remarkably, in the presence of CoQ_{10}, the khat-induced GSH suppression was reversed in the organs under study. Acute exposure (12 days) to manganese alone resulted in significant suppression of GSH in the brain (P<0.05), liver (P<0.05), spleen (P<0.05) and heart (P<0.05). In the lungs, there was a marginal suppression of GSH that was not statistically significant. Importantly, the manganese-induced GSH suppression in all the organs under study was abrogated in the presence of CoQ_{10}. Additionally,

significant GSH suppressions were noted following simultaneous exposure to khat (chronic) and manganese (acute) in the brain ($P<0.05$) liver ($P<0.05$), spleen ($P<0.001$), lungs ($P<0.001$) and heart ($P<0.05$) (Figure 4.2 A-E). In the presence of CoQ_{10}, the GSH suppression following simultaneous exposure to khat and manganese was reversed in all the organs under study.

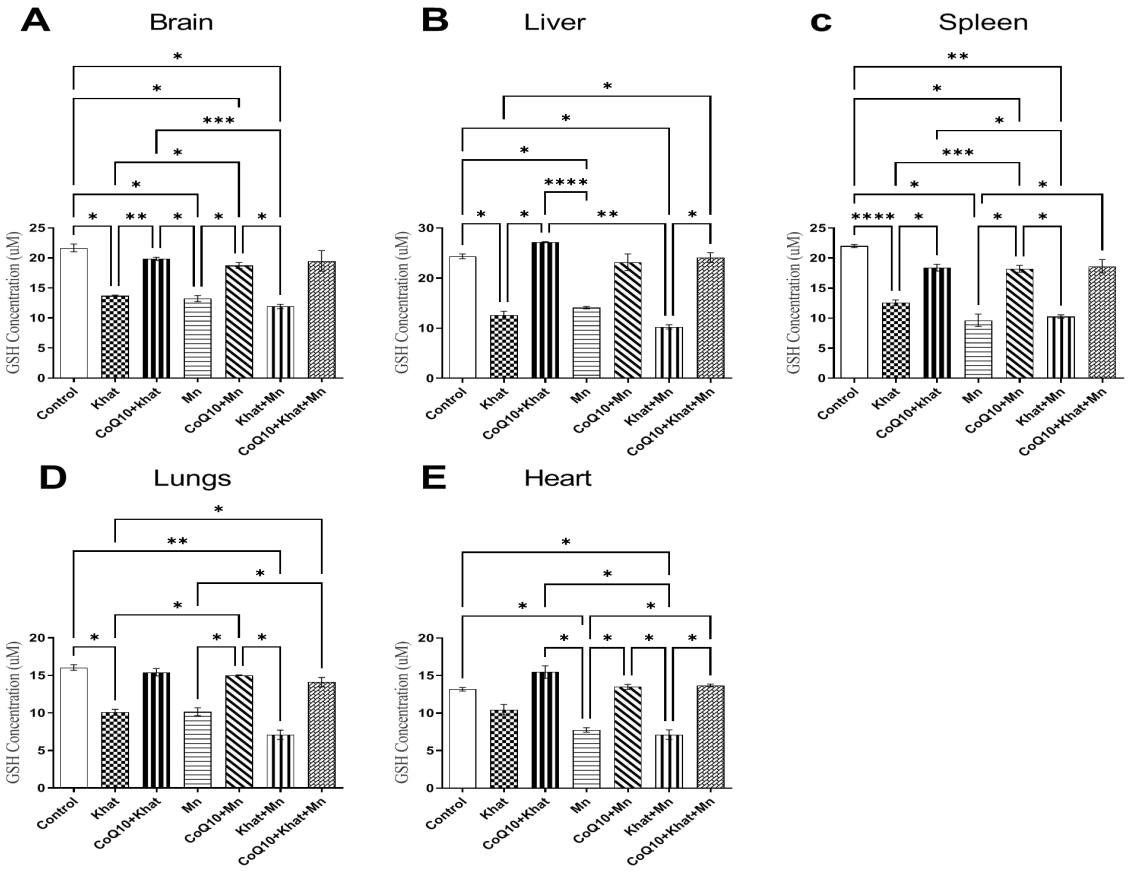


Figure 4.2: Effect of chronic khat and/or acute manganese administration on cellular GSH concentration in mice. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. GSH units expressed in micromoles/g (µmol/g). GSH units expressed in micromoles/g (µmol/g)

4.3.3 CoQ_{10} abrogated khat-induced inflammation at 90dpt

Subchronic exposure to khat resulted in inflammation as denoted by the significant elevation of the pro-inflammatory cytokine tumor necrotic factor-alpha (TNF-α) (Fig 4.3A). Importantly,

this elevation was nullified in the presence of CoQ_{10}. The level of another pro-inflammatory cytokine interferon gamma (IFN-γ) was unaffected following subchronic exposure to khat (Fig 4.3B). An additional finding showed that there was a suppression of the anti-inflammatory cytokine interleukin-10 (IL-10) that was not statistically significant was noted (Fig 4.3C). Remarkably, CoQ_{10} showed a tendency to reverse the IL-10 suppression. An analysis of the ratios of the pro-inflammatory cytokines versus the anti-inflammatory cytokines (TNF-α:IL-10) revealed that the ratio was higher; signaling khat-induced inflammation (Fig 4.4A).

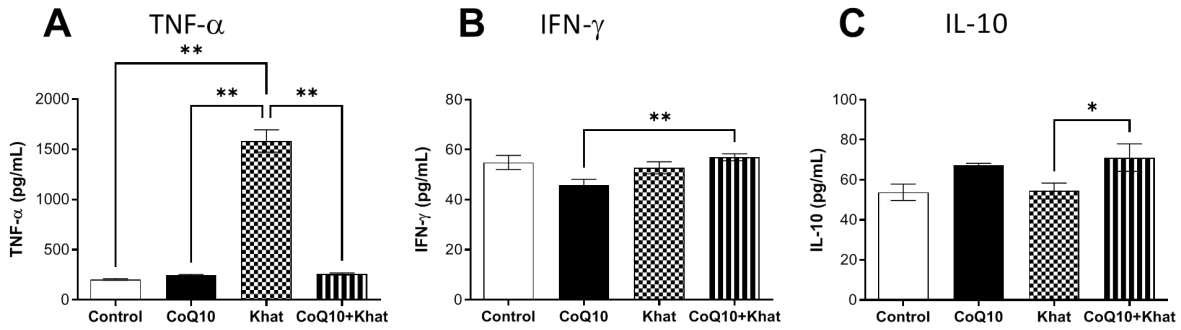


Figure 4.3: The effect of subchronic khat exposure on the levels of the cytokines Tumor Necrosis Factor-alpha (TNF-α), Interferon gamma (IFN-γ), and Interleukin-10 (IL-10). Units pg/mL: Picograms per milliliter. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

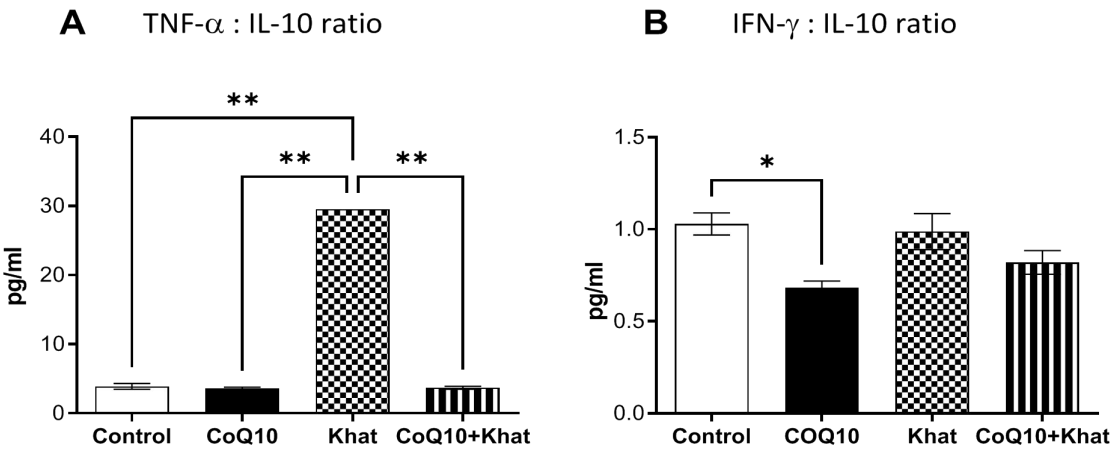


Figure 4.4: The effect of subchronic khat exposure on the levels of the cytokines Tumor Necrosis Factor-alpha (TNF-α), Interferon gamma (IFN-γ), and Interleukin-10 (IL-10). Units pg/mL: Picograms per milliliter. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10. GSH units expressed in micromoles/g (µmol/g)

4.3.4 Coenzyme-Q_{10} supplementation abrogated khat and/or manganese-induced inflammation

Co-exposure to khat and manganese induced a significant elevation of the pro-inflammatory cytokines tumor necrosis factor–alpha (TNF-α) (P<0.001) and interferon gamma (IFN-γ) (P<0.05) (Fig. 4.5A and B), denoting khat and manganese-induced inflammation. Remarkably, the khat and/manganese-induced elevation of this cytokines was normalized in the presence of CoQ_{10} (Fig. 4.5A and B). The level of the anti-inflammatory cytokine interleukin-10 (IL-10) was suppressed in a manner that is not statistically significant when khat and/or manganese were administered separately (Fig. 4.6C). In the presence of CoQ_{10}, the khat and manganese-induced IL-10 suppression was reversed. In another unique observation, simultaneous exposure to khat for 132 days and manganese for 12 days resulted in a significant elevation of the same

cytokine (IL-10). However, this novel finding was reversed in the presence of CoQ_{10}. The higher ratios of the pro-inflammatory versus anti-inflammatory cytokines (Fig. 4.6D and E), further indicate khat and manganese-induced inflammation when the two were either administered separately or simultaneously.

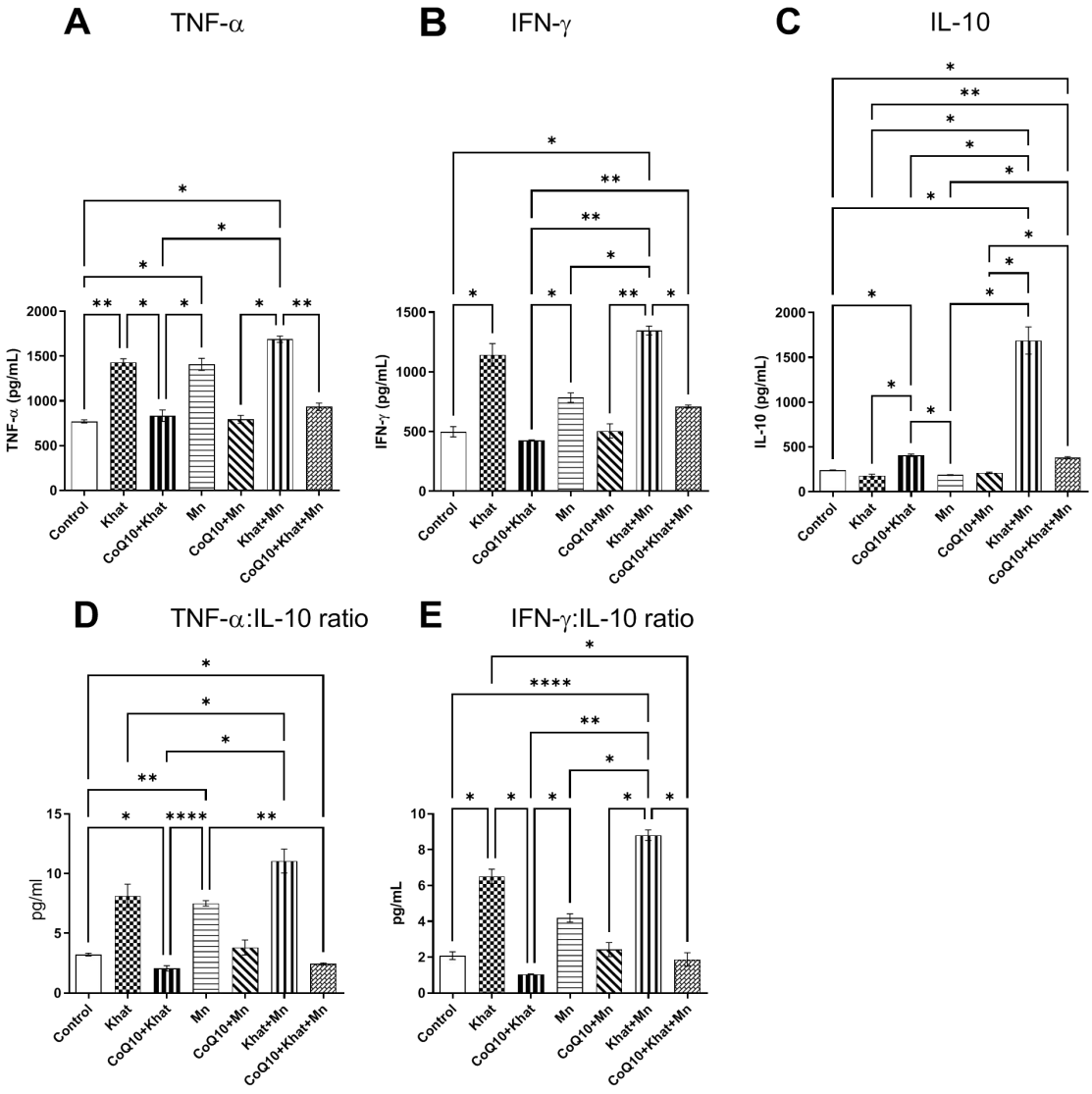


Figure 4.5: The effect of chronic khat and/or acute manganese exposure in mice on the levels of the cytokines Tumor Necrosis Factor-alpha (TNF-α), Interferon gamma (IFN-γ), and Interleukin-10 (IL-10). Units pg/mL: Picograms per milliliter. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

4.3.6 Khat induced an enhanced elevation of the TNF-α following subchronic exposure

Upon a comparative analysis of the impact of subchronic versus chronic khat exposure, it was observed that pro-inflammatory cytokine TNF-α produced was not statistically different (Fig 4.7A). The opposite effect was observed with the pro-inflammatory cytokine IFN-γ, where it was significantly elevated when mice were chronically exposed to khat compared to subchronic phase ($p<0.00001$) (Fig 4.7B). Additional findings in the current objective show that the levels of the anti-inflammatory cytokine IL-10 were elevated by a higher margin when the mice were chronically exposed to khat ($p<0.00001$) (Fig 4.7C). A further analysis of the ratio of the pro-inflammatory to anti-inflammatory cytokines with respect the durations of khat exposure in mice show that: there was a higher ratio of TNF-α: IL-10 during subchronic exposure to khat, and a higher ratio of IFN-γ: IL-10 when mice were chronically exposed to khat.

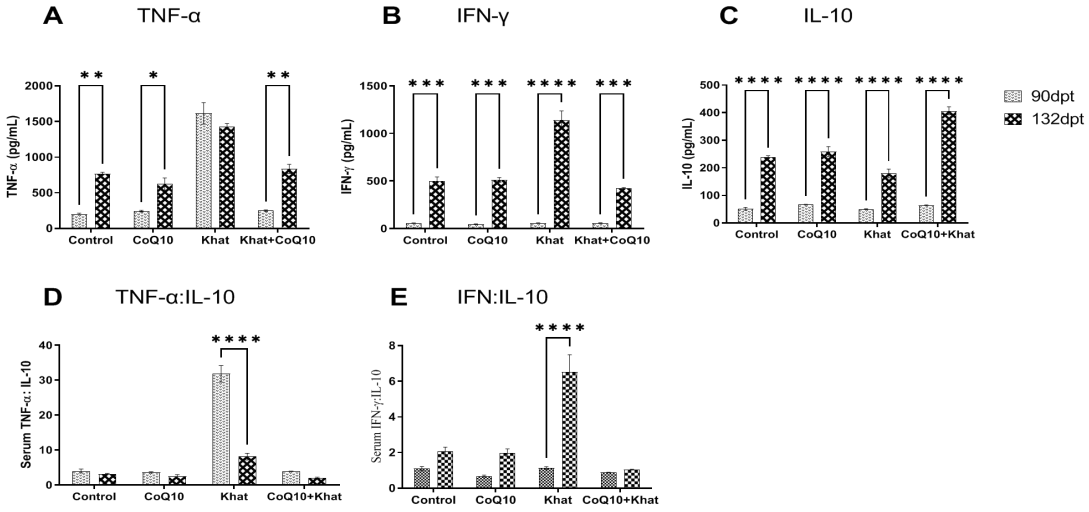


Figure 4.7: The comparison of subchronic versus chronic khat exposure in mice on the levels of the cytokines Tumor Necrosis Factor-alpha (TNF-α), Interferon gamma (IFN-γ), and Interleukin-10 (IL-10). Units pg/mL: Picograms per milliliter. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

4.4 DISCUSSION

While oxidative stress and inflammation can be highlighted as some main mechanisms behind khat-induced and manganese-induced toxicities separately, there is still a paucity of data on the impact of simultaneous exposure to khat and other environmental toxins such as manganese with regard to these two mechanisms. Understanding how these two biological mechanisms are affected by subchronic and chronic exposure to khat and manganese may generate vital information to khat users and the general public. Outcomes of the present study demonstrate for the first time the putative role of CoQ_{10} against exposure to khat as well as co-exposure to other environmental toxicants such as manganese. In the current objective, oxidative stress and inflammation were investigated for their role in khat and manganese-induced toxicities following subchronic and chronic exposure. For oxidative stress determination, the levels of reduced glutathione (GSH) were assayed in the brain, liver, spleen, heart and lungs. Reduced glutathione (GSH) is one of the most abundant antioxidant systems in mammals (Mishra *et al.,* 2005). Importantly, this key antioxidant is ubiquitously distributed in several tissues where it functions to protect organs against oxidative damage (Dukhande *et al.,* 2009; Mishra *et al.,* 2005). Therefore, measurement of cellular GSH is one of the most reliable ways to determine the cells antioxidant capacity as well as the level of cellular oxidative damage.

Subchronic exposure to khat (90dpt) resulted in significant GSH elevations in the brain and the spleen. The noted khat-induced GSH elevations in the two organs (brain and spleen), denotes induction of oxidative stress via increase in GSH following khat challenge. Of note, there is a common assumption that the breakdown of the alkaloid fractions of khat yields charged metabolites during metabolism of khat, hence, resulting in a net increase of cellular oxidative damage (Ali *et al.,* 2015; Al-Qirim *et al.,* 2002). The significant finding showing

khat-induced oxidative stress in the current objective of this study may validate the latter assumption that metabolism of the alkaloid fraction yields toxic metabolites leading to organ toxicity. Worthy of note is that the psycho-active components of khat constitutes the alkaloid fraction of khat (Kalix, 1996).

Additional findings in regards to oxidative stress show that there was depletion of GSH in the liver following both subchronic and chronic exposure to khat; this shows a severely overpowered antioxidant capacity in hepatocytes. This is a critical finding in liver toxicity following exposure to chemical xenobiotics, including acetaminophen toxicity (Fu *et al.,* 2018). Notably, during extreme toxicity to the liver, GSH is normally depleted. Hence, the GSH depletion following khat exposure may signal oxidative damage induced by khat. This outcome in the current study is of great significance since there has been growing evidence of khat-induced liver damage and auto-immune hepatitis in humans (Riyaz *et al.,* 2014; Alsalahi *et al.,* 2012). Therefore, oxidative stress could be among the major contributors to the liver pathology. As an additional finding, the khat-induced suppression of liver GSH was heightened during the subchronic phase (90dpt) when compared to the chronic phase. Thus, the khat-induced oxidative stress observed in the subchronic phase (90dpt) of the current study demonstrates the potential of khat to initiate tissue pathology via oxidative stress even with subchronic exposure.

Numerous studies have shown that khat is associated with the induction of oxidative stress. However, the role of CoQ_{10} in assuaging the khat-induced oxidative stress has not been previously investigated. In this study, CoQ_{10} appears to abrogate the khat-induced oxidative damage in the organs following subchronic exposure to khat. This may be attributed to the clearly established role of CoQ_{10} as a potent antioxidant (Mirmalek *et al.,* 2016; Rashid *et al.,* 2014). Despite the fact that CoQ_{10} has a potent antioxidant capacity, a recent finding showed

that CoQ_{10} reduces mitochondrial alteration by inhibiting the up regulation of manganese superoxide dismutase (MnSOD) and expression of the heme oxygenase genes (Lee *et al*., 2014). In addition, findings of previous experiments have a clear correlation between CoQ_{10} supplementation and oxidative damage protection, neuroprotection and immunomodulation (Nyariki *et al.,* 2019; Rashid *et al.,* 2014).

Further findings in the present objective revealed significant suppression of GSH in the brain, liver, spleen and lungs following chronic khat exposure. The same pattern of GSH suppression was observed following acute exposure to manganese with the exception of the lungs. Of significance, is the fact that there appeared to be enhanced suppression following simultaneous exposure to khat and manganese in the lungs and the heart. The implication of this finding is that, long-term khat users may suffer severe oxidative damage in their lungs and heart when exposed to excess levels of manganese in the environment. It is important to note that manganese has the capacity to induce tissue damage via oxidative stress (Chen *et al.,* 2018; Milatovic *et al.,* 2009; Isaac *et al.,* 2007). Another remarkable finding is that CoQ_{10} dramatically reversed the chronic khat-induced and manganese-induced GSH suppression. This finding shows putative role of CoQ_{10} against subchronic khat-induced oxidative damage. Several studies have shown the putative role of CoQ_{10} in neutralizing oxidative stress conditions induced by other toxins (Belardinelli *et al.,* 2008; El-Laithy *et al.,* 2018; Xing *et al.,* 2004). However, one of the most profound finding in the current study is the positive effect of CoQ_{10} in abrogating oxidative damage resulting from both subchronic and chronic exposure to khat as well as simultaneous exposure to khat and manganese. The findings additionally demonstrate the putative role of CoQ_{10} against oxidative damage following co-exposure to the two toxins.

Cytokines are critical in modulating the host's immune response. Exposure to toxins and xenobiotics potentially affects the immune system. In the current objective, the effects of subchronic as well as chronic exposure to khat on the immune system was experimentally determined. In addition, the impact of manganese separately and in combination with khat was determined. During the subchronic phase, there was a significant khat-induced increase in the levels of the pro-inflammatory cytokine TNF-α, with a marginal increase in IFN-γ. The marked khat-induced rise in the level of TNF-α, could be attributed to a previous finding that khat also triggers apoptosis through TNF-α activation of caspase (Lu *et al.,* 2017). In the presence of CoQ_{10}, the khat-induced increase in TNF-α was nullified in the present study. This significant outcome corroborates previous findings on the immunomodulatory effect of CoQ_{10} on TNF-α (Nyariki *et al.,* 2019; Zhai *et al.,* 2017; Neyrinck *et al.,* 2015)**.** An additional finding showed that the levels of the anti-inflammatory cytokine IL-10 were suppressed in a manner that is not statistically significant following subchronic exposure to khat. Systemic imbalance of pro- and anti-inflammatory response due to khat administration was observed. Remarkably, co-administration of khat with CoQ_{10} showed balance between the pro- and anti-inflammatory cytokines, thus this equilibrium can be attributed to the anti-inflammatory capacity of CoQ_{10} previously reported (Schmelzer *et al.,* 2008). While there is sufficient data available demonstrating the potential anti-inflammatory effect of CoQ_{10}, there still exist information gap in the actual mechanism behind this outcomes. However, in one of the few studies attempting to decipher the mechanism of CoQ_{10} in modulating inflammation it was shown that CoQ_{10} inhibits expression of the IL-6 and TNF-α (Sohet *et al.,* 2009).

The impact of the treatments following chronic exposure to khat and/or acute exposure to manganese on the immune system was additionally investigated by changes in the pro-

inflammatory and anti-inflammatory cytokines. Consequently, chronic khat and/or acute manganese induced significant elevations in the levels of the pro-inflammatory cytokines tumor necrosis factor –alpha (TNF-α) and interferon gamma (IFN-γ).

With regard to inflammation, a comparative analysis of the inflammatory responses following subchronic versus chronic exposure to khat was performed. Consequently, there was a significant elevation of the pro-inflammatory cytokine IFN-γ during the chronic phase. However, the levels of TNF-α were unaffected when this comparison (subchronic vs chronic) was performed. Despite the fact that there might not be a clear explanation behind this noble finding, it is clear that the pathway leading to khat-induced proliferation of the pro-inflammatory cytokine IFN-γ is a slow process. Nonetheless, there is sufficient evidence that khat initiates inflammation via elevation of the two cytokines through two independent processes: the production of TNF-α by activation of the mitogen activated protein kinases (MAPK) cascades (Dirhem *et al.,* 2013), and the rise of IFN-γ via the nuclear-kappa-light-chain-enhancer of activation of B-cells (NFkB) pathway (Murdoch *et al.,* 2011). It is also important to note that CoQ_{10} modulates inflammation by inhibiting the expression of the genes involved in the two pathways (Zhai et al., 2017; Lee *et al*., 2014).

Beyond reasonable doubt, findings from the present study show that exposure to khat and/or manganese induces deleterious events in a mammalian system via oxidative stress and inflammation. Moreover, the injuries were exacerbated following simultaneous exposure to khat and manganese. Perhaps the most profound finding is that in the presence of CoQ_{10}, the effects following khat and/or manganese exposure were abrogated. Therefore, the current study shows that CoQ_{10} may be a useful intervention strategy against khat and/or manganese-induced toxicities.

CHAPTER FIVE

THE ROLE OF CoQ_{10} SUPPLEMENTATION ON ORGAN PATHOLOGY FOLLOWING KHAT AND MANGANESE CO-EXPOSURE

5.1 INTRODUCTION

Numerous scientific reports have demonstrated the role of khat in the induction of multiple organ pathologies (Abdul-Mughni *et al.,* 2018). Other than the brain, which is affected by the psycho-active components of khat (cathine and cathinone), the other two most affected organs include the liver and kidney (Alsalahi *et al.,* 2012; Roelandt *et al.,* 2011). Despite the widespread information on the toxic nature of khat, there still exists gap in regard to the extent of toxicity following chronic exposure to khat. In addition, the majority of khat users reside in urban polluted areas; this exposes them to environmental pollutants such as manganese. This necessitates the need for studies geared towards generating information on extent of damage to the vital organs of people chronically exposed to khat and residing in urban areas. The present objective sought to determine the impact of both subchronic and chronic exposure to khat on liver and the kidney. The impact of simultaneous exposure to khat and manganese, an environmental metal toxin was also investigated. Finally, the impact of CoQ_{10} on the khat and manganese-induced hepatotoxicity and nephrotoxicity was evaluated.

Manganese is a vital component of the manganese superoxide dismutase, hence, required in trace amounts (Isaac *et al.,* 2007). It should be noted that urban areas are densely populated and have higher levels of heavy metal-driven pollution. Notably, most khat chewers reside in urban areas; hence, they are exposed to higher levels of pollutants such as manganese. Manganese exposure is via contaminated air and drinking water (Santamaria, 2008). Both khat

and manganese have been reported to separately induce systemic damages (Fan *et al.,* 2020; Assefa *et al.,* 2018; Santamaria, 2008; Al-Zubairi *et al.,* 2003). Therefore, it is plausible to make an assumption that khat users living in urban areas are vulnerable to heightened toxicities following khat and manganese co-exposure.

As earlier mentioned, the liver and the kidney are some of the target organs of khat and manganese toxicity (Fan *et al.,* 2020; Abdul-Mughni *et al.,* 2018; Alsalahi *et al.,* 2012). Previous studies have shown that both khat and manganese independently induce an elevation of the liver enzyme markers alanine transferase (ALT), aspartate transferase (AST), gamma glutamyltransferase (GGT) and bilirubin, with histopathological findings showing regions of hepatic hyperplasia, hepatic fibroblast proliferation and vacuolation (Abdul-Mughni *et al.,* 2018; Alsalahi *et al.,* 2012). Similar patterns of organ toxicities have been reported in the kidney following separate khat and manganese exposure in previous studies. Essentially, khat induced periglomerular fibroblast proliferation and renal congestion in studies done in mice Abdul-Mughni et al., 2018; Alsalahi et al., 2012;).

Given that there have been limited efforts towards finding appropriate remedies against-induced toxicities, the present study sought to determine the role of coenzyme-Q_{10} (CoQ_{10}) against chronic exposure to khat and acute exposure to manganese. Additionally, the role of CoQ_{10} in the modulation of simultaneous exposure to khat and manganese was evaluated. Worthy of note is that, CoQ_{10} is an abundant endogenous antioxidant in mammals that declines with age (Belardinelli *et al.,* 2008). CoQ_{10} in the present study was informed by promising findings that show its potency against neurological conditions, oxidative damage, inflammation and hematological perturbations (Fuller *et al.,* 2006;;Xing *et al.,* 2004; Matthews *et al.,* 1998).

Previous studies demonstrated strong ameliorative effect against chemical drug-induced toxicities (Mwaeni *et al*., 2021; Gao *et al*., 2016; Beal *et al.*, 1998).

5.2 MATERIALS AND METHODS

5.2.1 Experimental design for subchronic exposure to khat

To simulate subchronic exposure to khat, all mice under khat treatment were exposed to khat for 90 days. Forty (40) Swiss albino mice randomly divided into four groups were used in this study. Group one was the control which free access to mice pellets and clean water *ad-libitum* only; group two was given CoQ_{10} at a dose of 200 mg/Kg body weight, group three was administered with 1500mg/Kg body weight of crude khat extract and group four received 1500mg/kg body weight of khat extract and 200mg/Kg body weight of CoQ_{10}. CoQ_{10} was administered daily for two weeks prior to the start of khat treatment to boost its systemic concentration as shown in previous studies (Rashid *et al.,* 2014). CoQ_{10} was administered one hour prior to the administration of khat for group four that received both khat and CoQ_{10}.

5.2.2 Experimental design and dosage for chronic exposure to khat

To simulate chronic exposure to khat, all mice under khat treatment were exposed to khat for 132 days. Seventy Swiss Albino mice aged 3-4 weeks were divided into seven groups. The first group was the control group that had access to clean water ad-libitum; the second group was given was given 200µl of (3g/mL) of khat extract per kg body weight; the third group was given free access to manganese (Mn^{++}dissolved in water to make a concentration of 4g/liter); the fourth group was given 200µl of (3g/mL) of khat extract per kg of body weight and was given free access to manganese; the fifth group was co-administered with 200µl of CoQ_{10} (200mg/kg b/w) and 200µl of (3g/mL) of khat extract per kg body; the sixth group was given 200µl of

CoQ_{10} (200mg/kg b/w) and free access to manganese; finally, the seventh group was co-administered with 200µl of CoQ_{10} (200mg/kg b/w) and 200µl of (3g/mL) of khat extract per kg body and had free access to manganese water. The mice were housed in clean plastic cages at room temperature and a 12 hour light/dark cycle; with free access to mice pellets.

5.2.3 Preparation of khat extract and manganese

Thirty (30) grams of fresh khat leaves were chopped into small pieces and then homogenized in distilled water (30gms/10ml) after which filtration was done and the resulting supernatant was stored at -4°C. Two hundred microliters from the filtrate (200 µl/kg body weight) was used for intragastric administration into mice on khat treatment (Graziani *et al.,* 2008). Manganese (Mn) was prepared by dissolving 4 grams/liter of manganese (II) tetrahydrate (Loba Chemie, Mumbai, India) in one liter (4g/L) of deionized water. This preparation was available to the mice ad-libitum following the protocol by Elbetieha (Elbetieha *et al.,* 2002). Notably, administration of manganese was done in the last twelve days to the termination of the experiment for group three and four. In group four, both khat and Mn^{2+} were administered to the termination of the experiment on day 132.

5.2.4 Preparation of coenzyme-Q_{10}

CoQ_{10} solution (Now Foods, Bloomingdale, IL, USA) was prepared fresh on each day of experiment by dissolving it in olive oil. A dose of 200mg/Kg was selected for CoQ_{10} treatment in the current study. The choice of the dosage was based on a previous finding that 200mg/kg of this supplement is sufficient to cross the blood brain barrier and offer neuroprotection (Matthews *et al.,* 1998).

5.2.5 Sample collection

After 12 hours from the last day of treatment (90dpt for subchronic exposure and 132dpt for chronic exposure), mice were euthanized with ketamine and blood was drawn via cardiac puncture. The blood samples were left at room temperature for 30 minutes then centrifuged for 5 minutes at 10,000 rpm and 4℃ for immunological and serological assays. The organs (kidney, liver, brain and spleen) were extracted for biochemical and histopathological analysis.

5.2.6 Biochemical assays of liver biomarkers

Serum alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), aspartate Amino transferase (AST), direct bilirubin (DBIL) and total bilirubin (TBIL) were measured using automated analyzer (COBAS Integra-400 plus analyzer, Roche, Basel, Switzerland).

5.2.7 Histopathological analysis

The extracted organs (brain, liver, kidney and speen) samples were fixed in 4% formalin, dehydrated in absolute ethanol and finally embedded in paraffin. The prepared tissues were then sectioned at 5µ with subsequent staining using hematoxylin and eosin for microscopic examination. The sections were then examined for pathology and images taken under a light microscope (Zeis Axio scope).

5.2.8 Statistical analysis

Statistical analysis was done using Graphpad prism software package (Version 5.0). One way ANOVA was done to compare the treatment groups with controls. For internal comparisons, Turkey's post-hoc test was used. The results were given as a mean ± with significance set at $p<0.05$.

5.3 RESULTS

5.3.1 CoQ_{10} supplementation nullified khat-induced elevation of liver biomarkers AST, ALT, GGT, total bilirubin and creatinine

Subchronic exposure to khat resulted in significant elevations of the liver biomarkers aspartate aminotransferase (AST) ($p<0.05$) (Fig 5.1A), alanine aminotransferase (ALT) ($p<0.05$) (Fig 5.1B) and gamma glutamyl aminotransferase (GGT) ($p<0.001$) (Fig 5.1 D), total bilirubin ($p<0.001$) (Fig 5.1E) and the kidney biomarker creatinine ($p<0.05$) (Fig 5.1 F); signaling khat-induced hepatotoxicity and nephrotoxicity. Additional findings showed that the ratio of AST to ALT was high further confirming khat-induced liver damage (Fig 5.1 C). In the presence of CoQ_{10}, the elevation was abrogated.

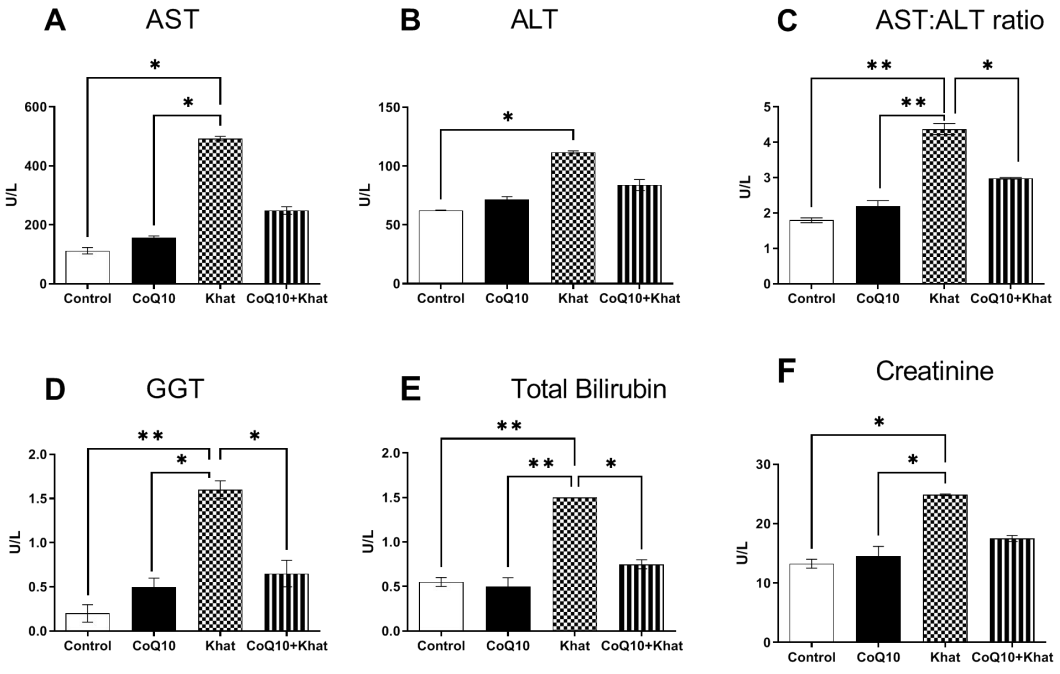


Figure 5.1: Effect of subchronic exposure to khat and/or CoQ_{10}, on the liver and kidney biomarkers. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

5.3.2 CoQ_{10} nullified khat and manganese-induced elevations of liver and kidney biomarkers

The liver enzyme biomarkers aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly elevated when the mice were exposed to khat for 132 days and/or manganese for 12 days (Fig. 5.2 A and B, respectively), denoting khat-manganese-induced hepatotoxicity and nephrotoxicity. A significantly higher ratio of AST: ALT (Fig. 5.2C), providing further evidence for khat and/or manganese-induced toxicity. Interestingly, the khat and manganese-induced rise in the liver biomarkers was curtailed in the presence of CoQ_{10}. In a similar version, significantly higher levels of total bilirubin (Fig. 5.2E) and gamma glutamyltransferase (GGT) (Fig. 5.2D), were noted providing additional proof of liver toxicity following khat and/or manganese exposure in mice. Remarkably, in the presence of CoQ_{10} the levels of both bilirubin and GGT were normalized. Finally, there was kidney damage in mice exposed to khat and/or manganese (Fig. 5.2F) as denoted by the elevated levels of creatinine. Notably, CoQ_{10} suppressed the khat and/or manganese-induced injury. Co-administration of khat and manganese resulted in more significant elevation of all liver biomarkers namely-AST, ALT, GGT, total bilirubin and creatinine. Clearly, exposure to manganese exacerbated khat-toxicity and vice versa. The results also show that in the presence of CoQ_{10}, the khat and manganese-driven liver and kidney damage were ameliorated (Fig. 5.2A, B, C, E and F)

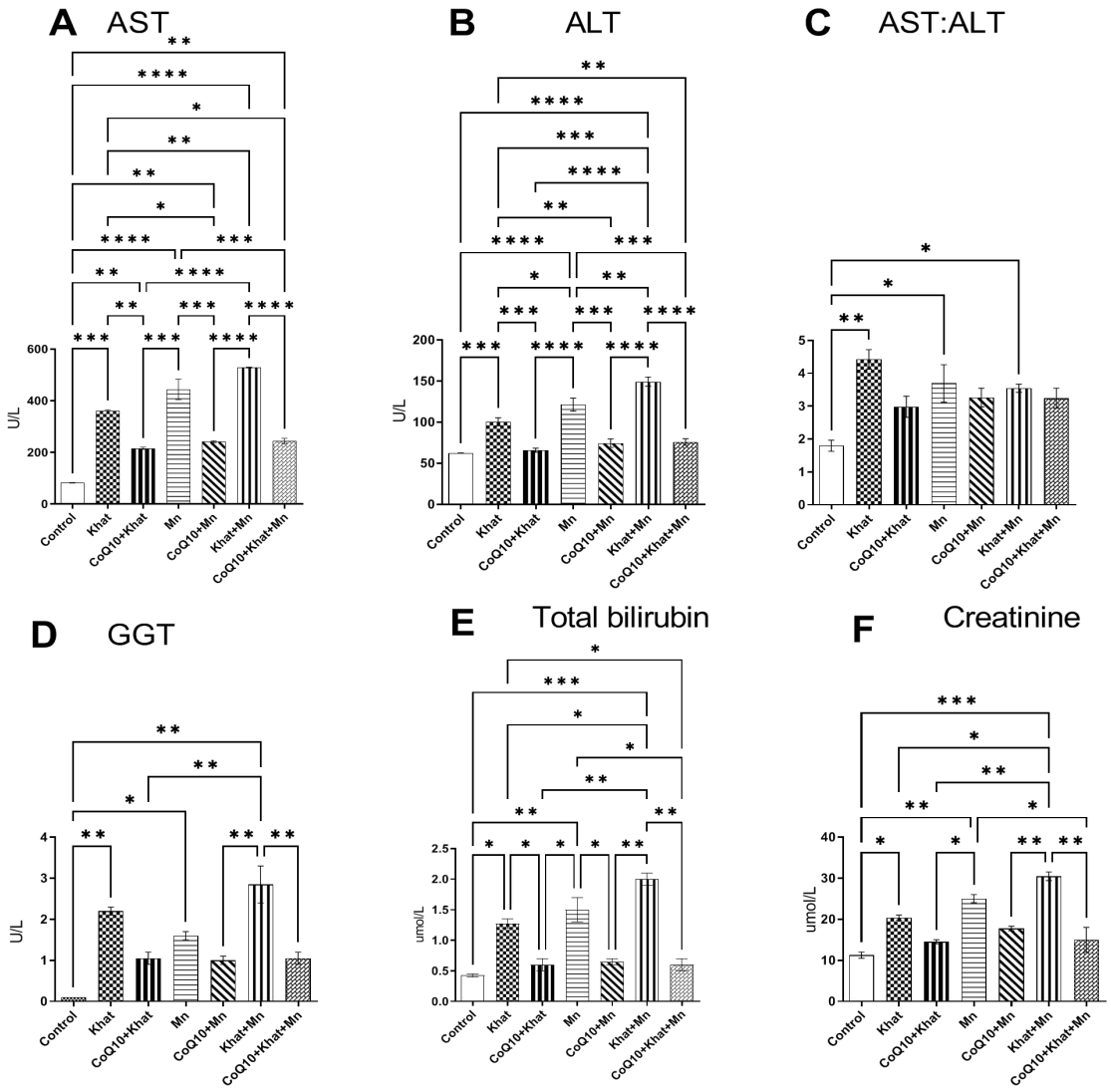


Figure 5.2: Effects of chronic khat and/or acute manganese administration on the kidney protein biomarkers in mice. The figure shows changes in the activity of A: AST (aspartate aminotransferase), B: ALT (Alanine aminotransferase), C: The ratio AST: ALT, D: total bilirubin, E: Gamma glutamyl aminotransferase (GGT) and F: creatinine. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

5.3.3 Enhanced liver injury following subchronic exposure to khat

A comparison of the effects of the subchronic versus chronic exposure to khat showed that the there was a significantly heightened elevation of the liver enzyme AST ($p<0.001$) (Fig 5.3A)

and the kidney biomarker creatinine ($p<0.0001$) (Fig 5.3E) during the subchronic phase. Additionally, there was a heightened elevation of ALT and total bilirubin that was not statistically significant (Figs 5.3B and D) during the same subchronic phase. On the contrary, the levels of GGT were significantly increased during the chronic phase ($p<0.05$) (Fig 5.3C).

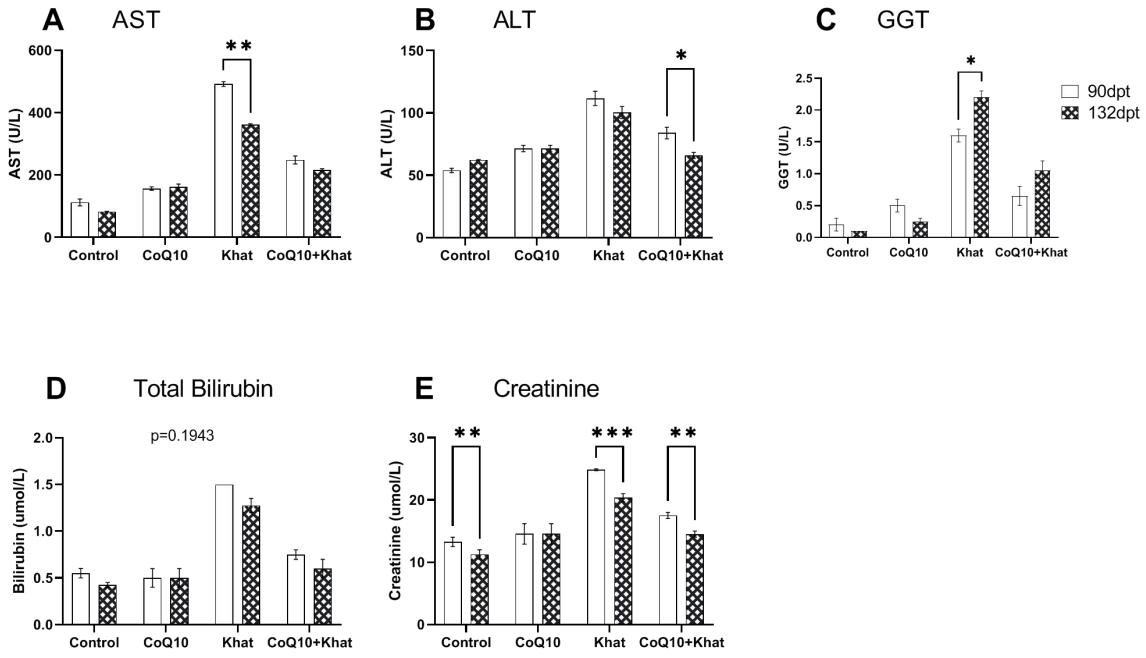


Figure 5.3: Effect of subchronic versus chronic exposure to khat and/or CoQ_{10}, on the liver and kidney biomarkers. Data was analyzed using one way ANOVA with Tukey post hoc test for multiple comparison. Level of significance *P ≤ 0.05; P**≤0.001***P ≤0.0001. n=10.

5.3.4 CoQ_{10} attenuated khat-induced liver organ injury

The liver sections from khat-treated mice showed multifocal areas of hepatic necrosis (indicated by arrows) and hepatocyte cytoplasm vacuolation (indicated by stars) (Fig 5.4b). Liver tissue sections from the control group revealed normal hepatocytes (Fig 5.4a) as well as mice co-treated with CoQ_{10} (Fig 5.4c).

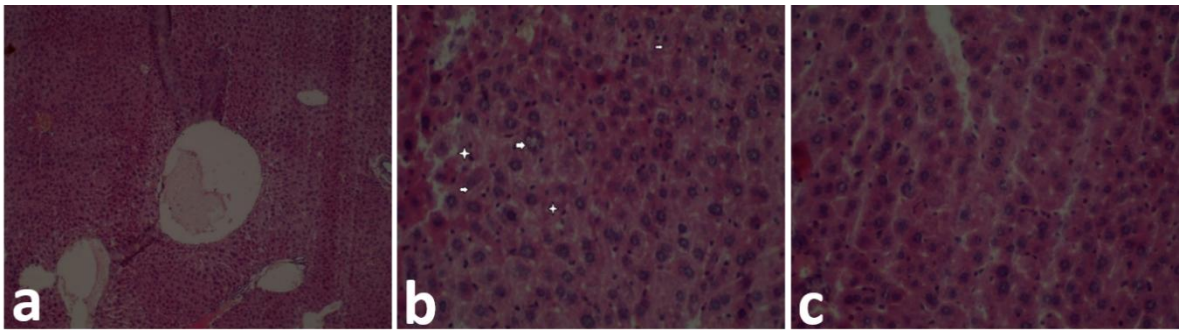


Figure 5.4: The figure shows the effect of khat and / or CoQ_{10} on the liver tissue. Mice orally administered with Coenzyme Q10 show mild liver damage as shown. (a) Normal liver section from control group, (b) Liver section from mice that received 1500mg/kg bwt of Khat orally showing focal hepatocyte necrosis (arrow) and hepatocyte cytoplasm vacuolation (star) (x100, H and E). (c)Normal Liver from mice treated with 200mg/kg of CoQ_{10} and 1500 mg/kg of body weight of khat (×100, H and E). The results clearly demonstrate that khat induced liver damage; and CoQ_{10} protected from such injury.

5.3.5 CoQ_{10} attenuated khat-induced kidney organ injury

There was tubular necrosis and tubular epithelium injury characterized by the vacuolar degeneration of the tubular epithelial cells in the khat-treated group (shown by the arrows) (Fig. 5.4b) in the kidney tissue section. Kidney sections from the control and the group co-treated with CoQ_{10} had normal kidney cellular architecture (Fig 5.5a and Fig 5.5c).

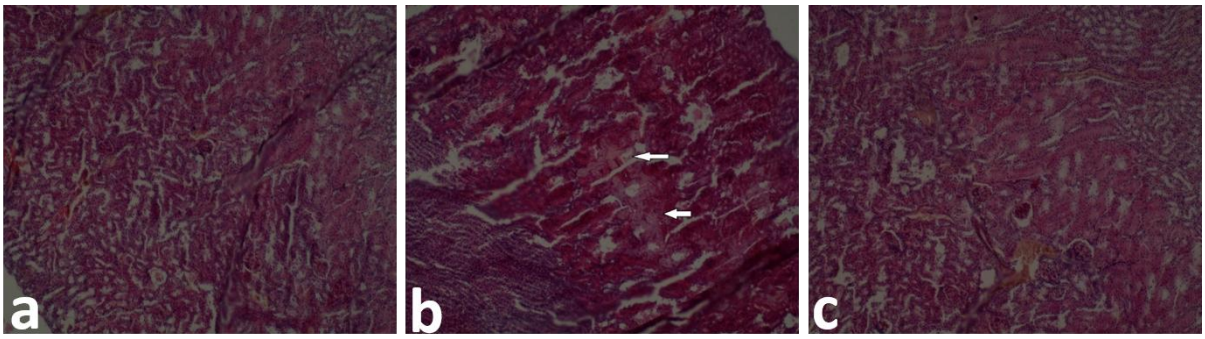


Figure 5.5: The figure shows the effect of khat and / or CoQ_{10} on the kidney tissue. (a) Normal Kidney of mice from control group (b) Focal areas of tubular necrosis in mice administered with Khat (arrows) and (c) Normal kidney of mice treated with 200mg/kg of CoQ_{10} and 1500 mg/kg of body weight of Khat. (Magnification: × 100, H and E). The results clearly show that khat extract induced kidney injury; and CoQ_{10} provided some protection from such damage.

5.3.6 CoQ_{10} assuaged chronic khat-induced liver damage

Histological analysis of the liver section revealed regions of bile duct hyperplasia (BD) and fibroblast proliferation in the liver of the khat exposed mice (Fig. 5.6b). Liver sections from mice co-exposed to khat and CoQ_{10} revealed diffuse hepatic fibrosis (Fig. 5.6c), and those of mice exposed to manganese alone revealed hepatic regions with congestion of hepatic blood vessels and multi-focal areas of hepatic fibroblast (Fig. 5.6d). In the CoQ_{10}+manganese group, the liver showed congestion of hepatic blood vessel without hepatic fibrosis (Fig. 5.6e). The liver sections from mice co-exposed to khat and manganese revealed localized areas of fibroblast proliferation and fibrosis (Fig. 5.6f). The group of mice exposed to CoQ_{10}, khat and manganese showed congestion of hepatic blood vessels (Fig. 5.6g).

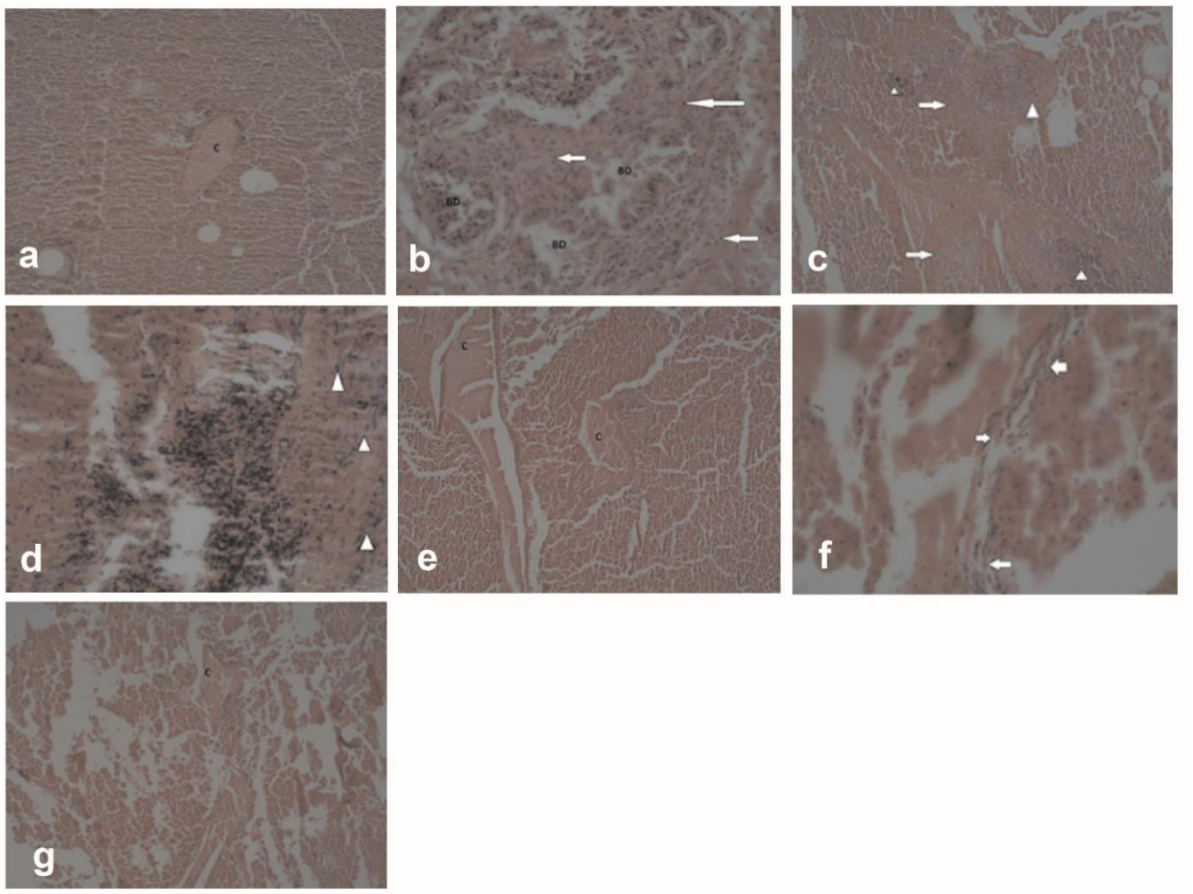


Figure 5.6: The effect of khat and/or manganese on the liver tissue. (a) Normal liver tissue from the control group showing normal tissue with congestion of hepatic blood vessel (Magnification: x100, H and E); (b) Liver section from khat group showing focal areas of bile duct hyperplasia (indicated "BD") and fibroblast proliferation and connective tissue proliferation (arrow) (Magnification: x100, H and E); (c) Liver section from mice exposed to CoQ_{10} and khat showing diffuse hepatic fibrosis (arrow) and kupfer's cells infiltration (arrow head) (Magnification: x100, H and E); (d) Liver section from mice exposed to manganese showing congestion of hepatic blood vessels and multi focal areas of hepatic fibroblasts proliferation (arrow head) (Magnification: x160, H and E); (e) Liver section from mice co-exposed to CoQ_{10} and manganese showing congestion of hepatic blood vessels (indicated by "C") (Magnification: x160, H and E); (f) Liver section from mice co-exposed to khat and manganese showing localized areas of fibroblast proliferation (arrow) (Magnification: x160, H and E); (g) Liver section from mice simultaneously exposed to CoQ_{10}, khat and manganese showing congestion of hepatic blood vessels (indicated by "C") (Magnification: x160, H and E).

5.3.7 Exposure to khat and/or manganese induced kidney organ injury

Kidney sections from mice chronically exposed to khat (132dpt) showed regions of periglomerular fibroblast proliferation and tubular epithelial cell swelling (Figure 5.7b). Kidney sections from mice co-exposed to khat and manganese revealed regions of periglomerular fibroblast proliferation, tubular epithelial cell swelling and congestion of renal blood flow; denoting severe kidney damage (Figure 5.7f). Similar findings were noted in kidney sections simultaneously exposed to khat and manganese and treated with CoQ_{10} (Figure 5.7g).

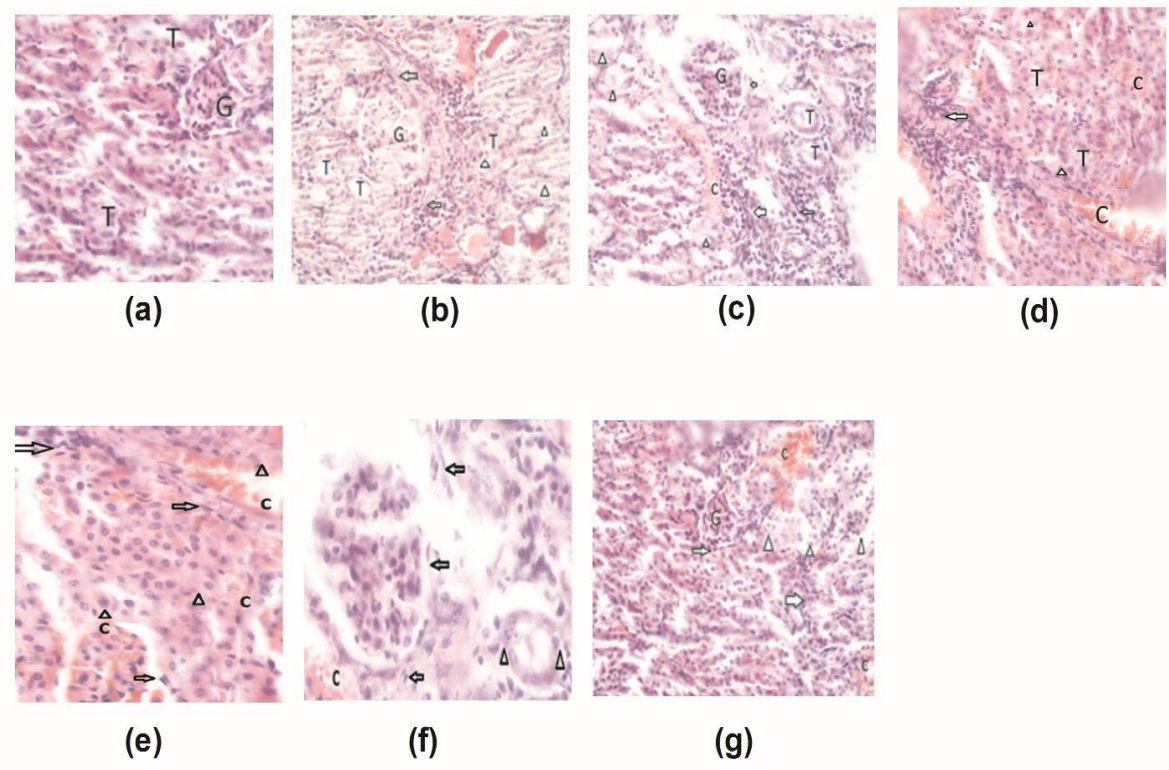


Figure 5.7: the effect of khat and/or manganese on the liver tissue. (a) Normal kidney tissue from the control group showing normal tissue (Magnification: x100, H and E); (b) Kidney section from khat group showing periglomerular fibroblast proliferation (arrow) and tubular epithelial cell swelling (arrow head) (Magnification: x100, H and E); (c) Kidney section from mice exposed to CoQ_{10} and khat showing periglomerular fibroblast swelling (arrow), tubular epithelial cell swelling (arrow head) and congestion of renal blood vessels (indicated by "C") (Magnification: x100, H and E); (d) Kidney section from mice exposed to manganese showing periglomerular fibroblast proliferation (arrow), tubular epithelial cell swelling (arrow head) and congestion of renal blood flow (indicated by "c") (Magnification: x160, H and E); (e) Kidney section from mice co-exposed to CoQ_{10} and manganese showing periglomerular fibrosis

(arrow), tubular epithelial cell swelling (arrow head) and congestion of renal blood vessels (indicated by "c") (Magnification: x160, H and E); (f) Kidney section from mice co-exposed to khat and manganese showing periglomerular fibrosis (arrow), tubular epithelial cell swelling (arrow head) and congestion of renal blood vessel (indicated by "C") (Magnification: x160, H and E); (g) Kidney section from mice simultaneously exposed to CoQ_{10}, khat and manganese showing congestion of renal blood vessels (indicated by "C"), periglomerular fibrosis (arrow) and tubular epithelial cell swelling (arrow head) (Magnification: x160, H and E).

5.4 DISCUSSION

Findings from this study clearly demonstrate that when khat and manganese are present at the same time, their individual toxicities are significantly increased. This finding may have profound implications for khat consumers, who reside in urban areas heavily polluted with heavy metals as a result of pollution and chemical industrial activities including mining. Of great significance, was the finding that CoQ_{10}, a powerful antioxidant, anti-inflammatory and immunomodulatory molecule significantly ameliorated khat and manganese-induced organ toxicities.

There is insufficient data on the effect of simultaneous exposure to khat and other environmental toxins such as manganese on organ pathology. Moreover, there are no studies focused on finding interventions that can alleviate khat-induced toxicities. Findings from the present study demonstrate for the first time the putative role of CoQ_{10} in ameliorating khat and manganese-induced toxicities.

Investigation of the liver and kidney biomarkers is of profound importance in determining the extent of liver and kidney damage induced by chemical toxins (Aldenborg *et al.,* 2006). In the current study, an assay for the liver enzyme or protein biomarkers such as aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT) and total bilirubin was done. For kidney function, the level of creatinine was measured. Consequently, subchronic exposure to khat (90dpt) resulted in significant elevation of the liver enzymes AST, ALT, GGT and total bilirubin. This outcome clearly shows that subchronic exposure to khat induces liver damage. The outcome further corroborates findings from previous studies following subchronic exposure to khat (Abdul-Mughni *et al.,* 2018; Pantano *et al.,* 2016; Alsalahi *et al.,* 2012). In addition the damage to the liver, the increased

levels of ALT points to the possibility of khat-induced toxicity in the other organs given that ALT is ubiquitously distributed in other organs such as the heart and the muscles (Aldenborg *et al.,* 2006; Civili, 1990). The higher ratio of AST: ALT following subchronic to khat exposure further confirmed khat-induced liver damage.

Subchronic exposure to khat resulted in increased levels total bilirubin. The implication of this finding is that the khat-induced bilirubin increase may signal an underlying khat-induced breakdown of the red blood cells (RBCs); since bilirubin is a product of RBC breakdown (Ismaeel *et al.,* 2014). Importantly, this assumption is reinforced by the earlier findings indicating that the levels of RBCs were suppressed as well as the percentage hematocrit when mice were sub chronically exposed to khat.

A profound finding in this study was that in the presence of CoQ_{10}, the khat-induced elevations of liver enzyme markers was abrogated. The role of CoQ_{10} in modulating the khat-induced liver pathology may partly be attributed to its role as a powerful antioxidant and anti-inflammatory (Garrido-Maraver *et al.,* 2014; Fuller *et al.,* 2006). Notably, oxidative stress and inflammation are some of the key mechanisms by which khat initiates toxicity (Abdelwahab *et al.,* 2018; Al-Qirim *et al.,* 2002). The CoQ_{10}-driven nullification of khat/manganese-induced elevation of bilirubin may have vital implication on RBCs. Possibly, CoQ_{10}, via its antioxidant capacity could protect RBCs from the khat and manganese-induced toxicity (Fibach *et al.,* 2008).

Measurement of creatinine levels has continued to be used to gauge the health status of the kidney (Oladipo *et al.,* 2016). Higher levels of creatinine are usually perceived as warning signs of impending kidney damage. Subchronic exposure to khat resulted in elevated levels of creatinine in mice in the current study; signaling possible khat-induced kidney damage. A

notable finding here was the fact that in the presence of CoQ_{10}, the khat-induced elevation of creatinine was down-regulated. This shows the protective role of CoQ_{10} against khat-driven nephrotoxicity.

Further investigations using histology indicated khat-induced hepatocellular necrosis. This could be attributable to the role of khat in activation of apoptotic mechanisms leading to hepatocellular cell death (Onyango *et al.,* 2020; Lu *et al.,* 2017). Additionally, this phenomenon could partly account for the reduction in the relative organ weight of the liver observed in the current study. An additional finding showed that there was khat-induced hepatocyte vacuolation. This finding corroborates a report from a previous study where exposure to khat for 90 days resulted in degenerative vacuolation in new Zealand rabbits (Abdul-Mughni *et al.,* 2018). Though there is still no clear mechanism behind this phenomenon, it is assumed that the various toxins in khat accumulate and cause vacuole expansion (Riyaz *et al.,* 2014; Alsalahi *et al.,* 2012). The role of CoQ_{10} in abrogating the khat-induced histopathological events in the liver was also demonstrated.

A histopathological analysis of the kidney tissue following subchronic exposure to khat, revealed focal areas of tubular necrosis. This pathological process was ameliorated in the presence of CoQ_{10}. Subchronic exposure to khat resulted in liver and kidney pathologies that were abrogated in the presence of CoQ_{10}. These findings show that CoQ_{10} may be a potent remedy against hepatotoxicity and nephrotoxicity following subchronic exposure to khat.

Despite the widespread literature on the deleterious effects following subchronic exposure to khat, there is still a paucity of data on the effects following chronic exposure to the psycho-stimulant herb. For this reason, the current study additionally sought to determine the impact of chronic exposure to khat on the liver and the kidney. To simulate chronic exposure to

khat, mice were given khat for 132 days. Chronic exposure to khat resulted in liver and kidney damage as indicated by the significant elevations of the liver biomarkers AST, AST, GGT and total bilirubin. Acute exposure to manganese equally resulted in significant elevation of liver and kidney damage biomarkers. In the presence of CoQ_{10}, the khat and manganese-induced elevations of the liver and kidney enzymes/proteins was abrogated. The implication is that CoQ_{10} supplementation prevented khat-induced hepatotoxicity and nephrotoxicity.

To decipher physiological and biochemical complications of co-exposure, mice chronically exposed to khat were acutely administered with manganese on day 120. Chronic exposure to khat followed by acute exposure to manganese resulted in heightened hepatotoxicity and nephrotoxicity as denoted by the enhanced levels of AST, ALT, GGT, creatinine and total bilirubin. The implication of this outcome to khat users is that, repeated consumption of khat and concurrent exposure to excess levels of manganese could result in severe hepatotoxicity and nephrotoxicity. This is of central importance given that a majority of the khat users live in urban areas heavily polluted with manganese and other toxic metals. The extent of toxicity following simultaneous exposure to khat and manganese for khat users residing in the polluted areas for a larger part of their lives is still unknown and warrants further investigation. However, it is plausible to make an assumption based on our findings that the latter scenario, has far reaching implications in terms of toxicity.

Further investigations revealed a CoQ_{10}-driven protection against khat and manganese toxicity when co-administered. Possibly, CoQ_{10} modulates pathways leading to khat and manganese-induced toxicities. In the current research, there was clear evidence that khat and manganese partly induced their toxicities via oxidative stress and inflammation. Since manganese is excreted through the biliary system, damage to the liver leading to reduced

capacity of the liver to excrete manganese potentially results in manganese accumulation in the liver. The consequence of this is manganese-induced liver damage via oxidative stress since excess manganese accumulation inhibits the mitochondrial superoxide dismutase responsible for protecting the cell against oxidative damage (Michalke *et al.,* 2014).

The histological findings from the chronic phase of the study were indicative of khat and/or manganese-induced liver and kidney damage. Histological sections from the mice liver for khat and/or manganese treatment revealed focal areas of bile duct hyperplasia and fibroblast proliferation. Notably, hepatic fibroblast proliferation is a common feature during chronic chemical-induced liver injury (Steven, 2019). On the other hand, liver sections from mice exposed to the three treatments (CoQ_{10}, khat and manganese) had a normal cellular distribution of hepatocytes with congestion of hepatic blood vessels. The kidney sections revealed regions of periglomerular fibrous proliferation, tubular epithelial cell swelling and congestion of renal blood flow for mice on khat and manganese.

Findings from the present study show that exposure to khat and/or manganese induces hepatotoxicity and nephrotoxicity. Moreover, the injuries were exacerbated following simultaneous exposure to khat and manganese. A notable finding is the protective effect of CoQ_{10} against the hepatotoxic and nephrotoxic effects of khat and/or manganese in the subchronic and chronic phase. These findings provide remarkable opportunities for further studies on the possibility of developing a nutritional or pharmacological intervention to protect from khat and manganese-driven organ toxicity among khat consumers.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 GENERAL DISCUSSION

There has been increasing reports showing growing use of khat in the horn of Africa and the Arabian region (Mihretu *et al*., 2017). In Kenya, it is common to see youths gathered in certain neighborhood while chewing khat leaves. This signals a growing substance abuse crisis, given the increasing reports of khat-induced toxicities (Al-Qirim et al., 2002; Mohammed et al., 2014). In the present study, the effects of khat on physiological, biochemical and organ pathology were determined. In addition, the effects following co-exposure to khat and manganese were elucidated. In efforts to find appropriate strategies against khat-induced toxicities, the present study additionally sought to find the potential ameliorative role of CoQ_{10} against khat and/or manganese-induced toxicities.

Consequently, negative physiological and biochemical changes following subchronic and chronic exposure to khat were noted as shown by the marked increase in brain weight and spleen weight following subchronic and chronic exposure to khat. In addition, a notable decrease in the body weight of mice exposed to khat subchronically and chronically was registered. The heightened increase in the weight of the brain following chronic exposure to khat further shows heightened toxicities associated with prolonged exposure to khat.

Further findings showed khat-induced anemia following subchronic exposure as shown by the depleted RBC and HCT following exposure to khat. A significant finding in this study is that there was a heightened HCT suppression following chronic exposure to khat; signaling

severe khat-induced toxicity following prolonged exposure. In the presence of CoQ_{10}, the RBC and HCT suppression following subchronic and chronic exposure to khat was abrogated. This demonstrates the protective role of CoQ_{10} on the hematopoietic system. Additionally, supplementation with CoQ_{10} nullified the khat-induced elevation of WBC subtypes and khat-induced depletion of platelets further shows the protective role of CoQ_{10} on the hematopoietic system. Worthy of note is that, there is sparse data on the role of CoQ_{10} against khat-induced toxicities. These significant outcomes show that khat use has far reaching implications on khat consumers with pre-existing blood abnormalities.

The compromised reflex and preservation following both subchronic and chronic exposure to khat in the current study shows the effect of khat on the central nervous system. Despite the fact that this outcome corroborates previous findings (Gashaw *et al*., 2014), the significance of the present of the present study is that finding that damage to the CNS persisted during both subchronic and chronic exposure.

In the present study, oxidative stress and inflammation were evaluated for their role in khat-induced and manganese-induced toxicities. Consequently, there was khat-induced oxidative stress following subchronic and chronic exposure, as indicated by the increased GSH levels in the brain and the spleen and decreased GSH levels in the liver. While this finding may not be new, additional findings in the present study demonstrate that there was a heightened oxidative damage following chronic exposure to khat and co-exposure to khat and manganese. Of significance to note is that, manganese potentially induces oxidative damage (S. F. Ali *et al*., 1995; Milatovic *et al*., 2009). One of the most astounding finding was that in the presence of CoQ_{10}, the khat-induced oxidative stress was nullified.

Khat-induced increase in the pro-inflammatory cytokine TNF-α was noted following subchronic exposure in the present study. This shows khat-induced inflammation. This finding was replicated following chronic exposure with heightened levels of IFN-γ during this phase. In addition, heightened levels of TNF-α were noted following simultaneous exposure to khat and manganese. Importantly, CoQ_{10} appeared to abrogate the khat and/or manganese-induced inflammation. This novel outcome shows the putative role of CoQ_{10} against khat and/or manganese-induced inflammation for the first time. It additionally demonstrates the heightened dangers khat users are exposed to when simultaneously exposed to khat and manganese.

In the present study, khat-induced liver and kidney pathologies were recorded as denoted increased liver and kidney biomarkers AST, ALT, GGT and creatinine. This trend was repeated following chronic exposure to khat, with additional findings showing severe liver pathology following simultaneous exposure to khat and manganese. Importantly, these findings additionally demonstrate the possibility of heightened toxicity following simultaneous exposure to khat and manganese. Findings from the histological analyses of the liver sections revealed regions of hepatocellular necrosis and hepatocyte vacuolation following exposure to khat, reinforcing the earlier findings on biomarkers. In the presence of CoQ_{10}, the khat and/or manganese-induced liver and kidney pathology was abrogated.

6.2 CONCLUSIONS

Consumption of khat is harmful causing serious injury to the CNS/brain and other vital organs such as liver/kidney. In addition, consumption of khat negatively affected hematopoiesis, immune function and induced inflammation and oxidative damage. Manganese, when administered separately and when co-administered resulted in severe neurological deficits at physiological and biochemical level. Just like khat, manganese too interfered with hematopoietic process; evident with deranged levels of RBCs, WBCs and other blood cells. This points to a possibility of lethal khat-drug interactions; especially for khat users on medication that interfere with blood clotting cascades and those that affects hematopoiesis. Further findings showed that co-exposure to khat and manganese results in severe toxic effects, with a potential for serious organ damage; especially for liver and kidney. Lastly, CoQ_{10} blocked khat and manganese-driven sequelae.

6.3 RECOMMENDATIONS

The public should be well educated on the negative impacts of khat use. This should be made an important public health issue. In addition, further investigations on CoQ_{10}'s molecular basis for protection from khat/manganese should be done to conclusively establish its role in mitigating khat and manganese-driven biochemical toxicities.

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APPENDICES

Appendix 1

Publication 1

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Heliyon

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Research article

Coenzyme Q_{10} nullified khat-induced hepatotoxicity, nephrotoxicity and inflammation in a mouse model

Chepukosi Kennedy [a,b], Patrick Okanya [a,b], James Nyabuga Nyariki [a,b], Peris Amwayi [a,b], Ngalla Jillani [e], Alfred Orina Isaac [c,d,*]

[a] Department of Biochemistry and Biotechnology, Technical University of Kenya, P. O. Box 52428, 00200 Nairobi, Kenya
[b] School of Biological and Life Sciences, Technical University of Kenya, P. O. Box 52428, 00200 Nairobi, Kenya
[c] Department of Pharmaceutical Sciences and Technology, Technical University of Kenya, P. O. Box 52428, 00200 Nairobi, Kenya
[d] School of Health Sciences and Technology, Technical University of Kenya, P. O. Box 52428, 00200 Nairobi, Kenya
[e] Institute of Primates Research, P.O. Box 24481, Karen, 00502, Nairobi, Kenya

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ABSTRACT

Ethnopharmacological relevance: The consumption of khat (*Catha Edulis*, Forsk) is on the rise despite the much publicized associated deleterious health effects. How chemicals present in khat, affect various physiological and biochemical processes requires further scrutiny. A clear understanding of these processes will provide an avenue for countering khat-driven negative effects using appropriate pharmacological and/or nutritional interventions.

Aim of the study: The current study investigated the effect of khat on vital physiological and biochemical processes such as oxidative stress, inflammation and immune responses and the role of Coenzyme-Q_{10} (CoQ_{10}), a potent antioxidant and anti-inflammatory, in modulating any negative effects due to khat exposure.

Methodology: Three (3) weeks old forty (40) Swiss albino mice were randomly assigned into four treatment groups ($n = 10$). The first group was the control that was not administered with khat or CoQ_{10}. The second group received 200 mg/kg body weight (b/w) of CoQ_{10}, while the third group received 1500 mg/kg b/w of khat extract and finally the forth group was co-treated with 200 mg/kg b/w of CoQ_{10} and 1500 mg/kg b/w of khat extract. The experiment was conducted for 90 days after which samples were collected for physiological and biochemical analyses.

Results: The effects of khat and CoQ_{10} on the weights of brain, liver, kidney and spleen was determined. Administration of khat decreased the levels of RBCs and its subtypes (MCV, MCH, RDW-SD and RDW-CV), a clear indicator of khat-induced normochromic microcytic anemia. White blood cells (lymphocytes, monocytes, neutrophils and eosinophil) which are vital in responding to infections were markedly elevated by khat. Moreover, these results provide evidence for khat-induced liver and kidney injury as shown by increased biomarkers; AST, ALT, GGT and creatinine respectively. Standard histopathological analysis confirmed this finding for khat-driven liver and kidney injury. Further studies showed evidence for khat-induced inflammation and oxidative stress as depicted by increased levels of the pro-inflammatory cytokine TNF-alpha and elevation of GSH in the brain, liver and spleen. Remarkably, this is the first study to demonstrate the potential of CoQ_{10} in ameliorating khat-induced negative effects as outlined. CoQ_{10} supplementation restored the khat-induced reduction in RBC subtypes, and was protective against liver and kidney injury as shown by the appropriate biomarkers and standard histopathology analysis. The other significant finding was the CoQ_{10}-driven normalization of GSH and TNF-α levels, indicating a protective effect from khat-driven oxidative stress and inflammation respectively.

Conclusion: From this study, we conclude that CoQ_{10} may be useful in nullifying khat-driven deleterious events among chronic khat users.

Appendix ii

Publication 2

Toxicology and Environmental Health Sciences
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ORIGINAL ARTICLE

Manganese exacerbated chronic khat-induced neurological deficits, inflammation and organ toxicity in a mouse model

Kennedy W. Chepukosi[1] · James N. Nyariki[1] · Ngalla E. Jillani[4] · Patrick W. Okanya[1] · Alfred Orina Isaac[2,3]

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Abstract

Objective This study sought to determine whether chronic exposure to khat (*Catha Edulis*, Forsk) increases the vulnerability to the toxic effects of manganese (Mn^{2+}), when co-exposed.

Methods Three (3)-week-old forty (40) Swiss albino mice were randomly divided into four groups ($n = 10$). The various groups received khat and manganese separately or both. The experiment was conducted for 132 days to mimic chronic exposure to khat, with manganese administration in the last twelve days.

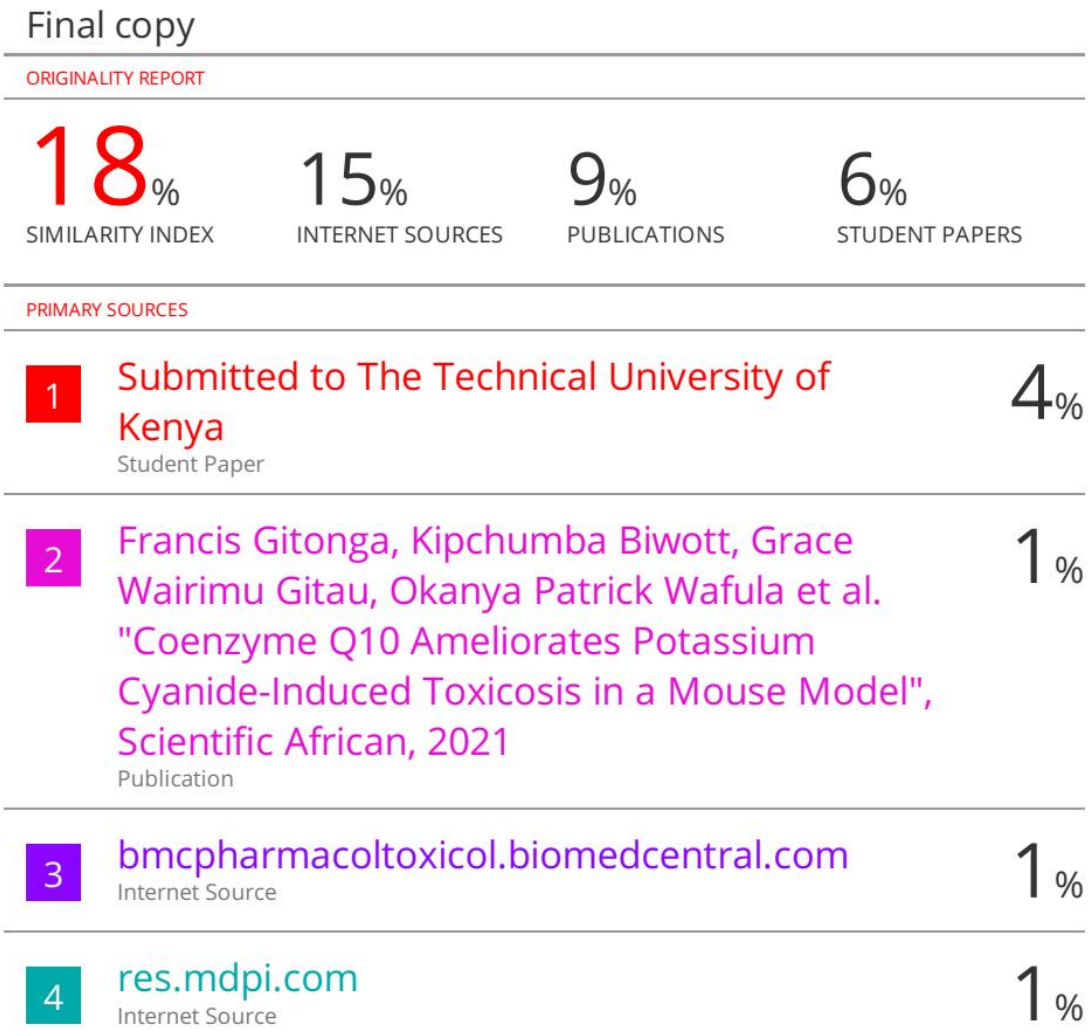
Results Khat-induced neurological deficits were markedly pronounced on co-exposure with manganese. Notably, deficits in motor performance, touch escape and aggression were deepened by manganese. Co-exposure (khat + Mn^{2+}) induced more profound changes in hematological indices such as suppression of RBCs, low hematocrit and hemoglobin levels. Manganese enhanced khat-induced depletion of a very powerful antioxidant, glutathione (GSH) in the brain, liver, heart and lung tissues. Exposure to khat and/or manganese led to significant elevations in the pro-inflammatory cytokines—tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-Ɣ), with a concomitant suppression of the anti-inflammatory cytokine and interleukin 10 (IL-10). Similarly, there was enhanced suppression of IL-10 following co-exposure (khat + Mn). Khat-induced hepatotoxicity and nephrotoxicity were exacerbated by co-exposure.

Conclusions In conclusion, acute exposure to manganese appears to aggravate neurological deficits and other multiple organ toxicities driven by chronic exposure to khat.

Keywords *Catha edulis* · Pollution · Metal toxicity

Appendix iii

Plagiarism Report



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