Renal effects of crude khat *(Catha edulis* F.) *e*xtract when administered alone and concomitantly with gentamicin in rats

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This is to certify that the thesis prepared by **Zewdneh Shewamene**, entitled: *Renal effects of Crude Khat (Catha edulis F.) extract when administered alone and concomitantly with gentamicin in Rats* and submitted in partial fulfillment of the requirements for the Degree of Master of Science (Pharmacology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Khat use has been reported to produce significant acute and chronic toxic effects including oxidative damage of cellular macromolecules such as DNA, lipids and proteins contributing to the development of several pathologies, notably cancer, nephrotoxicity, hepatotoxicity and neurodegenerative diseases.

Although various studies have been carried out on the pharmacological actions; the effect of khat induced changes in the redox status of kidney and other tissues has not yet been worked out in details. The aim of this study was therefore to investigate whether khat has a direct or permissive role in causing nephrotoxicity. Sixty four healthy Sprague Dawely rats were divided into eight experimental groups of eight animals and khat was administered in different doses (100 mg/kg, 200 mg/kg and 400 mg/kg orally) for ten days alone and two days before and eight days in combination with gentamicin (100 mg/kg, intraperitoneally). Following administration, animals were killed by light ether anesthesia and blood and renal tissue were used to measure renal markers, including creatinine, blood urea nitrogen, antioxidant enzymes as well as markers for lipid peroxidation using established protocols.

Administration of khat at high dose (400 mg/kg) significantly caused marked renal dysfunction as evidenced by increased serum creatinine ($p<0.001$), blood urea nitrogen ($p<0.001$), and lipid peroxidation ($P<0.001$), whereas renal superoxide dismutase and catalase enzymatic activities were decreased ($p<0.001$) compared to control animals. Furthermore, disturbed renal indices by gentamicin were considerably accentuated by high dose (400 mg/kg) of crude khat extract when given concurrently with gentamicin. Khat alone or with gentamicin was also found to alter renal histopathology, normalized kidney weight and body weight of rats with increasing dose. In conclusion, khat at high dose alone or with gentamicin is able to induce renotoxicity in rats.

Key words: creatinine, blood urea nitrogen, superoxide dismutase, catalase, khat, rat

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Acronyms

ARF – Acute renal failure

ATL – Ascending thin limb

BUN – Blood urea nitrogen

CHF – Chronic renal failure

DCT – Distal convoluted tubule

DTL – Descending thin limb

GRF – Glomerular filtration rate

JGA – Juxtaglomerular apparatus

LH – Loop of Henle

LPO – Lipid peroxidation

MDA – Malondialdehyde

ROS – Reactive oxygen species

SOD – Superoxide dismutase

TAL – Thick ascending limb

TBARS – Thiobarbituric acid reactive substance

WHO – World health organization

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1. Introduction

1.1. The renal anatomy and physiology

Human kidneys are paired, bean-shaped organs situated in a retroperitoneal position on the posterior aspect of the abdominal cavity. The renal system consists of kidney, ureter, bladder and urethra as well as associated blood vessels. The kidney is covered by a fibrous capsule which is further surrounded by perinephric fat and then by the perinephric fascia which also enclose the adrenal gland. The kidneys of an adult man weigh approximately 120 to 170 g each and those of an adult woman weigh slightly less and are somewhat smaller. The kidney is composed of the cortex, medulla, renal sinus and pelvis (Bissinger, 1995; O'Callaghan, 2009).

The cortex, the outer most part contains the glomeruli, proximal and distal tubules, cortical collecting ducts, and peritubular capillaries of the nephrons. The middle part of the kidney, the medulla, contains the renal pyramids, straight portions of the tubules, loops of Henle (LH), vasa recta, and terminal collecting ducts. The renal sinus and pelvis compose the innermost portion of the kidney (Fig 1). The nephron, the basic urine forming unit of the kidney, consists of an initial filtering component called the renal corpuscle and a longer tubular portion that extends out from the renal corpuscle, reabsorbing and conditioning the filtrate (Bissinger, 1995; Vander et al., 2001).

Each kidney has over a million nephrons. Each renal corpuscle contains a compact tuft of interconnected capillary loops called the glomerulus and a fluid-filled capsule, Bowman's capsule, into which glomerulus protrudes. As blood flows through the glomerulus, a portion of the plasma filters into Bowman's capsule that is separated from the fluid in Bowman's space by a filtration barrier (Vander et al., 2001).

The renal tubule is a very narrow hollow cylinder made up of a single layer of epithelial cells that differ in structure and function along the tubule's length. It consists of proximal tubule, LH and distal tubules. The proximal tubule is continuous with Bowman's capsule and makes a tortuous path until finally forming a straight portion that dives into the renal medulla. The tubular cells are tall, columnar epithelial cells with many microvilli, a high

surface area, and a well developed luminal endocytic apparatus (Vander et al., 2001; Robert et al., 2007).

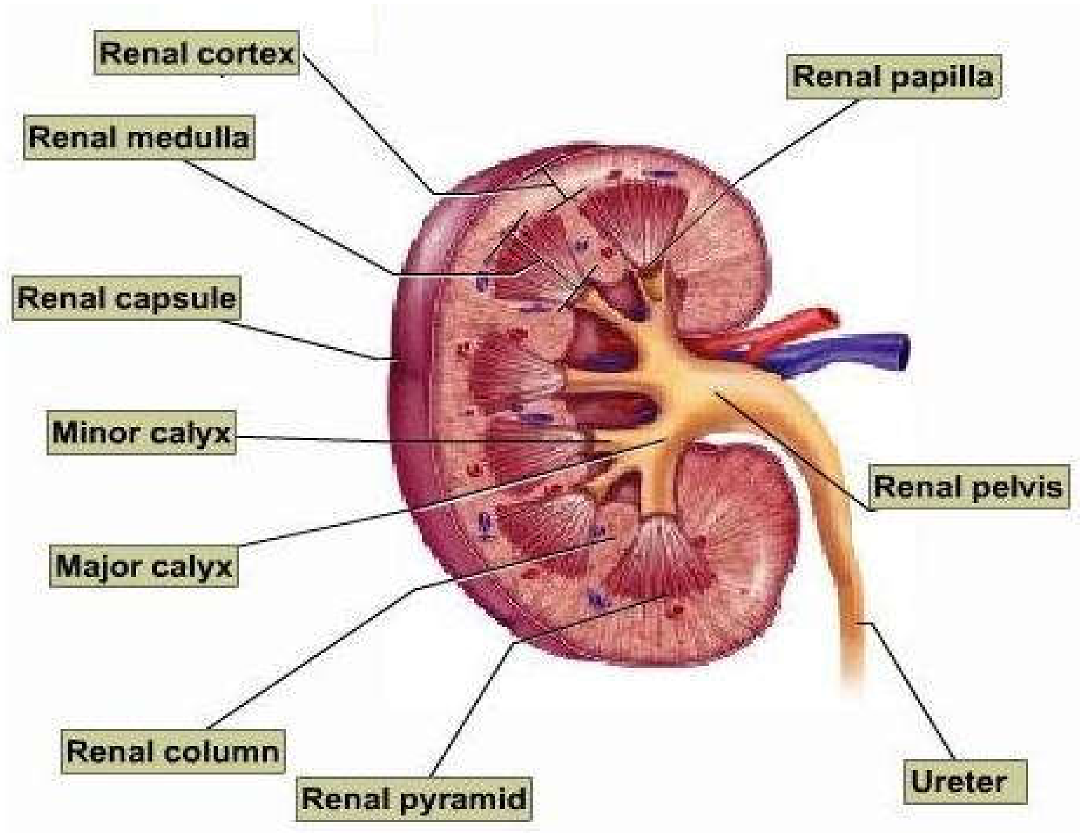

Fig 1: Renal pyramids and blood vessels (Robert et al., 2007)

Between the outer and inner strips of the outer medulla, the tubule abruptly changes morphology to become the descending thin limb (DTL), which penetrates the inner medulla, makes a hairpin turn, and then forms the ascending thin limb (ATL). At the juncture between the inner and outer medulla, the tubule once again changes morphology and becomes the thick ascending limb (TAL). Together the proximal straight tubule, DTL, ATL and TAL form the LH. The DTL is highly permeable to water, yet its permeability to NaCl and urea is low. In contrast, the ATL is permeable to NaCl and urea but is impermeable to water. The TAL actively reabsorbs NaCl but is impermeable to water and urea. Approximately 25% of filtered Na^+ is reabsorbed in the LH, mostly in the

TAL, which has a large reabsorptive capacity. Approximately 65% of filtered Na^+ is reabsorbed in the proximal tubule and since this part is highly permeable to water, reabsorbtion is essentially isotonic (Robert et al., 2007).

Near its end, the ascending limb of each LH passes between the afferent and efferent arterioles of that loop's own nephron containing with the afferent arteriole via a cluster of specialized columnar epithelial cells known as the macula densa. The wall of the afferent arteriole contains secretory cells known as juxtaglomerular (JG) cells. The combination of macula densa and JG cells is known as the juxtaglomerular apparatus (JGA). The macula densa is strategically located to sense concentration of NaCl leaving the LH and regulate renin release (Vander et al., 2001)

Approximately 0.2 mm past the macula densa, the tubule changes morphology once again to become the distal convoluted tubule (DCT). Like the TAL, the DCT actively transports NaCl and is impermeable to water. Since these characteristics impart the ability to produce dilute urine, the TAL and the DCT are collectively called the diluting segment of the nephron (Jackson, 2006). Several distal tubules empty into each collecting tubule and the collecting tubules join to form collecting ducts. The collecting tubule has two different cell types: the principal cells, which reabsorb Na^+ and secrete K^+ via sodium and potassium channels and the intercalated cells, which are involved mainly in H^+ secretion. In this portion of the nephron, the movement of ions and water is regulated by the mineralocorticoid aldosterone and antidiuretic hormone, respectively (Rang et al., 2006)

The kidneys play the central role in regulating the water concentration, inorganic-ion composition, and volume of the internal environment. It is also involved in the excretion of metabolic waste products such as urea, uric acid, creatinine as well as some foreign chemicals, such as drugs, pesticides, and food additives. During prolonged fasting, the kidneys synthesize glucose from amino acids and other precursors and release it into the blood. Further, the kidneys act as endocrine glands, secreting at least three hormones: erythropoietin, renin, and 1, 25-dihroxyvitamin D3 (Vander et al., 2001).

1.2. Renal pathology

1.2.1. Acute renal failure

Acute renal failure (ARF) is defined as an abrupt decrease in renal function sufficient to result in retention of nitrogenous waste products (blood urea nitrogen, BUN and creatinine) in the body. Although there is unanimity of opinion regarding this general definition, there is no consensus regarding the magnitude of elevation of serum creatinine and BUN sufficient to ascribe a diagnosis of ARF. Moreover, there is a nonlinear relationship between decreasing glomerular filtration rate (GFR) and rising serum creatinine concentration in individuals with a normal basal serum creatinine. Thus, in individuals with a normal basal serum creatinine, significant decrease in GFR is often associated with either slight or modest increase in serum creatinine concentration. Also, not only renal elimination, but also rate of production and volume of distribution are significant determinants of serum creatinine concentration (Robert et al., 2007).

1.2.2. Chronic renal failure

Unlike ARF, which is temporary, chronic renal failure (CRF) is long term and, in most cases, is irreversible. When the kidneys fail, dialysis or a kidney transplant is needed to support life and people can live for decades with dialysis and/or kidney transplants. This is extremely serious and can eventually lead to a total shut down of the kidneys (end stage renal failure). Without proper treatment, to remove the wastes and fluids from the bloodstream, this condition is fatal. When the kidneys fail then fluids and toxins begin to accumulate in the bloodstream. As the fluids begin to build up, the patient may become puffy and swollen in the face (edematous), and they may experience fatigue (Vander et al., 2001; Robert et al., 2007)).

Most symptoms of CRF are not apparent until kidney disease is in an advanced stage. The most common causes of CRF include diabetes mellitus, chronic inflammation of the kidneys' glomeruli (glomerulonephritis), hypertension, kidney cancer, kidney stones, systemic lupus erythematosus, and sickle-cell anemia (Robert et al., 2007).

1.2.3. Gentamicin induced nephrotoxicity

Kidney is a common target for the toxic xenobiotics due to its capacity to extract and concentrate toxic substances to its large blood flow share and an unequal interarenal distribution of drug metabolizing enzymes (Kacew and Bergeron, 1990; Werner and Costa, 1995). Accordingly, nephrotoxics may cause direct tubular injury, interstitial nephritis, decreased renal perfusion, primary glomerulopathy and obstructive nephropathy (Werner and Costa, 1995).

Several studies suggested that aminoglycosides are transported across the apical membrane of proximal tubular cells by pinocytosis after binding of the aminoglycosides to receptors located there (Just et al., 1977). The initial points of attachment are the acidic phospholipids mainly phosphatidylserine, an abundant acidic phospholipid on brush borders, since modulation of the membrane content in these phospholipids results in commensurate changes in uptake (Molitorin and Simon, 1985).

Quickly thereafter, as a second step, aminoglycosides are transferred to the transmembrane protein megalin, with which they become internalized in endosomes (Moestrup et al., 1995). Megalin, a giant endocytic receptor abundantly expressed at apical membrane of renal membrane of proximal tubules, plays an important role in binding and endocytosis of aminoglycosides in proximal tubular cells. Megalin antagonists have been developed such as cytochrome C, which hold promise as prospective therapeutic agents for preventing or minimizing the iatrogenic tubular damage induced by gentamicin (Nagai and Takano, 2004).

Hydroxyradicals are strong mediators of tissue injury as they are involved in oxidation of a wide variety of biomolecules, leading to cell membrane injury and protein degeneration (Ali, 1995). Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical (Walker et al., 1999).

Due to the accumulation of aminoglycoside in the proximal tubule lysosomes, impaired function of these organelles is suggested to be an important mechanism of nephrotoxicity. Aminoglycosides lead to an extensive dysfunction of these organelles through inhibition of the activities of the enzymes and the alteration of the properties of the lysosomal membrane permeability (Morin et al., 1980). Animals and clinical observations support the hypothesis of the mechanism of lysosomal damage is inhibition of phosphatidylinositol phospholipase C, causing a phospholipidosis within the proximal tubular lumen and eventually an enrichment of lipid material (myeloid bodies) in the lysosomes themselves (Hostetler and Hall, 1982; Werner and Costa, 1995).

Simmons et al. (1980) showed that mitochondrial injury is involved in the pathogenesis of gentamicin nephrotoxicity as the drug compromises oxidative phosphorylation impairing cellular energy production (Kaloyanides et al., 1980). Besides, gentamicin competes with magnesium for monovalent cations. This leads to increased mitochondrial membrane permeability resulting in mitochondrial swelling and alterations in mitochondrial respiratory function (Weinberg and Humes 1980).

1.3. The experimental plant: Khat

Khat (*Catha edulis* Forsk) is a shrub or small to medium-sized evergreen tree that belongs to Celastraceae family and cultivated mainly in the Yemen and East African Countries. It was first described by Peter Forskal (1736-1763), a Swedish botanist, on his journey with his friend Karsten Niebuhr to Egypt and Yemen. Karsten Niebuhr named khat as *Catha edulis* forskal in memory of his friend Peter Forskal (Al-Motarreb *et al*., 2002a).

The shrub grows to a height of 6 meters and the leaves are leathery, glossy, brownish green, with serrated edges, arranged in an alternate fashion on the straight branches (Fig 2). The young shoots and leaves are the parts chewed for their psychoactive properties (Cox and Rampes, 2003).

There are several names for the plant, depending on its origin: chat, qat, qaad, miraa, mairungi, muhulo, hagigat, cat, Catha, gat, tohai, and muraa. The dried leaves of khat are

known as Abyssinian tea or Arabian tea (Cox and Rampes, 2003; Ishraq, 2004). In Ethiopia, it is commonly known as "chat" and has other local names such as Aweday, Beleche, Abo mismar, Gelemso, Wondo and others based on place of cultivation. It is claimed that the Aweday, which is cultivated in Harar highlands of Eastern Ethiopia is the most potent and expensive among the local brands and hence chosen for export (Belew *et al*., 2000; Gebissa, 2008). Aweday khat, most costly local brand in Ethiopia, is collected from its natural habitat (Harar) for the purpose of this study

Fig 2: Photograph *of Catha edulis* Forsk (Khat)

1.3.1. Purpose of khat chewing

Chewing the leaves of khat is a social habit in Yemen and East African countries. People chew fresh khat leaves daily on a regular basis mainly in the afternoon although some people start to chew khat in the morning. Social gatherings like wedding parties, funerals and at election time have made khat chewing more popular (Al-Motarreb *et al*., 2010).

Euphoria, alertness and central nervous system stimulation induced by cathinone, the main active constituent derived from khat chewing, makes this habit popular among large

numbers of society (Al-Motarreb *et al*., 2010). Khat chewing was linked to the body's physical health many centuries ago and was mentioned as a medicine by an Arabic physician, Abu Al-Rihan Bin Ahmed al-Baironi (973-1051 AD), in his book P*harmacy and therapeutics Art* (El-Tahir, 1990). It had been used for the management of obesity and depression due to its central stimulant effect (Al-Attas, 1981). Many factors play a role in the extension to its social use in society; easy transportation from village to city khat market, the availability of cheap khat making it affordable for all and afternoon free time because work officially finishes at 2:00 pm in the Yemen. People also believe that khat helps them to work more effectively, particularly with manual work, due to increased energy and alertness (Al-Motarreb *et al*., 2010).

1.3.2. Prevalence of khat chewing

Information on the prevalence of khat use in the general population is scarce. Khat use is highly prevalent in East African and Middle Eastern countries, in particular in Yemen (Manghi *et al*., 2009). Figures for the number of chewers of individual countries is mainly anecdotal, however, surveys have been performed to determine the exact incidence of khat chewing among specific cohorts (Al-Motarreb *et al*., 2010).

Khat is freely available in Ethiopia and is a highly valued export commodity. Khat mainly cultivated in the eastern part of Ethiopia, but it also started to grow in all parts of the country. The number of khat chewers has significantly increased and khat consumption has become popular in all segments of the Ethiopian population (Selassie and Gebre, 1996). Prevalence of life time khat chewing in four colleges of the Amhara region was 26.7% (Kebede, 2002), 54.9% around Butajirra (Alem *et al*., 1999) and 46% among staff of Jimma University, Oromia region (Gelaw and Haile Amlak, 2004). There is evidence that khat use in Ethiopia is more prevalent in ethnic communities with a tradition of khat use but is now becoming an every-day drug for the general population (Alem *et al*., 1999; Belew *et al*., 2000).

In South-western Uganda, use and perception of khat was studied among 130 students, 35 law enforcement officials and 16 transporters. In this sample, 32% had experience with chewing khat and 20% were still using khat. The authors concluded that knowledge of

khat is becoming widespread in Uganda and that its consumption is increasing especially among youths and young adults (Ihunwo et at., 2004).

In a population based survey in Yemen, 86% percent of the males and 50% of the females were khat users. Out of these 88% of females chew khat for the mere reason to attend social events (Basunaid. *et al*., 2008). Another document from national population survey in the same year showed khat chewed daily by a high percentage of the adult population in Yemen. It has been estimated that about 80% of Yemeni men and 60% of women chew Khat (Marwan and Mohannad, 2008). In the UK, 75 male Yemeni adults reported chewing up to 3 bundles of khat per week of which 39% were assessed as dependent (Kassim and Croucher, 2006).

A pill containing extract of khat leaves known as “Hagigat” has been sold to Israeli drug users (Bentur et al., 2008). It is also spreading to non-ethnic users in the UK. Khat is illegal in the USA, Canada, and many European countries including Finland, Ireland, France, Switzerland, Norway and Sweden (Al-Motarreb *et al*., 2010).

1.3.3. Constituents of khat

Khat contains three main phenylpropylamine alkaloids; S-(-)-cathinone (S-α-aminopropriophenone), norpseudoephedrine (cathine) and norephedrine. Cathinone is the main psychoactive constituent in khat, but it is very unstable and rapidly decomposes into norpseudoephedrine and norephedrine as the leaves and shoots dried (Kalix, 1992). But very recent studies demonstrated that cathinone persists in dried khat for a time frame of several years, and simple drying techniques are an effective means to preserve seized khat, which is an evidence for long term storage (Chappell and Lee, 2010).

The metabolism of cathinone to cathine involves reduction of the ketone group to an alcohol, a fairly common metabolic pathway in humans, catalyzed by liver microsomal enzymes. Only 7% or less of the absorbed cathinone is excreted unchanged in the urine, and is mainly excreted in the form of norephedrine and cathine (Cox and Rampes, 2003). Cathinone has a mean terminal elimination half-life of 1.5 - 4.5 h; for cathine it is approximately 5 h. The amount of norephedrine excreted in urine is much higher than the

amount ingested, indicating that cathinone is also metabolized to norephedrine (Feyissa and Kelly, 2008).

The presence of amphetamine and caffeine in khat has been excluded, but as a result of structural similarity, cathinone has been termed as 'natural amphetamine' (Dhaifalah and Santavy, 2004). This similarity between cathinone and amphetamine suggested that the two substances might have the same mechanism of action. Amphetamine produces its effects by activating neurotransmission mediated by the catecholamines; noradrenaline and dopamine, in particular by releasing these neurotransmitters from their physiological storage sites. Cathinone is capable of releasing dopamine, noradrenaline and serotonin from synaptic terminals (Kalix, 1996).

Khat also has another group of alkaloids, the cathedulines, identified as K1, K2, K6 and K15 from the Kenyan khat, E2, E4, E5 and E8 from Ethiopian khat and Y1 from Yemeni khat. Cathedulines are thought to be of less significance compared to the 14 phenylpropylamines with regard to stimulant effects, but could probably play a role in inducing other effects in humans (Al-Motarreb et al., 2002a). In addition to alkaloids, there are glycosides, tannins and terpenoids in khat which include merucathinone, ethereal oils, sterols, triterpenes, flavonoids and ascorbic acid (Al-Motarreb *et al*., 2002a; Al-Hebshi *et al*., 2005).

1.3.4. Pharmacological effects of khat

Because of the psychostimulant effect, khat is used as a recreational drug by many people. Hence, used in formal meetings (khat sessions) where the participants are engaged in discussions and maintain social contact. During such sessions the leaves and the bark of the plant are chewed slowly over several hours and the juice of the masticated leaves is swallowed, but not the residues (Toennes et al., 2003). Similar to psychostimulants, khat ingestion produces several central nervous system effects, including increased motor stimulation, euphoria, and a sense of excitement and energy (Kalix, 1996: Nencini et al., 1998).

Cathinone like 3,4- methylendeioxymethamphetamine (MDMA, 'ecstasy') and amphetamine exerts pronounced behavioral effects including euphoria, excitability, anxiety, irritability, hyperactivity, restlessness and insomnia (Cox and Rampes, 2003). The central nervous activity of cathinone is qualitatively and quantitatively similar to that of amphetamine (Kalix, 1984). Although khat does contain active constituents thought to be similar to amphetamine, khat and amphetamine were shown to produce different effects on some behavioral parameters. Amphetamine appeared to be stronger in producing stereotyped behaviors than khat. By contrast, khat (200 mg/kg) superseded amphetamine in producing memory deceits and anxiety. These observations suggest that khat and amphetamine might not be parallel in their behavioral effects, raising the possibility that the two agents could differ in central pathways that they activate to produce their effects, or that constituents of khat unrelated to amphetamine might be responsible for the observed differences (Bedada and Engidawork, 2010).

While the nature of khat dependence remains under active debate, there is accumulating evidence indicating the existence of a withdrawal syndrome and a low level of tolerance. Withdrawal symptoms usually include inertia, nightmares, trembling, depression, sedation and hypotension (Cox and Rampes, 2003).

Regular khat chewing is associated with elevated mean diastolic blood pressure. Khat chewing by human volunteers increases blood pressure which coincides with elevated plasma levels of cathinone (Brenneisen *et al*., 1990). Vasoconstriction from electrical field stimulation is potentiated and claimed to arise from enhanced noradrenaline release (Kalix, 1992). There is probably a significant cardiac component to the increase in blood pressure after chewing khat through tachycardia, since the increase in blood pressure and heart rate were reduced by the beta adrenoceptor antagonist, atenolol (Hassan *et al*., 2005). Subchronic administration of khat extract was found to increase blood pressure and cardiac biomarkers as a result of myocardial cell death (Al-Motarreb *et al.,* 2010; Admassie and Engidawork, 2011).

Recently, the khat chewing habit has changed and many chewers extend their chewing time into the evening, sometimes until midnight. This has been associated with a

change in the circadian rhythm of presentation with acute myocardial infarction. The most common time for presentation with major cardiovascular events, such as acute myocardial infarction and sudden death, is in the early morning and after waking and rising (Al-Motarreb *et al.,* 2010). This diurnal rhythm is associated with increased sympathetic outflow and circulating catecholamines producing increase in heart rate, blood pressure, myocardial contractility and oxygen demand soon or after rising. However, there is a shift in this diurnal rhythm of acute myocardial infarction amongst khat chewers, where a greater proportion presented in the evening compared with non-khat chewers (Al-Motarreb *et al*., 2002b).

Recently, khat chewing showed to significantly decrease subjective feeling on hunger and increase the sensation of fullness but had no effect on ghrelin and peptide YY levels. It was therefore, concluded that the anorxigenic effect of khat may be secondary to central mechanism mediated via cathinone (Murray et al., 2008). High plasma levels of the anorectic hormone, leptin, have been found 4 h after a heavy khat chewing session (400g). This hormone may then contribute to the decreased appetite and body weight observed in khat chewers (Al-Dubai *et al*., 2006)

Other reported acute and chronic effects of khat include low birth weight in babies of khat chewing women, reduced sperm count and motility, increased risk of myocardial infarction and liver problems (Al-Qirim et al., 2002; Abdulwaheb et al., 2007; Admassie and Engdawork, 2011; Mohammed and Engidawork, 2011).

1.3.5. Khat chewing and the kidney

Regular khat chewing in human may cause kidney damage, as total serum protein levels were reduced in khat consumers, while the levels of urea and creatinine were greatly increased. Aside from these biochemical changes, other studies have reported histopathological changes in both livers and kidneys of treated rats (Al-Motarreb et al., 2002a: Al-Qirim et al., 2002).

In another study on rats, consumption of crude khat extract or its alkaloid fraction produced an oxidative stress by altering the activities of free radical metabolizing/scavenging enzyme systems. Flavonoids present in khat are found to

enhance free radicals scavenging enzyme activities like glutathione and catalase if given alone (Al-Qirim et al., 2002). This finding seemed khat may damage different tissues including kidney by inducing oxidative stress.

Nephrotoxic and hepatotoxic effects have been shown in vivo after khat administration to New Zealand white rabbits (Al-Habori *et al*., 2002; Al-Mamary *et al*., 2002). The generation of free radicals and oxidants is seriously implicated in khat toxicity as oral administration of khat in rats was associated with decreased serum free radicals metabolizing enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Al-Zubairi et al., 2003).

In spite of the vast published data on the pharmacology and chemistry of khat, toxicological studies with laboratory animals and human toxicity reports particularly on renal system are still very scarce. Moreover, the effect of khat induced redox changes particularly in the kidney has not yet been worked out in details. The scarcity of published reports calls on the imperative need for more exhaustive laboratory studies regarding redox changes induced by khat consumption in order to generate a sufficient body of knowledge. This study therefore attempted to investigate whether khat has a direct or permissive role in causing nephrotoxicity. The study is also believed to provide an impetus to initiate further study on the effect of khat and its constituents in different tissues.

2. Objectives

2.1. General objective

This study aimed at investigating whether crude khat (*C. edulis* F.) extract has a potential to cause and accentuate kidney damage in normal and gentamicin administered rats, respectively.

2.2. Specific objectives

- To examine the effect of crude khat extract on serum creatinine and BUN levels.
- To examine the effect of crude khat extract on renal oxidative markers; Superoxide dismutase (SOD) and Catalase activities as well as Malondialdehyde (MDA) level.
- To examine renal histopathological changes associated with administration of crude khat extract.
- To investigate whether crude khat extract has a permissive role on gentamicin induced renotoxicity by monitoring biochemical and oxidative markers as well as histopathological changes.
- To assess the dose dependent body and kidney weight changes after administration of crude khat extract when given alone and with gentamicin.
- To propose a possible mechanism for the renototoxic effect, if any, of crude khat extract.

3. Materials and Method

3.1. Chemicals

Analytical grade chemicals and solvents were used for this study. Chloroform (Sigma Aldrich, Germany), diethyl ether (Fisher Scientific limited, UK), formalin (El Naser Pharmaceutical Chemicals Co., Egypt), gentamicin (SPCL, China), glacial acetic acid (Fisher Scientific limited, UK), sodium chloride (BDH laboratory supplies, England), Tween 80 (Research lab-fine Chem industries, Mumbai) were purchased from local markets. The assay kits for serum creatinine and BUN were acquired from Roche-cobas, Switzerland. SOD, catalase and MDA assay kits were imported from Nanjing NianChen Bioengineering Institute, China

3.2. Experimental animals

Sixty four (32 male and 32 female) healthy Sprague Dawley rats (170 – 210 g) bred in the animal house of School of Pharmacy, Addis Ababa University were used for the experiment. The rats were housed in polypropylene cages and maintained under room temperature (22-25 ºC) and 12 h light and dark cycle. Standard pelletized feed and tap water were provided *ad libtum.* All animals were handled according to internationally accepted guidelines (ILAR, 1996) and the protocol was approved by the School of Pharmacy Ethics Committee.

3.3. Collection of the plant material

Bundles of fresh khat shoots and small branches were purchased (2000g) fresh at a local market from Aweday, its natural habitat, 525 km South East of Addis Ababa, Ethiopia. The fresh bundles were packed in plastic bags and transported in an icebox to the School of Pharmacy, Addis Ababa University. The plant was identified by a taxonomist and a voucher specimen (ZS001) was deposited at the National Herbarium, College of Natural Sciences, Addis Ababa University. The fresh leaves were immediately kept at -20ºC till the time of extraction.

3.4. Extraction of khat

Extraction was performed as described elsewhere with slight modification (Connor et al., 2002: Bedada and Engdawork, 2010). Briefly; the freeze-dried plant was finely minced, weighed and placed in Erlenmeyer flasks (400 g per flask) wrapped with aluminum foil to avoid light induced decomposition. Chloroform (150 mL) and diethyl ether (450 mL) (1: 3 v/v) were added to cover the minced leaves. The resulting mixture was stirred using a rotary shaker (New Brunswick Scientific Co, USA) at 120 rpm and 20ºC under dark condition for 24 h.

The content was later filtered through folded filter paper. The filtrate was again passed through a round filter paper with the help of a mini filter pump. The organic filtrate collected in this way was pooled together, kept in wide mouth amber bottles and placed in a hood for 24 h to remove the organic solvents. The fraction was left overnight in a deep freezer and then lyophilized using freeze dryer (Christ 100400 Bioblock Scientific, France). The yield was calculated and found to be 1.02% which was similar with previous works (Abdelwahib *et al*., 2007; Admassie and Engidawork, 2011; Mohammed and Engidawork, 2011).

3.5. Grouping and dosing of animals

Animals were divided into 8 experimental groups of 8 (four male and four female) each. The first group served as control (CON) and given vehicle (Tween 80, 2%v/v in water) orally. The second, third and fourth groups received crude khat extract at three different doses of 100 mg/kg (K100), 200 mg/kg (K200) and 400 mg/kg (K400) orally for ten days. The fifth group (GEN) were treated with gentamicin for eight days at a dose of 100 mg/kg, intraperitoneally (Parlakpinar *et.at*., 2006). The rest of the groups received crude khat extract at three different doses 100 mg/kg (GK100), 200 mg/kg (GK200) and 400 mg/kg (GK400) orally for two days before and eight days concomitantly with gentamicin (100mg/kg intraperitonaelly).

The rats were weighed on alternate days and the last known weight was used for dose calculation. The final day body weight was used for the calculation of body weight change and expression of normalized kidney weight (Annie *et al*., 2005; Harlalksa *et al*.,

2005). The dose for the khat extract were selected from previous reports (Abdelwahib *et al.*, 2007; Bedada and Engidawork, 2010; Mohammed and Engidawork, 2011).

3.6. Sample collection

Twenty-four hour after the last treatment (Parlakpinar *et al.*, 2005), the animals were slightly anesthetized with ether inhalation and bilateral prilumbal vertical incisions were made and blood samples were collected with cardiac puncture for BUN and serum creatinine determination. The blood samples were left at room temperature for 30 min for coagulation and then centrifuged at 3000 rpm for 15 min at 4°C (Centurion Scientific Ltd K240R, UK) to separate serum. The serum was stored at -20°C for 48 h (Aftron AFF 545, Denmark) until subjected for analysis of creatinine and BUN levels. Immediately after cardiac puncture, animals were killed with high dose of ether inhalation and both kidneys were removed. Right kidneys were rinsed in chilled saline, decapsulated, blotted on a filter paper and quickly weighed. Then, it was homogenized in ice-cold saline in volume of nine times of its weight to yield 10% (w/v) tissue homogenate and stored at -20°C until it was analyzed for SOD and catalase activities as well as MDA level. The left kidneys were fixed in 10% formalin for histopathological examination (Al-majed *et al.*, 2002)

3.7. Biochemical analysis

3.7.1. Serum creatinine and blood urea nitrogen

The concentration of serum creatinine and BUN were measured by Cobas integra 400 (Roche, Switzerland) using commercial kits according to the manufacturer's protocol. Creatinine level was determined by Jaffe's reaction without deproteinization where the samples were subjected to react with picrate in alkaline pH forming a yellow-red color with maximum absorbance at 512 nm. For measurement of BUN level, kinetic test with urease and glutamate dehydrogenase were used. Urea in the sample was hydrolyzed by urease forming ammonia that in turn reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase and reduced nicotinamide adenine dinucleotide (NADH) to produce L-glutamate. The rate of decrease in the NADH concentration is directly

proportional to the urea in sample that can be determined by measuring the absorbance at 340 nm. The BUN was calculated from urea using a formula BUN (mg/dl) = urea*0.467.

3.7.2. Determination of total superoxide dismutase activity

The total SOD activity was determined using a reaction system consisting of xanthine and xanthine oxidase that produces $O_2^{\cdot-}$. The $O_2^{\cdot-}$ oxidizes hydroxylamine forming nitrite, which colors amaranth by the color developer and can be assayed at 550 nm (Unic model 2100 spectrophotometer). During the assay, 50 ml of the 10% tissue homogenates were mixed well with the reaction system on a vortex mixer (Labnet S0100-230V, Labnet International Inc., USA) and incubated in 37°C water bath (Oakton Stable Temp WD-1250-15, USA) for 40 min. The formation of superoxide radical and nitrite was inhibited by SOD in the samples reducing the intensity of the amaranth color as well as the absorbance upon addition of the color developing agent. The total SOD activity in the sample was calculated and expressed as U/mg protein. One unit of SOD activity is defined as the amount of SOD that will produce 50% inhibition of oxidation of hydroxylamine induced by xanthine and xanthine oxidase at 37°C in 1mg/ml protein concentration of tissue homogenate.

3.7.3. Determination of catalase activity

Catalase activity was measured based on the provided manufacturer procedure that relies on the reaction of enzyme in the presence of an optimal concentration of H_2O_2. The rate of dismutation of H_2O_2 to H_2O and O_2 is proportional to the concentration of catalase. Briefly, 50ul of 10% renal homogenates of rats were mixed well with a known concentration of H_2O_2 on a vortex mixer and incubated at 37°C in a water bath for exactly 1min. Ammonium molybdate was added to the mixture to quench the reaction and react with the remained H_2O_2 forming a stable colored complex. The absorbance of the complex was measured at 405 nm. Finally, the catalase catalytic activity of the tissue samples was calculated and expressed as U/mg protein. One unit of catalase catalytic activity is defined as the amount of enzyme that will decompose 1µmol H_2O_2 per second at 37°C in 1mg protein of tissue homogenate.

3.7.4. Determination of malondialdehyde level

The amount of lipid peroxides was calculated as thio-barbituric acid reacting substance (TBARS) such as MDA, formed from the breakdown of polyunsaturated fatty acids, and considered as an index for the peroxidation reaction. The level of MDA in renal homogenates was assayed based on thio-barbituric acid (TBA) method, where MDA undergoes condensation reaction with TBA generating red product that has a maximum absorption peak at 532 nm. Briefly, tissue homogenates (10%) were well mixed with TBA reaction system in test tubes on vortex mixer and then test tubes were sealed with aluminum foil with a hole stung with a needle. The mixtures were incubated at 95°C in a water bath for 40 min, cooled with flowing water and then centrifuged at 4000 rpm for 10 min. The supernatants were carefully pippeted into quartz cuvete (Exactaoptech, Germany) to read the absorbance of the red color at 532 nm and MDA level determined.

3.8. Morphometric Analysis

3.8.1. Body and kidney weight changes

Body weight of all animals before and after the experiment was taken and the difference was expressed as body weight change. The weight of both right and left kidneys of each rat was measured at the end of treatment after sacrificing the animal. For standardization, total weight of both kidneys/100g body weights was determined (Erdem *et al*., 2000).

3.8.2. Histopathology examination

The kidney tissues were embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin for slide preparation. Then, the slides were coded and examined by a pathologist, who was blind to the treatment groups via light microscopy for assessment of histopathological changes such as interstitial inflammation, tubular necrosis and nuclear disintegration (Ulutas *et al*., 2006).

3.9. Statistical analysis

All data were presented as mean ± standard error of the mean. The analysis was performed by one way ANOVA followed by Tukey's multiple comparison tests. Level of significance was set at $p < 0.05$ and SPSS data analysis software version 19 was used for data processing.

4. Results

4.1. Biochemical analysis

4.1.1. Serum creatinine and blood urea nitrogen levels

Khat treatment at doses of 100 mg/kg and 200 mg/kg did not affect levels of both BUN and creatinine, as there were no significant differences between CON and K100 as well as K200 rats. By contrast, K400 rats displayed a significantly greater creatinine (54.2%, $p<0.001$) and BUN (30.2%, $p<0.001$) levels compared to CON rats. Furthermore, GEN rats showed significantly elevated creatinine (110.2%, $p<0.001$) and BUN (142.2%, $p<0.001$) levels compared to CON rats. Compared to K100, K400 rats exhibited significantly greater creatinine (44.4%, $p<0.001$) and BUN (28.7%, $p<0.001$) levels. In addition, K400 rats showed a significant increase in creatinine (37.8%, $p<0.01$) and BUN (24.8%, $p<0.05$) levels compared to K200. Significant differences were not observed between K100 and K200 rats in terms of both creatinine and BUN (Table 1).

Table 1: Effects of crude khat extract on serum creatinine and blood urea nitrogen levels in rats.

Groups	Serum creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
CON	0.59 ± 0.02	19.92 ± 0.34
K100	0.63 ± 0.02	20.15 ± 0.42
K200	0.66 ± 0.05	20.79 ± 0.40
K400	0.91 ± 0.02^{a3b3c2}	25.94 ± 0.53^{a3b3c1}
GEN	1.24 ± 0.03^{a3b3c3d3}	$48.25 \pm 0.97^{a3b3c3d3}$

Values are mean ± SEM. n = 8; ^{a} compared to CON; ^{b} compared to K100; ^{c} compared to K200; ^{d} compared to K400. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$. (CON: control; K100, khat 100 mg/kg; K200, khat 200 mg/kg; K400, khat 400 mg/kg; GEN, gentamicin 100 mg/kg).

To assess whether khat-induced increase in the serum markers was comparable to that of gentamicin, analysis was performed between GEN and all khat treated rats. The result revealed that GEN rats exhibited significantly higher creatinine (96.8%, $p<0.001$; 87.9%, $p<0.001$; 36.3%, 0.001) levels compared to K100, K200 and K400 rats, respectively. Similarly, BUN levels significantly increased in GEN rats compared to K100 (139.5%, $p<0.001$), K200 (132%, $p<0.001$) and K400 (86%, $p<0.001$) rats, respectively (Table 1).

In order to investigate the renal effect of khat when administered concomitantly with gentamicin (well known renotoxic agent), three doses of crude khat extract were given with gentamicin. The results indicated that significant increase was not observed in creatinine and BUN levels in GK100 and GK200 rats compared to GEN rats. However, administration of khat at a dose of 400 mg/kg concomitantly with gentamicin showed significantly elevated creatinine (25%, $p<0.001$) and BUN (19.9%, $p<0.001$) levels compared to gentamicin alone (Table 2).

Table 2: Effects of khat and gentamicin co-administration on serum creatinine and blood urea nitrogen levels.

Groups	**Serum creatinine (mg/dl)**	**Blood urea nitrogen (mg/dl)**
GEN	1.24 ± 0.03	48.25 ± 0.97
GK100	1.28 ± 0.03	49.42 ± 1.02
GK200	1.30 ± 0.02	49.94 ± 0.71
GK400	1.55 ± 0.06^{e3}	57.83 ± 0.79^{e3}

Values are mean ± SEM. n=8; ^{e} compared to GEN. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$. (GEN: gentamicin 100 mg/kg; GK100: gentamicin 100 mg/kg + Khat 100 mg/kg; GK200: gentamicin 100 mg/kg + Khat 200 mg/kg; GK400: gentamicin 100 mg/kg + Khat 400 mg/kg).

4.1.2. Effects on antioxidant enzymes

K100 and K200 rats had slightly decreased renal SOD and CAT enzymatic activities that were statistically insignificant compared to CON rats. By contrast, K400 rats revealed significantly decreased SOD (14.8%, $p<0.01$) and catalase (35%, $p<0.001$) enzymatic activities compared to CON rats. Significantly greater reduction in SOD (47.4%, $p<0.001$) and catalase (63%, $P<0.001$) activity was also noted in GEN compared to CON rats. Significant differences were not observed in terms of SOD and catalase activities between K100, K200 and K400 rats though slight reduction was depicted as the dose of khat increased (Table 3).

The reduction in SOD and catalase enzymatic activities following khat and gentamicin administration was also compared. GEN rats exhibited a significant decrease in renal SOD (45.3%, $p<0.001$; 43.3%, $p<0.001$; 38.3%, $p<0.001$) enzyme activity compared to K100, K200 and K400 rats, respectively. Furthermore, catalase activity significantly decreased in GEN rats compared to K100 (63.1%, $p<0.001$), K200 (59.6%, $p<0.001$) and K400 (43.1%, $p<0.001$) rats respectively (Table 3).

Table 3: Effects of crude khat on renal SOD and CAT enzymatic activities in rats.

Groups	**SOD (U/mg protein)**	**CAT (U/mg protein)**
CON	259.50 ± 7.56	17.69 ± 0.73
K100	249.49 ± 11.49	17.62 ± 0.56
K200	240.66 ± 6.97	16.20 ± 0.40
K400	221.17 ± 7.51^{a2}	11.50 ± 0.87^{a3}
GEN	136.43 ± 4.94^{a3b3c3d3}	6.54 ± 0.47^{a3b3c3d3}

Values are mean ± SEM. n=8; ^{a} compared to CON; ^{b} compared to K100; ^{c} compared to K200; ^{d} compared to K400. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$. (CON: control; K100, khat 100 mg/kg; K200, khat 200 mg/kg; K400, khat 400 mg/kg; GEN, gentamicin 100 mg/kg).

Table 4 illustrates the effect of crude khat extracts when given concomitantly with gentamicin on renal SOD and catalase activities. Compared to GEN rats, GK100 and GK200 had shown slightly reduced renal activity of SOD and catalase that failed to reach statistical significance. By contrast, GK400 groups revealed a significant reduction of renal SOD (25.7%, $p<0.05$) and catalase (49.4%, $p<0.01$) enzymatic activities compared to GEN rats.

Table 4: Effects of khat-gentamicin co-administration on renal SOD and CAT enzymatic activities in rats.

Groups	SOD (U/mg protein)	CAT (U/mg protein)
GEN	136.43 ± 4.94	6.54± 0.47
GK100	134.86 ± 3.23	5.72 ± 0.27
GK200	126.08± 8.23	4.60 ± 0.18
GK400	101.33 ± 3.48^{e3}	3.31 ± 0.46^{e3}

Values are mean ± SEM. n=8; ^{e} compared to GEN. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$. (GEN: gentamicin 100 mg/kg; GK100: gentamicin 100 mg/kg + Khat 100 mg/kg; GK200: gentamicin 100 mg/kg + Khat 200 mg/kg; GK400: gentamicin 100 mg/kg + Khat 400 mg/kg).

4.1.3. Effects on lipid peroxidation

As it can be seen from Fig 3, K100 rats showed a slightly increased MDA level compared to CON though statistical significance was not reached. MDA level increase, however, was significantly greater for K200 (41.5%, $p<0.05$), K400 (103.8%, $p<0.01$) and GEN (141.2%, $p<0.001$) compared to CON rats. In addition, the MDA level elevation produced by GEN rats was significantly greater than K100 (119.2%, $p<0.001$), K200 (70.3%, $p<0.001$) and K400 (18.3%, $p<0.01$) rats. After concomitant administration of khat and gentamicin, the renal tissue lipid peroxidation status was also investigated. Accordingly, while no apparent difference was observed with the other doses, khat at a

dose of 400 mg/kg displayed a significant increase (38.6%, p<0.001) in MDA level when compared with gentamicin alone.

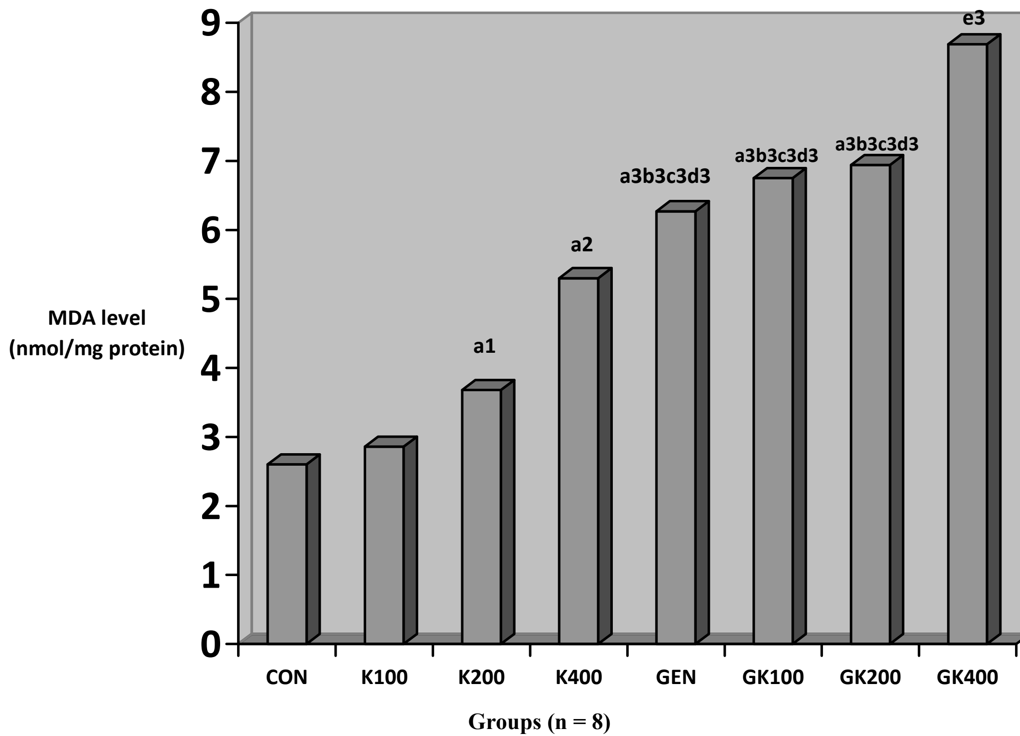

Fig 3: Effects of crude khat extract alone and with gentamicin on renal lipid peroxidation.

Values are mean ± SEM. n=8; [a] compared to CON, [b] compared to K100, [c] compared to K200, [d] compared to K400, [e] compared to GEN. [1]p<0.05; [2]p<0.01; [3]p<0.001. (CON: control; K100: khat 100 mg/kg; K200: khat 200 mg/kg; K400: khat 400 mg/kg; GEN: gentamicin 100 mg/kg; GK100: gentamicin 100 mg/kg + Khat 100 mg/kg; GK200: gentamicin 100 mg/kg + Khat 200 mg/kg; GK400: gentamicin 100 mg/kg + Khat 400 mg/kg).

4.2. Morphometric analysis

4.2.1. Effects on body weight change and normalized kidney weight

At the end of the experiment, body weight change was determined for each group of animals in percent. The results revealed that K100 did not show significant body weight change compared to CON rats. However, K200 ($p<0.05$), K400 ($p<0.01$) and GEN ($p<0.001$) groups revealed significant body weight loss compared to CON. Moreover, body weight loss following gentamicin treatment was significantly greater ($p<0.001$) compared to all groups of khat treated rats.

Treatment with crude khat extract at a dose of 400 mg/kg produced a significant ($p<0.05$) increase in normalized kidney weight compared to CON. GEN rats revealed significantly increased kidney weight gain ($p<0.001$) compared to CON and all khat groups. Animals treated with khat 100 mg/kg and 200 mg showed slight kidney weight gain without statistical significance as compared to the control rats (Table 5). Significant body weight loss or kidney weight gain was not observed between K100, K200 and K400 rats.

Table 5: Effects of crude khat extract on body weight change and normalized kidney weight

Groups	Body weight change (%)	Kidney weight (gm) /100g body weight
CON	2.83±0.48	0.70 ± 0 .01
K100	2.22±0.69	0.73 ± 0.01
K200	0.32±0.46^{a1}	0.74 ± 0.01
K400	-4.96±0.28^{a2}	0.82 ± 0.01 ^{a1}
GEN	-10.36±57 ^{a3b3c3d3}	0.89 ± 0.01^{a3b3c3d3}

Values are mean ± SEM. n=8; ^{a} compared to CON, ^{b} compared to K100, ^{c} compared to K200, ^{d} compared to K400. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$. (CON: control; K100: khat 100 mg/kg; K200: khat 200 mg/kg; K400: khat 400 mg/kg; GEN: gentamicin 100 mg/kg).

As illustrated in Table 6, concomitant treatment of khat at a dose of 400 mg/kg with gentamicin induced significantly greater ($p<0.01$) body weight loss compared to gentamicin alone. Treatment with plant extract at a dose of 400 mg/kg along with gentamicin significantly increased ($p<0.01$) kidney weight gain caused by gentamicin alone.

Table 6: Effects of crude khat extract with gentamicin on body weight change and normalized kidney weight

Groups	Body weight change (gm)	Kidney weight (gm) /100g body weight
GEN	-10.36±0.57	0.89 ± 0.01
GK100	-10.85±0.86	0.89 ± 0.02
GK200	-11.75±0.84	0.90 ± 0.01
GK400	-15.13±1.37 ^{e3}	1.00 ± 0.02 ^{e2}

Values are mean ± SEM. n=8; ^{e} compared to GEN. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$. (GEN: gentamicin 100 mg/kg; GK100: gentamicin 100 mg/kg + Khat 100 mg/kg; GK200: gentamicin 100 mg/kg + Khat 200 mg/kg; GK400: gentamicin 100 mg/kg + Khat 400 mg/kg).

4.2.2. Histopathological studies

Histopathological examination of the kidneys of rats treated with khat alone or with gentamicin revealed marked degenerative changes compared to the control animals. Observed changes include mild to moderate interstitial inflammation, hypertrophied glomerular capillaries, injured dilated Bowman's capsule and vascular congestion.

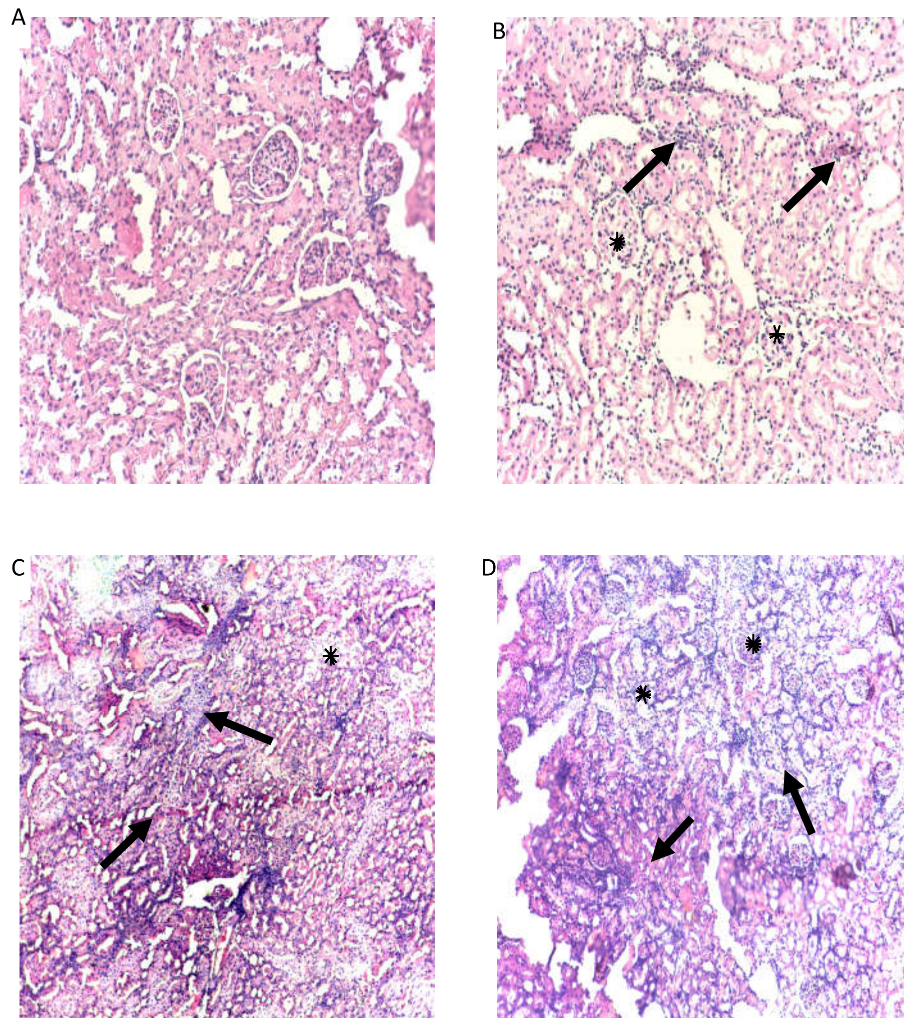

Fig 4. Photomicrographs of stained renal tissues for CON, K400, GEN and GK400 rats. The kidneys of the control rats showed normal renal parenchyma with normal histo-architecture (Fig 4A). On the other hand K400 rats showed mild to moderate interstitial inflammation (arrows), hypertrophied glomerular capillaries and injured dilated Bowman's capsule (*). GEN rats showed marked infiltrative inflammatory cells and vascular congestion (arrows) and vacuolar degeneration of the tubules (*) (Fig. 4C). Furthermore, GK400

rats revealed more extensive and marked infiltrative inflammation, complete destruction of glomerular capillaries (arrows), degeneration of the tubules and Foamy appearance in the tubular epithelial cells (*) (Fig 4D), H&E, x 40.

5. Discussion

In this study the effect of khat administration alone and with gentamicin was studied in terms of alteration of renal markers, including creatinine, BUN, antioxidant enzymes as well as markers for lipid peroxidation. In addition, histopathological changes such as presence of inflammation, tubular degeneration, hyaline casts and vascular congestions were evaluated. Rats with gentamicin induced nephrotoxicity was well documented as it can provide an excellent model of acute renal failure to test different compounds or extracts which could have renoprotective roles (Ali, 1995).

5.1. Biochemical changes

In the present study, administration of khat at high dose (400 mg/kg) had significantly increased serum creatinine and BUN levels, suggesting that khat use may impair renal function by reducing the ability of kidneys to excrete these products. These effects perhaps may originate from changes in the renal blood flow and glomerular filtration rate induced by khat treatment (Kalix, 1984). Al- Motarreb and Broadley (2003) reported that khat chewers experience an increase in heart rate, body temperature, sweating and cold extremities which dictate presence of peripheral vasoconstriction caused by khat chewing. On the bladder, khat chewing produced a fall in urinary flow rate, an effect that has been shown to be inhibited by the selective α_1-adrenoceptor antagonist, indoramine, and therefore attributed to activation of this receptor subtype (Nasher et al., 1995). Peripheral vasoconstriction following khat administration as described above would explain the raised serum creatinine and BUN levels.

Compared to khat, gentamicin treatment significantly resulted in a marked elevation of both creatinine and BUN levels. Thus, it is possible to say that alteration of these renal indices caused by khat treatment is relatively mild to moderate compared to gentamicin. To investigate whether khat plays a permissive or pro-aggravation renotoxic role, both agents were given concomitantly and high dose of khat (400 mg/kg) accentuated gentamicin induced rise in creatinine and BUN levels. Hence, elevation of these markers following high dose of khat (alone and with gentamicin) clearly shows that khat has a direct a renotoxic potential rather than a permissive role. Gentamicin induced oxidative

stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by causing renal vasoconstriction (Walker and Shah, 1987).

ROS generated following gentamicin treatment may also impair the expression of endothelial nitric oxide synthase (eNOS), whereas superoxide anion may scavenge nitric oxide, thereby reducing the amount of the endogenous vasodilator in the vasculature (Conger, 1999). The accentuating role of khat when given with gentamicin may partially be attributed to its constituents that could possibly mediate similar effects as that of gentamicin.

SOD is the predominant if not the primary defense against free radicals which catalyze the dismutation of superoxide radicals to hydrogen peroxide that in turn is removed by catalase or glutathione peroxidase (Maritim et al., 2003). There are three forms of SOD in mammalian tissues: copper zinc SOD, manganese SOD and extracellular SOD, together contributing to the total SOD activities (Young and Woodside, 2001). In agreement with previous observations (Karadeniz et al., 2008), the present study also indicated gentamicin induced oxidative stress, as shown by significant decrease in kidney catalase and SOD activities. Exhaustion of enzymatic renal oxidative defense mechanisms along with enhanced ROS generation could result in oxidative damage in gentamicin treated rats (Karahan et al., 2005).

Khat administration at high dose (400 mg/kg) significantly decreased both renal SOD and catalase enzyme activities. At this dose, khat significantly aggravated the decline in renal catalase and SOD enzymatic activities induced by gentamicin suggesting that the extract is able to generate free radicals or directly inhibit synthesis of antioxidant enzymes. This finding is similar to recent studies in which administration of khat extract or its alkaloid fraction were shown to alter the activities of the free-radical metabolizing/scavenging enzyme system (Al-Qirim et al., 2002; Al-Hashem et al., 2011). Flavonoid fraction of khat has no effect on SOD activity (Al-Qirim et al., 2002) which suggests that alkaloids in crude khat extract are responsible for renotoxic effects

The peroxidation of lipids gives rise to a number of secondary products, MDA being the principal and most studied one. This aldehyde is a highly toxic molecule and has been considered as more than just a marker of lipid peroxidation (Rio et al., 2005). The rationale of MDA as a biomarker relies on that it is solely derived from lipid peroxides and changes in MDA concentration reflects changes in lipid oxidation level (Lykkesfeldt, 2007). Baliga et al. (1999) documented that gentamicin cause lipid peroxidation in the kidneys via ROS generation.

The results in the present study clearly indicated that intragastric administration of khat at doses of 200 mg/kg and 400 mg/kg had shown accelerated lipid peroxidation in the renal tissues as reflected by an increase in MDA level. Additionally, gentamicin induced elevation of renal MDA level was significantly accentuated by khat 400 mg/kg co-treatment possibly by augmenting ROS generation by gentamicin. Although khat at a dose of 200 mg/kg did not reduce antioxidant enzymes to a significant level as presented above, it elevated the MDA level significantly. This could possibly suggest that khat may induce lipid peroxidation by mechanisms other than reduction of antioxidant enzymes.

Although khat contains alkaloid and flavonoid constituents, the toxic effects of crude khat extract in this study may suggest that overwhelming effects of alkaloids since flavonoids play a protective role (Al-Qirim et al., 2002; Al-Zubairi et al., 2003). Al-Hashem et al. (2011) reported similar finding that the toxic effect of khat extract on hepatic and renal functions may be related to lipid peroxidation as indicated by a significant increase in lipid peroxidation biomarkers (TBARS). By contrast Al-Zubairi et al. (2003) suggested that khat chewing may not provoke lipid peroxidation, and hence may have some antioxidative property as khat contains polyphenolic (proanthocynidines) constituents that play a role as antioxidants. The result of this study might be an indicator that flavonoids contained in khat at high dose might induce pro-oxidant effect rather than antioxidative role.

5.2. Morphologic pathology

Severe body weight loss was observed in this study following gentamicin treatment; however the normalized kidney weight was significantly increased. This finding is consistent with other reports (Ali et al., 1992; Erdem et al., 2000; Harlalka et al., 2007). Gentamicin induced weight loss may be related to direct renal tubules injury and/or increased catabolism. Injury of the renal tubules leads to subsequent loss of the tubular cells that take part in renal water reabsorbtion. This is accompanied by loss of water, leading to dehydration and loss of body weight (Ali et al., 1992). The increase in the normalized kidney weight of gentamicin treated rats probably resulted from the edema that was caused by drug induced acute tubular necrosis (Erdem et al., 2000).

The same effect as that of gentamicin were found in rats treated with crude khat extract reaching the significance level at a doses of 400 mg/kg. Concomitantly treated animals with gentamicin and khat 400 mg/kg had also shown significantly severe body weight loss and increased normalized kidney weight gain. The fact that renal injury by gentamicin involves ROS and crude khat extract has aggravated oxidative stress may rationalize the accentuation of the gentamicin-induced body weight reduction and normalized kidney weight gain. The reduction in body weight may also be ascribed to khat induced delay in intestinal absorption that contributes to some degree of malnutrition. In a randomized controlled trial, Heymann et al. (1995) reported a delay in gastric emptying after chewing khat, which was attributed to the sympathomimetic action of the cathinone. Moreover, Gunaid et al. (1999) showed khat to prolong whole gut transit and Makonnen (2000) reported that khat produced constipation in mice and an antispasmodic action on guinea-pig isolated ileum.

The loss of body weight in this finding as a result of khat treatment can also be substantiated by increased plasma leptin level that leads to loss of appetite (Al-Dubai et al., 2006). The role of ghrelin and peptide YY (PYY) in khat induced weight loss has been excluded (Murray et al., 2008).

The present study revealed that khat at higher dose (400 mg/kg) induced histopathological changes as evidenced by mild renal interstitial inflammation, hypertrophied glomerular capillaries and injured dilated Bowman's capsule (Fig 4B). The same dose of crude khat extract worsens gentamicin induced histopathological (Fig. 4C) changes (more extensive and marked infiltrative inflammation, complete destruction of glomerular capillaries, degeneration of the tubules and Foamy appearance in the tubular epithelial cells (Fig 4D) than gentamicin alone.This finding was in line with previous studies. For example, Al-Hashem et al. (2011) reported that microscopic examination of the kidneys of khat treated rats showed major changes including invasion of infiltrative inflammatory cells, hypertrophied glomerular capillaries, dilated Bowman's capsules, cytoplasmic vacuolar degeneration as well as complete cytoplasmic vacuolization of tubular cells.

6. Conclusion

In the present study, gentamicin administration caused marked renal dysfunction as evidenced by increased serum creatinine, blood urea nitrogen, and lipid peroxidation and decreased SOD and CAT activities. Administration of khat at higher dose was shown to cause renal damage. Furthermore, disturbed renal indices by gentamicin were considerably accentuated by high dose (400 mg/kg) of crude khat extract. Khat, alone or with gentamicin was also found to alter renal histopathology, normalized kidney weight and body weight of rats.

Hence, it could be concluded that use of khat at higher dose may cause an oxidative stress leading to renal injury either by depleting anti-oxidative mechanisms or by enhancing pro-oxidant components of tissues. More reasonably, khat may be able to perturb both protective and damaging mechanisms by which a cell has to balance in order to escape from oxidative stress.

7. Recommendations

- It will be appropriate to experiment long term effects of crude khat extract on kidney.
- Laboratory studies on different tissues has to be conducted in order to ascertain whether khat toxicity is tissue specific or more general
- Isolations of khat components thereby investigating their respective effects will provide a body of knowledge about the effect of khat on renal tissues.
- Experimental study should be conducted to rule out the collective effect of pesticides with crude khat extracts.
- Comparative studies on the different varieties of khat leaves available in the market should be performed in order to rule out the effect of geographical variation on composition of khat constituents.

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