The first historical reference to *Catha edulis* Forsk (Celastraceae) appeared in a chronicle on Amda Seyon, who reigned Abyssinia from 1314-1344 when Abyssinian tea was noted as a popular drink in Abyssinia and Aden (Gordon *et al*., 1961; Al-Meshal *et al*., 1983). This plant is known by different local names and is grown wild in Afghanistan, Uganda, Rawanda, Burundi, Zaire, Tanzania, Zambia, South Africa and South West Arabia (Greenway, 1947; Clarke, 1960).

There is a presumed antiquity of the *C. edulis* leaves, shoots and roots’ use as a native medicine for several illnesses. It used to aid the treatment of genitourinary diseases and is known to afford protection against malaria (Peter, 1952). Masai and Kipsigi people used it to treat gonorrhea (Glover *et al*., 1966). Khat infusion is used to administer to the invalids (MerABI, 1929). The dried leaves of khat are sometimes also smoked (Margetts, 1967). Leaves and roots are said to be of use in influenza, while roots alone suffice for stomach troubles (Bally, 1938; Bally, 1945; Githens, 1948). In South Africa it is a common remedy for cough, asthma and other chest diseases (Voster, 1974). In Saudi folk medicine it is used as antipyretic, CNS stimulant and in asthma (Ageel *et al*., 1987).

Apart from the native medicinal importance, the wood of *C. edulis* is used for building huts and as paper pulp (Greenway, 1917). However, the main reason why Khat leaves have entered the international arena is for its abuse. The widespread habit for chewing fresh leaves and shoots of Khat in Southern Arabia and Africa (Macioli and Parrinello, 1967) is more than a recreational habit reinforced by psychostimulant and euphorizing effects of Khat. The habit has a deep rooted social and cultural tradition (Nencini *et al*., 1978). Altogether each person takes some 100 to 200 g Khat leaves (Kalix and Braenden, 1985); however, in certain locations addicts are found chewing up to 2.5 kg every day (Peter, 1952). Khat produces central stimulation, ensuing elation, alertness, and euphoria, reminiscent of amphetamine action. The tendency of many Khat users to obtain their daily supply by any means is clear manifestation of psychic dependence and the World Health Organization has classified it as a ‘drug of abuse’, which can produce mild to moderate psychic dependence. This psychic dependence has damaging effects of
both social and economical aspects (Nahas, 1981). For example the sexual disturbance due to Khat may endanger family life (Macioli and Parrinello, 1967; Halbach, 1972), while considerable loss of man hours decreased productivity and loss of agriculture land for its cultivation may lead to serious economic imbalance (WHO, 1980a; Krikorian and Getahun, 1973).

Today several million people living in East Africa and South West part of the Arab peninsula are exposed to the Khat menace. Several attempts have been made to restrict Khat production and consumption in the past (Halbach, 1972). However, psychic dependence on this natural recreational drug is widely spreading, even to the parts where it is not grown (Nencini et al., 1989).

Although millions of people are exposed to the habit of Khat chewing but serious and detailed investigations on the toxicity of Khat are lacking. Previous reviews on this subject are mainly concerned with pharmacological aspects of Khat (Kalix and Braendon, 1985; Kalix, 1984; Al-Meshal et al., 1985; Attef et al., 1997) and the information on its toxicity is still scattered in literature. In this study an attempt has been made to present the toxicity of Khat with some background and the role of its phytoconstituents, which will provide renewed insight in approach to the serious Khat abuse.

Toxicity of Khat

1. General toxicity

   The desirable effects of Khat leaves include relief from fatigue, increased alertness, feeling of elation and heightened self-confidence. It is effective in blunting sensation of hunger (LeBraas and Fretillere, 1965), sense of well being and increase of libido (Nencini et al., 1978).

   Khat induces mild euphoria, excitement, accompanied by episodes of logorrhoea and verbal aggressivity. In larger doses, the wakefulness passes on to drowsiness and deep slumber. Insomnia is a corollary effect of Khat use. The chronic use of Khat may cause cerebral hemorrhage, hypertension and sometimes nausea and vomiting (Hassan et al., 2000; Al-Motarreb et al., 2002; Halket et al.,

   "..."
Extensive Khat use may cause positioning with neurological symptomatology (Khattab and Amer, 1995), loss of articulations, neuromuscular incoordination and collapse, hyperesthesia (Connor et al., 2000), hypothermia, twitching, spasticity, convulsions and death (Margetts, 1967; Halbach, 1972; LeBraas and Fretillere, 1965; Heisch, 1945).

The patients of schizophrenia are frequent users of Khat, which aggravate the intensity of schizophrenic problems (Luqman and Donowski, 1976). Such effects have also been noticed following chronic ingestion of amphetamines (Heisch, 1945). Khat chewing is reported to cause periodontal disease and mucosal lesions and disorders of upper gastrointestinal tract. It is known to cause constipation accompanied by hemorrhoids, paralytic ileus and cirrhosis of the liver. The extremities of the user felt cold and complained of drafts followed by palpitation and tachycardia (Halbach, 1972; Luqman and Donowski, 1976; Hughes, 1973).

The symptoms induced by Khat stimulate alcoholic intoxication (Maresova, 1967; Britton, 1939). It dilates pupils of the eye, excites the whole nervous system and causes a staring look (Halbach, 1972). In Kenya, Khat chewing was found to induce maniac psychosis and poisoning (Carothers, 1945; Dhadphale et al., 1981; Alem and Shibre, 1997; Jager and Sireling, 1994).

2. Phytoconstituents of Khat and their toxicity

Katin and celestrine were the first reported alkaloids of Khat (Fluckiger and Gerock, 1987; Mosso, 1893). Later cathin, cathinine and cathidine were isolated (Stockman, 1912; Cais et al., 1975). Wolfes (1930) believed (+)-norpseudoephedrine to be the active constituent of Khat. Studies by the United Nations Narcotic Laboratories finally confirmed (-)-cathinone [(-)-S-O-amino propiophenone] having S-configuration as that of (+)-amphetamine to be responsible for the activity of Khat (UN, 1975; Knoll, 1979; Yanagita, 1979; Schuster and Johanson, 1979; Rosecrans et al., 1979; WHO, 1980b; Kohli and...
Fig. 1 Khat Shrub

Fig. 2 Khat Leaves
Goldberg, 1982). The percentage yield of celastraceus alkaloids (cathedulins) is known to alter significantly on geographical location (Al-Meshal et al., 1985).

The known phytoconstituents of Khat are alkaloids, flavonoids, tannins, sterols/triterpenes, volatile bases, ethereal oils, ascorbic acid, resins and sugars (Quedan, 1972; Crombie, 1980; Gellert et al., 1981; Szendrei, 1983; El-Kiey et al., 1968) etc.

Tannins were found to damage liver and adrenal glands, and Khat polyphenolic compounds presumed to increase the probability of esophageal cancer (Alles et al., 1961; Mortan, 1979; Mortan, 1980; Schorno et al., 1982). The periodontal disease, mucosal lesions, gastric problems and constipation among the Khat users have also been attributed to high tannin content of this plant (Halbach, 1972; Luqman and Donowski, 1976; Hughes, 1973; Maresova, 1967). However, the relative properties of tannins have been found to vary substantially among different types of Khat (Fiedler and Hildebrand, 1954).

The presence of the known antioxidant ascorbic acid in Khat is reported to act as an antidote to amphetamine like substances (Krikorian, 1984). However, Khat variants are known to differ in ascorbic acid contents (Kalix and Braenden, 1985).

The phenylalkylamines are pharmacologically known to act like amphetamines (Kalix, 1994; Kalix, 1996; WHO, 1980a; Szendrei, 1980; Nencini, 1980; Zelger et al., 1980; Zelger and Carlini, 1981; Kalix, 1981). Although pharmacological studies have been undertaken on (-)-cathinone (Kalix and Braenden, 1985; Johanson and Schuster, 1981; Velterio and Kalix, 1982; Kalix, 1983) but little is known about its toxicity. Khat alkaloids (-)-cathinone, (+)-cathinone, (-)-N-formylnorephedrine and (+)-N-formylnorephedrine, at an acute dose of 5 to 30 mg/kg (i.p.) were found to produce a varying degree of hypoprothrombinemia with corresponding response on fibrinogen level in rats (Tariq et al., 1987a). The hypertensive state often observed in chronic Khat chewers may be due to increased viscosity of blood produced by khat alkaloids. Cathinone is reported to cause reduction in cardiac glycogen and serum
triglycerides (Al-Meshal et al., 1987a). In a comparative study of cathinone and amphetamine at a dose of 5, 10 and 15 mg/kg on brown adipose thermogenesis in rats both the compounds produced significant dose dependent increase in intracapsular brown adipose tissue and rectal temperature. However, amphetamine was found to be three times more potent as compared to cathinone (Tariq et al., 1989a).

Al-Meshal (1987b) reported mitodepressive effect of (-)-cathinone on *Allium cepa* root tips at a concentrations of 0.5-1.0 percent. The treatment was found to induce significant condensation and clumping of chromosomes, sticky metaphases and anaphase bridges.

Cathinone was found to be unstable and decomposed to form a dimer (3,6-dimethyl-2, 5-diphenylpyrazine), and fragments such as benzaldehyde, ethylamine and 1-phenyl-1,2-propandione. These compounds have also been isolated from Khat (WHO, 1980b). The degradation of cathinone may occur both *in vivo* as well as *in vitro* and the activity of cathinone may be partially or fully related to these compounds.

Alkaloids are known to react with nitrites in acidic media to produce carcinogens (Hecht et al., 1978). Cathinone may undergo nitrosation by reacting with nitrites of the body fluid and act as carcinogen. The cold nitrosation of cathinone is found to produce methyl phenyl ketone while hot nitrosation leads to the formation of 2,5-dimethyl-3,6-diphenylpyrazine and 6-hydroxy-propiophenone (Schorno, 1979). These reactions are reported to be catalyzed by some phenolic compounds present in plants (Pignatelli et al., 1982; Nakamura and Kawabata, 1981). It is interesting to note that all these compounds are present in Khat leaves.

*C. edulis* is known to be a rich source of flavonoids. Kaempferol, quercetin, myricetin, dihydrmyricetine dihydromyricetin-3-0-rhamnose and glycosides of myricetin, quercetin and dihydromyricetin have been isolated from its leaves (El-Sissi and Abdalla, 1966; Gellert et al., 1981; Al-Meshal et al., 1986). Pamuku et al (1980) have reported a high incidence of ileal tumors in rats fed a diet containing 1000 ppm of quercetin for 1 year. However, most reports (Ambrose et al., 1952;
Hirono et al., 1981; Saito et al., 1980; Mirino et al., 1982) show no significant increase in tumor frequencies. Quercetin was found to be mutagenic in *Salmonella typhimurium* strains by the spot (100 μg per disk) and plate (50 μg - 2.5 mg per plate) incorporation methods. Flavonols (quercetin, kaempferol and galangin) were shown to be mutagenic in mammalian cells *in vitro* at levels of 5-75 μg/ml (Carver et al., 1983; Amacher et al., 1979; Meltz and Gregor, 1981). However, no consistent increases were observed in the micronucleus frequency of bone marrow or peripheral blood erythrocytes from mice treated with quercetin (Mac Gregor et al., 1983).

The observations of Khat revealed its adverse effects on human beings. The experiments on animals conducted so far are suggestive of its acute toxicity (Mack, 1995), mutagenicity, teratogenicity and also possible carcinogenicity (Gunaid et al., 1995; Awange and Onyango, 1993; Soufi et al., 1991; Kassie et al., 2001).

3. Metabolism of Khat and its active principle

The literature on the metabolism of *C. edulis* is scanty. Maitai and Mugera (1975) observed the presence of d-norpseudoephedrine in the urine of human subjects (range 30-40%) who had ingested Khat material.

In a study on the metabolism of cathinone (the active alkaloid of Khat), Brenneisen et al (1986) reported its conversion to amino alcohol metabolites by stereospecific keto reduction. The main metabolite of (-) cathinone was identified to be R/S-norephedrine. The metabolite of (+) cathinone was found to be (-) norpseudoephedrine.

4. Biochemical effects

Said (1967) reported inhibition of brain amino-oxidase and effect on blood sugar level on administration of chloroform extract of Khat leaves to busca rabbits. The results of the two week treatment with ethanolic extract of leaves at a dose of 200 mg/kg, revealed reduction in serum glucose, alkaline phosphatase and increase in total bilirubin, acid phosphatase and lactate dehydrogenase in rats (Tariq et al.,
1983). The decrease in serum glucose was attributed to significant decrease in food intake or due to endocrinological disorders (Ahmed and el-Qirbi, 1993). The increase of bilirubin and decrease in alkaline phosphatase activity was related to hepatic inflammation and increase in LDH was associated to congestion and cellular infiltration in myocardium observed in the histopathological studies. The changes in cholesterol, total protein, albumin, uric acid, SGOT, SGPT were not significant in the same study.

Chloroform extract of Khat leaves was found to inhibit RNA and DNA synthesis in the neurons of 7 and 19 days chick embryo (Hammouda, 1971, 1972). Subcutaneous administration of chloroform ether extract of the leaves reduced the concentration of RNA, DNA and total proteins in liver and brain in the dose levels of 0.5-1.0 g/kg in rats (Hondt et al., 1984). Chloroform extract of leaves also inhibited protein, DNA and RNA biosynthesis in KB, IBR (Greenway, 1947) and XP2Bi cells at varying concentrations (20-100 ng/ml) indicating its cytotoxicity and mutagenicity to mammalian cells (Al-Ahdal et al., 1988).

5. Morphological abnormalities and visceral toxicity

Acute toxicity studies in mice and rats revealed no mortality of animals up to a single oral dose of 2 g/kg body weight of ethanolic extract of Khat leaves. The histopathological studies on the two weeks Khat treated rats, revealed congestion and cellular infiltration in myocardium (Tariq et al., 1983).

In a study on chronic toxicity (Maitai, 1977) rats fed with Khat mixed in ground commercial pellets (concentrations of 1-50%), were observed to consume less food and retardation in growth. Catarrhal inflammation of increasing severity was noticed in glandular stomach and duodenum. Histopathological studies revealed mostly vacuolation type degenerative changes. Centrolobular and coagulative necrosis was also observed in liver and kidney, in addition to hemorrhagic gastritis and duodenitis. Based on the published data regarding the toxicity of tannins in rats (Godsi, 1965) the lesion in stomach and duodenum were attributed to the high contents of tannins in Khat (El-Sissi and Abdalla, 1966).
The study on the mean organ weights heart, lungs, kidney, spleen and liver showed an increase in weight at lower doses and decrease in weight at higher doses of khat. The tumors mostly appeared at axilla, chest, cervical region and abdomen. The histopathological examination revealed reactive hyperplasia in lymph nodes, lymphoid tissues and areas of necrosis; necrotic areas in subcutaneous tissues showed a lot of polymorphs and scattered inflammatory changes in muscles. The skin tumors and eye lesions observed in the Khat group were also observed in a parallel study on (-) cathinone (Tariq et al., 1989b). Thus, the skin tumors and eye lesions were attributed to the presence of (-)-cathinone.

6. Reproductive toxicity

In North Yemen where Khat chewing is common, infant and childhood mortality rates were found to be equal or exceeding 50% till 1976. Varying effects were observed on Khat chewers like effects on their sex-life, but in most, a delay in ejaculation phase was observed. Many Khat chewers reported a spontaneous secretion of spermatial fluid. Poor lactation has been reported in khat chewing lactating mothers (Luqman and Donowski, 1976).

The low production and cessation of sperms in the seminiferous pathway under the effect of dietary Khat was attributed to DNA replication deficiency (Hammouda, 1978; el-Shoura et al., 1995).

7. Teratogenicity and congenital effects

Investigations on the development of chick embryos, mother hen fertility and hatchability with the chloroform extract of Khat demonstrated a remarkable retardation of organ development, inhibition of limb bud formation and congenital abnormalities (Kamel et al., 1966; Hammouda et al., 1969; Islam et al., 1994).

A number of congenital abnormalities such as degeneration of blastoderm, retardation of heart development and stoppage of the process of cranial flexure were observed in the treatment of white leg horn eggs with chloroform extract of Khat (Kamel et al., 1967). The effect of Khat on RNA of the neurons of 7 and 19
days chick embryo showed fragmentation of the RNA content of 7 days neurons and the RNA in the advanced stage of the 19th day neuron was found to be seriously disintegrated and degenerated. This effect on RNA with the subsequent accumulation of these bodies on the cell periphery was due to disturbance of the rate of protein synthesis (Hammouda, 1971).

The DNA content in the nucleus was found highly diminished after the treatment of khat for seven days. On the other hand DNA of the advanced neuron of 19-day treatment was reported severely disintegrated and even resorbed. The changes in DNA content were attributed to the differences in the chromatid number and the physiological changes (Hammouda, 1972).

8. Mutagenicity

Recreational drugs such as alcohol, LSD and marijuana are clastogenic and present a potential hazard to man (Sax and Sax, 1966; Doorance et al., 1970; Bick, 1970; Hondt et al., 1980). Narcotic Khat was also investigated for its cytological and genetic effects. In a study on its mitodepressive effect in the meristematic region of Allium cepa root tips (Kabarity and Malallah, 1980), aqueous extract of Khat leaves produced a drastic reduction in mitotic index.

This mitodepressive effect induced by Khat was accompanied by imbalance in the frequencies of mitotic phases. The chloroform extract of Khat leaves was found to be mutagenic and cytotoxic in bacteria (Al-Ahdal et al., 1988).

Hondt et al (1984) found that Khat extract in different doses increased the chromosomal aberrations, which include gaps, breaks, centrometric attenuation and centric fusions. The mitotic index was reduced in all the treatments indicating cytotoxic activity of Khat.

Aberrations in the bone marrow were mostly shown to be of structural type. The methanolic extract of Khat leaves at a dose of 125, 250 and 500 mg/kg in mice significantly increased the frequency of micronucleated polychromatic erythrocytes and induced bone marrow depression in bone marrow cells. It also induced significant chromosomal aberrations in the testis such as aneuploids.
autosomal univalent, univalent of the sex chromosomes and polyploids (Tariq et al., 1987c). Khat extract was also found to increase the percentage of sperms with abnormal head forms and achromosome (Qureshi et al., 1988).

Prolonged exposure (6 weeks) of mice to the extract also resulted in the induction of total and partial sterility in males and significant post-implantation loss in the dose range of 50 to 200 mg/kg. These studies suggested that cathinone might partially or totally be responsible for the mutagenic activity and sterility induced by Khat (Tariq et al., 1990).
Stress:

Any environmental factor that can significantly modify an animal’s biological responses resulting into stress is called a stressor. The stressors may be physical, physiological or psychological in nature. Physical conditions of the environment such as temperature, light and sound directly affect the internal environment of an organism, and create disturbances in the structural and chemical composition of the body. The resistance offered by the body against these conditions is termed as physical stress. If the organism has the capacity to adapt and combat these conditions, it survives otherwise death of the organism occurs. According to Selye (1956), stress is the nonspecific response of the organism to any demand made upon it. Stress, like anxiety, is a broad and general concept-describing organism’s reactions to environmental demands (Rabkin and Struening, 1976). Seyle (1976) described the sequence of pathological changes occurring in the animals following exposure to stressful stimuli. He named it as “General Adaptation Syndrome” (G.A.S.), which develops in three stages comprising of-

i. **Alarm reaction or Shock**, when the animal is initially exposed to stressor and must set up defenses to combat it.

ii. **Stage of resistance**, when the organism is able to adjust to the changed environment. The adaptation is optional at this stage.

iii. **Stage of exhaustion**, in this stage the acquired adaptation is lost and body succumbs to stress disorders.

Alarm reaction consisted of the triad of lymphothymic involution, gastrointestinal ulceration, and loss of cortical lipid and medullary chromaffin substance from the adrenals. If the effect of
stressful stimuli continues for a long period, the body develops the stage of resistance. However, in case the stress is sufficiently severe and prolonged and the body fails to adapt, it may lead to state of exhaustion in which the animal develops symptoms similar to those seen in the first stage.

Environmental stress has been found to invoke compensatory metabolic changes through modification of the quantity of enzymes that are normally rate limiting, under fine control or inducible by hormones (Ramasarna, 1978). The process of adaptation at the cellular level to chronic stress seems to occur by sequential changes in hormones, enzymes and metabolites leading to a new steady state.

Various theories (Selye, 1956; Mason, 1968) have been postulated to elucidate the physiological response of the organism to stress. but none of them have been totally satisfactory (Burchrfield, 1979). The most commonly accepted definition of stress as proposed by Selye (1956) is that it is anything, which causes an alteration of homeostatic state of the body.

Stress, in its medical sense, is essentially the rate of wear and tear in the body. The feeling of just being tired, jittery, or ill is subjective sensations of stress (Selye, 1956). Rabkin and Struening (1976) propounded that stress can be one of the component of any disease, not just as those designated as psychosomatic. The chronic disease may be etiologically linked with excessive stress, caused due to organization of modern technological societies (Dodge and Martin, 1970).

Dozens of stress models have been used to study the activity of the sympathetic-adrenomedullary system under stress. Forced immobilization is one of the well-explored models of stress in the rat.
This model was chosen in this study because it combines emotional stress (escape reaction) and physical stress (muscle work), resulting in both restricted mobility and aggression (Kvetnansky and Mikulaj, 1970).

**Stress and Biochemical Parameters:**

The relationship between stress, hormones and neurotransmitters is now well established. During stress increase in the activity of sympatho-hypothalamic-pituitary-adrenal system has been observed (Kvetnansky and Mikulaj, 1970). In response to stress, the hyperactive pituitary gland releases the tropic hormones, which in turn by acting on their target endocrine glands stimulate the synthesis and release of their respective hormones (Levi, 1967). Thus, due to stress the circulating levels of catecholamines, cortisol, ACTH, growth hormones, acetylcholine, and histamine have been found considerably enhanced (Glick et al., 1965; Berson and Yalow, 1968; Kvetnansky, 1972; Mikulaj et al., 1975; Pandey, 1976 and Kopin et al., 1980).

The metabolism of catecholamines is found altered in response to stressful stimuli (Subrahmanium, 1973; Schneider et al., 1974 and Serova et al., 1999). In various stressful conditions the activity of catecholamines synthesizing enzymes have been observed enhanced (Serova et al., 1999; Kvetnansky, 1976 and Rysanek et al., 1978). An increase in dopamine-β-hydroxylase (DBH) activity has been recorded following stimulation of sympathetic nervous system or exposure to stress (Sabban et al., 1995). The activity of MAO, the catabolising enzyme of catecholamines is decreased in hypophysectomized animals or after hydrocortisone treatment (Parvez and Parvez, 1973). Moreover, decreased activity of MAO in the rat brain have also been
Fig. 3 Cage used for the immobilization of rats

Fig. 4 Immobilized rat in the cage
reported after repeated immobilization stress (Bhagat and Horenstein, 1976; Kvetnansky, 1980).

The stress-induced hyperactivity of adrenal cortex was originally described by Selye (1946). According to Henry (1977), the psychological stress activates either the pituitary-adrenal-cortical system or the sympatho-adrenocortical system when the organism fails to compete with the situation, a state leading to depression. In this type of stress the ACTH and corticosteroid levels are augmented with unaltered catecholamine levels. Enhanced levels of corticosteroids have also been reported in variety of diseases (Polgar et al., 2002; Yeap and Hosking, 2002).

The plasma and urinary serotonin levels have been found increased after exposure to immobilization stress (Toh, 1969; Sarkar, 1978 and Hirvonon et al., 1978). Increase in urinary level of corticosterone, adrenaline, noradrenaline and decrease in dopamine excretion have been reported during immobilization stress (Sudo, Ayako et al., 1993).

Following immobilization stress an increase in blood pressure and heart rate have been observed (Rhee et al., 1989). The serum level of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) has been found increased after restraint stress (Pal et al., 1995). Studies have also shown that immobilization stress significantly decreases circulating levels of glucose (Quirce and Maickel, 1981). Lactate dehydrogenase (LDH), GOT and GPT have been proved as tumor markers for their changes in concentration in serum, liver and kidney (Vinitha et al., 1995).
Free Radicals:

Free radical is any species capable of independent existence that contains one or more unpaired electrons (An unpaired electron is one that occupies an atomic or molecular orbital by itself). The presence of one or more unpaired electrons causes the species to be attracted slightly to a magnetic field, and sometimes makes the species highly reactive. Radicals can easily be formed when a covalent bond is broken if one electron from each of the pair shared remains with each atom, a process known as hemolytic fission.

Heat, electromagnetic radiation or others can provide the energy required to dissociate the covalent bond.

Sources of radicals, such as photochemical or enzymic system, have been observed to inactivate enzymes, cause erythrocyte haemolysis, kill bacteria, degrade DNA, and destroy animal cells in culture.

Free radicals are of great importance in the mode of action of several toxic substances and in the process of inflammation (Chevion et al., 1982; De Vries, 1981; Do Campo and Mareno, 1984).

Radicals have been suggested to be involved in ischaemia and in degenerative arterial disease (Wilson, 1976).

Stress and Free Radical Metabolism:

Free radical reactions are ubiquitous in living beings because of the high chemical reactivity of the intermediates. Various pathways are known by which free radicals can mediate cellular toxicity. The action of free radicals on biological system has the potential for disturbing the balance of pro-oxidants and anti-oxidants.
An alteration in this balance in the favor of pro-oxidant is known as oxidative stress (Sies, 1985). To control and reduce the free radical induced cellular damage, the organism has a compensatory mechanism, which comprises the most important variables in controlling or preventing free radical reactions. These defenses include some naturally occurring antioxidants as well as exogenous agents that have been proved useful. Some of these are water soluble or confined exclusively to non-polar environment such as ascorbic acid (Vit C) and tocopherol (Vit E), respectively. The other antioxidants that have received maximum attention in biological systems include selenium and the thiol containing compounds like glutathione and the enzymes of glutathione cycle (Flohe et al., 1976; Kosower and Kosower, 1978). Antioxidants are divided into two main classes:

i. **Preventive antioxidants**: These antioxidants interfere with initiation of the free radical chain reaction, e.g. catalase and other peroxides and the chelators of metal ion.

ii. **Chain Breaking antioxidants**: They interfere with chain propagation. They comprise superoxide dismutase (SOD), which acts in the aqueous phase and reduces the superoxide anion.

Antioxidant and free radical scavenging system exists in the cell to protect against the damaging effects of free radicals produced as a part of normal cell respiration and other cellular processes such as inflammatory response (Flohe et al., 1973; Willson, 1980, 1983. Cohen, 1984, Tappel, 1984; Kaplotiwitz et al., 1985). The involvement of free radicals and free radical reaction have been observed in the etiology and development of a number of diseases.
Fig. 5: Biological antioxidant defense systems.
especially life limiting (Pryor, 1987). Role of reactive oxygen species have been reported in some emotional stress models (Guliaeva et al., 1988; Deviatkina et al., 1989; Sasnovsky and Kozlov, 1992) and in oxidative stress related diseases (Sies, 1991). Oxidative damage to lipid, protein and DNA in the brain has been observed during immobilization stress (Liu et al., 1996).

**Superoxide dismutase (SOD: EC 1.15.1.1)**

All aerobic organisms utilized \( \text{O}_2 \) and must have some mechanism by which they can minimize \( \text{O}_2 \) toxicity. One mechanism is the production of superoxide radical and its dismutation reaction, catalyzed by the enzyme superoxide dismutase (Harman, 1956, 1971). The superoxide anion is a free radical formed by one electron transfer to oxygen.

\[
\text{O}^{\cdot -} + \text{e}^{-} \rightarrow \text{O}_2
\]

Superoxide dismutase (SOD) catalyzes the dismutation between two moles of superoxide anion to yield one mole of oxidized product (oxygen) and one mole of reduced product (hydrogen peroxide) (Klug et al., 1972).

\[
\text{O}_2^{\cdot -} + \text{O}_2^{\cdot -} + 2\text{H}^{+} \rightarrow \text{O}_2 + \text{H}_2\text{O}_2
\]

This is analogous to the dismutation of hydrogen peroxide to oxygen and water catalyzed by catalase, electrostatic repulsion between two molecules of superoxide anion limit their approach to one another; SOD overcomes the barrier and greatly increases the dismutation rate (Fridovich, 1976, 1978).

Several forms of SOD have been identified since McCord and Fridovich first discovered the enzyme in 1969. They identified the
enzymatic activity associated with erythrocuprein, a copper-zinc protein of erythrocytes. The copper is associated with enzymatic activity, whereas the zinc is structural. Similarly, SOD activity is associated with a family of Cu-proteins, cerebrocuprein in brain and hepatocuprein of liver (Fried, 1979). In mammalian tissues, a second form exists in which manganese is the prosthetic group (Fridovich, 1976). In rats and mice the Mn SOD is localized to mitochondria, whereas Cu-Zu SOD is cytoplasmic. However, this distribution does not hold in other species.

Fried and Mandel (1975) indicated that very high levels of SOD activity are present in liver, while the adrenals, kidney and red blood cells have intermediate activity and lower activities were found in most other tissue including brain.

Superoxide dismutase (SOD) in various tissues of rats appears to protect against the toxic effects of oxygen free radical-generated by its further reaction with cellular component (McCord et al., 1971; Fridovich, 1975). It is the first enzyme of scavenger enzyme series to ameliorate the damage caused in cells by free radicals (Slater, 1984). Singlet oxygen and superoxide radical are potentially toxic to living cells as they can participate in the oxidation of cell macromolecules like protein, lipids etc. in case of leak from the original oxidation reaction (King et al., 1975). Superoxide anions are generated during interaction of molecular oxygen with flavins, NADH, glutathione peroxidase and catecholamines (Misra and Fridovich, 1972; Heikkila and Cohen, 1973). Immobilization stress induces antioxidant defense changes in the plasma of rats (Liu et al., 1994). Several workers have reported the role of oxygen free radical in restraint stress induced gastric lesions and the role of SOD in clinical studies on stress gastritis prophylaxis (Kayabali et al., 1994; Li and Zhang, 1993).
Catalase (EC 1.11.1.6):

Most purified catalases have been shown to consist of four protein subunits, each of which contains a haem (Fe (III)-protoporphyrin) group bound to its active site. Dissociation of the molecule into its subunits, which easily occurs on storage, freeze-drying, or exposure of the enzyme to acid or alkali, causes loss of catalase activity. The three-dimensional structures of catalase from beef liver and from the fungus *Penicillium vitale* have been determined by X-ray crystallography.

It exist to remove hydrogen peroxide within cells, catalyses the reaction:

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

Immobilization stress was found to decrease the activities of catalase and glutathione peroxidase in rats (Kostic *et al.*, 2000).

Glutathione:

Glutathione has been considered to function as biological antioxidant. Its role in the destruction of the free radicals has been reported (Sohal *et al.*, 1984). Several workers have also reported the role of glutathione in cellular protection during aging (Pruche *et al.*, 1991). Combined action of glutathione and superoxide-dismutase (SOD) form an integral component of cellular antioxidant defense (Munday and Winterborn, 1989). Enhanced level of lipid peroxides along with depletion of glutathione has been observed (Younes and Sieger, 1980; Katoh *et al.*, 1989). Glutathione inhibits the oxidative stress induced by different compounds like ascorbate, NADPH-BrCCl₃ and NADPH-Fe³⁺ (Wefers and Sies, 1988; Tampo and Yanaha, 1990). It plays a protective role in rats against toxic oxygen
species generated by hyperoxia (Van et al., 1985). Glutathione is essential for the repair process in brain exposed to oxidative damage by free radicals (Pellmar et al., 1992). Depletion of glutathione during immobilization stress, stimulate oxidants and oxidative damage thus leading to degenerative diseases of aging including brain-dysfunction (Liu et al., 1996). Some workers have implicated the loss of antioxidant glutathione in the pathogenesis of Parkinson’s disease (Drukarch et al., 1996). It has been reported that reduced glutathione (GSH) is decreased after intragastric feeding of khat in rabbits (Farag et al., 1989).

Glutathione-S-transferase (GST:EC 2.5.1.18):

Glutathione-S-transferase is a non-selenium dependent glutathione peroxidase (Sies et al., 1979). It was first identified in 1961 (Booth et al., 1961; Coombs and Stakelum, 1961). The enzyme was subsequently named glutathione-S-aryltransferase. Later on, several other GSTs were demonstrated depending upon the substrate specificity. Following types of GSTs have been described so far.

i. Glutathione-S-alkyltransferase, catalyzing the conjugation of a variety of alkylhalides with glutathione (Johnson, 1966).

ii. Glutathione-S-epoxide transferase, active towards the conjugation of variety of alkylhalides with glutathione (Boyland and Williams, 1965).

iii. Glutathione-S-alkene transferase, catalyzing the conjugation of unsaturated compounds with glutathione.

The enzymes are almost ubiquitous in nature, and GST activity has been identified in man, non-human primates, rats, mouse, hamster, guinea pig, chicken, cow, sheep, trout and shark
(Mannervik, 1985). The concentration of GST is, in general, high in mammals (upto 10% of cytosolic proteins in some organs), in other species (shark) the level of activity is quite low (Sugiyama et al., 1981). In addition, it is generally present in most mammalian organs.

Glutathione-S-Transferase (GST) was found reduced in rats with denervation of the liver, thus confirming the role of the peripheral nervous system (Spiridonov et al., 1989). It has a major role in the detoxification of oxyradicals and their products (Mannervik and Danielson, 1988). Brain GST plays an important role in the detoxification of potential toxicants through their conjugation and biotransformation (Booth et al., 1961; Boyland and Chasseaud, 1969; Dixit et al., 1980; Kubota et al., 1985). Greater accumulation of the toxic compounds inhibits the GST activity (Boylan and Chasseaud, 1969). GST has been also reported as tumor marker enzyme for detection of initiated cells during liver carcinogenesis (Tatematsu et al., 1988). 

Uric acid:

Uric acid can act as an antioxidant, both by binding iron and copper ions in forms that do not accelerate free radical reactions, and by directly scavenging oxidizing species such as singlet O₂, NOCl, and peroxyl radicals (Ames et al., 1981; Davies et al., 1986).

Uric acid also reacts with some oxidants in vivo (Grootveld and Halliwell, 1987). However, reaction of uric acid with certain oxidizing species, such as *OH or peroxyl radicals, can generate uric acid radicals that are themselves capable of doing biological damage e.g., by inactivating certain enzymes (Kittridge and Willson, 1984; Aruoma and Halliwell, 1989).
Immobilization stress can induce a hyperuricemic state in rats, and also increasing the purine catabolite by involvement of endogenous catecholamine (Yonetani et al., 1979), or by increasing xanthine oxidase activity in rats (Kovacheva and Ribarov, 1998).

**Cortisol:**

Cortisol is a corticosteroid hormone, which is secreted from the adrenal cortex. The hormones of the pituitary adrenocortical axis are involved in the regulation of functions of the central nervous system (Holsboerm, 1989). They not only coordinate the neuroendocrine processes to stress itself, but also affect psycho-physiological processes. In 1936 Selye observed that diverse noxious agents cause an enlargement of the adrenal cortex as a consequence of the "stress syndrome". Yates and Maran (1974) reported that a variety of stressful events cause a release of ACTH from the anterior pituitary. The secreted ACTH stimulates the synthesis of corticosteroids in the adrenal cortex. The elevated corticosteroid levels in plasma inhibit the further release of ACTH from the pituitary. In a series of elegant experiments, Harris (1948) demonstrated that the release of ACTH from the pituitary is regulated by a corticotropin-releasing factor (CRF) from the hypothalamus. The CRF synthesized in the hypothalamus reaches the pituitary by a private portal blood supply. It then stimulates the secretion of ACTH from the pituitary. CRF is a 41 amino acid peptide (Vale et al., 1981), and was thought to be the major, if not the sole means, of releasing ACTH from the pituitary. ACTH can also be released and regulated by catecholamines and other hormones (Axelrod and Reisine, 1984).

There have been a number of investigations using cortisol to assess the reaction of the pituitary-adrenocortical axis under various
conditions. Lundberg and Frankenhauser (1980) found increased cortisol levels in situations which were accompanied by boredom, impatience and tiredness (vigilence task). In situations characterized by a high controllability and predictability (self-placed RT task), Lehmann et al. (1992) reported an adrenocortical suppression. Furthermore, there is increasing evidence that cortisol modulates brain function in humans. This principal endogenous glucocorticoid in humans increases slow-wave sleep and decreases rapid-eye-movement sleep (Born et al., 1991). There is some evidence that heart rate changes are accompanied by cortisol changes dependent on personality. Furthermore, an increasing heart rate is related to increasing difficulty of a task (Eason and Dudley, 1970; Carrol et al., 1986).

**Transaminases (Aminotransferases):**

The transaminases constitute a group of enzyme that catalyzes the interconversion of amino acid and α-ketoacid by transfer of amino group. The α-ketoacid glutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino transfer reactions; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group.

Aspartate transaminase (GOT: EC 2.6.1.1) has been isolated from the thermophilic microorganism *Bacillus isothermophilus* (Bartsch Klaus et al., 1996). In viral hepatitis and other forms of liver diseases associated with hepatic necrosis, serum GOT and GPT (EC 2.6.1.2) are found elevated even before the clinical signs and symptoms of disease appear (e.g. jaundice). Five to ten fold elevations of the two enzymes occur in patients with primary or
metastatic carcinomas of liver, with GOT usually being higher than GPT (Vinitha et al., 1995). Rapid increase in the activities of the two enzymes in serum have been reported during restraint stress (Sun et al., 1995) and a slight or moderate elevations of both SGOT and SGPT activities may be observed after intake of alcohol and after administration of a variety of drugs (e.g. ampicillin) (Norbert, 1996).

**Creatine Kinase (CK: EC 2.7.3.2)**

This enzyme, also referred to as ATP-creatine N-phospho- transferase, catalyzes the reversible reaction:

\[
\text{Creatine} + \text{Creatine phosphate} \rightleftharpoons \text{H}_2\text{N—C—N—CH}_2—\text{COO} + \text{ATP} \rightleftharpoons \text{O—P—N—C—N—CH}_2—\text{COO} + \text{ADP}
\]

The concentration is very high in skeletal muscle and myocardium, appreciable amounts are found in the brain, tiny amounts are found in a few other organs, while none is found in the liver. Many studies have shown that CK values are high in patients with myocardial infarction, progressive muscular dystrophy and alcoholic myopathy, but normal in patients with hepatitis and other forms of liver disease. Isoenzymes of CK were detected by Roberts (Roberts, 1979). The physiochemical properties of CK found in extracts of the human heart, brain, and skeletal muscle differ. Enzyme in the brain (CK₁ or BB) moves most rapidly toward the anode; that in skeletal muscle (CK₃ or MM) moves most slowly. Myocardial extracts consist mainly of the MM and the MB (CK₂) isoenzymes.
The CK\(_1\) (BB) fraction of CK has been found to be present in significant concentration in the serum and pleural fluid of patients with carcinoma of the lungs and prostate (Petterson et al., 1981).

The users of Khat are exposed to a variety of other harmful substances such as tobacco, alcohol and tranquilizers (Macigo et al., 1995; Workneh, 1983; Zein, 1988). In the absence of systematic study on the exclusive effects of Khat in humans, the studies on clinical disorders conducted on abusers of Khat appears to be insufficient to present a correct estimate of Khat alone. The toxicity of Khat and its phytoconstituents still need thorough investigation, which will help to introduce effective control and relieve several millions of people from this pernicious habit. It would prevent deterioration of social and economical conditions in the concerned countries. Khat chewing is found to have a subjective effect on human beings (Kalix, 1987); also large number of medical problems has been reported in Khat Chewers (Kalix, 1990). Free radicals and free radical reaction have been observed in the etiology and development of a number of diseases, especially life limiting (Pryor, 1987). Even though, various studies have been carried out on the pharmacological actions of khat but the effect of khat or its constituents on stress induced changes in oxidant and pro-oxidant status in rats has not yet been worked out in details.

Repeated immobilization stress is a well-defined method for the production of chronic stress (Kvetnansky et al., 1970). It has also been shown to bring about antioxidant defense changes in the plasma of rats (Liu et al., 1994). Thus, immobilization stress was chosen in the present study as it combines emotional stress (escape reaction) and physical stress (muscle work), resulting in both restricted mobility and aggression (Kvetnansky and Mikulaj, 1970).
In light of above lacunae, the present study was undertaken to see the effect of khat and its main constituents, flavonoids/alkaloids alone and in the presence of stress (both Pre and Post exposure) in terms of alteration of circulating free radical metabolizing/scavenging enzymes like SOD, GST and catalase and the levels of uric acid, glucose, MDA, reduced glutathione, SGPT, SGOT, creatine kinase and cortisol on rats.

For analyzing the effect of khat on biomolecules under in vitro conditions, BSA, calf thymus DNA and plasmid DNA pBR322 were selected. Moreover, the effects of various free radical scavengers like sodium azide, potassium iodide, thiourea and sodium formate were also studied on the khat induced degradation of the above-mentioned protein and nucleic acids.