Generalized anxiety disorder (GAD) is a prevalent and impairing disorder, associated with extensive psychiatric and medical comorbidity. The current pharmacotherapies of anxiety are based on antidepressants and benzodiazepines. However, these drugs have limitations like slow onset of action, development of drug dependence, tolerance, amnesia and sedation. Therefore, the use of alternative therapies has increased substantially over the last decade. Kava is an intoxicating beverage made from the root extract of Piper methysticum and has been known as anxiolytic drug. The German health authorities banned kava extract containing products based on the suspicion of a potential liver toxicity. However, the risk-to-benefit ratio of kava extracts remains good in comparison to that of other anxiolytic drugs. To specialists in phytotherapy, this book provide detailed study to investigate the role played by amino acid neurotransmitters and acetylcholinesterase, in different rat brain areas, in the anxiolytic action of kava and the withdrawal effect of kava on them. Another aspect of this study is to demonstrate the effect of kava on some liver and kidney function parameters.

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Herbal Treatment of Anxiety

Anxiolytic action of Kava extract: implication of acetylcholine and amino acid neurotransmitters
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1. INTRODUCTION

1.1. Anxiety

Generalized anxiety disorder (GAD) is a prevalent and impairing disorder, associated with extensive psychiatric and medical comorbidity (Hidalgo et al., 2007). GAD increases the risk for subsequent depressive episodes and self-medicating with alcohol (Allgulander, 2012). Moreover, morbid anxiety influences the course of several somatic diseases, particularly neurological, cardiovascular, pulmonary, dermatological and endocrine disease (Allgulander, 2010).

According to a well-designed population-based prospective study in Norway (Engum, 2007), the risk of
counteracting type 2 diabetes was increased by base line anxiety or depression, even when adjusting for other well-known diabetic risk factors. In addition, several studies have indicated that anxiety is an independent risk factor for cardiac events. For example, Martens et al. (2010) have shown that significant more cardiac events occurred within an eight-year period in patients with coronary heart disease, in those who were anxious at base line compared to those were not. Moreover, chronic neuropathic pain, affecting a large portion of elderly people, is strongly associated with depression and anxiety. In a German population-based study, Beesdo et al. (2009) have indicated that chronic pain often preceded a diagnosis of GAD. Furthermore, Romera et al. (2010) have shown that painful physical symptoms frequently accompanied GAD in primary care. Sleep is of fundamental restorative importance to maintain health in most species, and disrupted sleep is one of the early indicators of relapse or exacerbation of
affective and psychotic disorders (Allgulander, 2012). Monti & Monti (2000) have indicated that at least every other GAD patient sleeps poorly, and has reduced sleep quality, reduced total sleep time and less time in sleep stages 3 and 4.

Generally, the risks of not treating anxiety, particularly risk of cardiovascular consequences, diabetes II, secondary depressive episodes and self-medication with alcohol, outweigh the risks of serious drug adverse effects (Allgulander, 2012).

1.2. Implication of amino acid neurotransmitters in anxiety

Amino acid neurotransmitters are schematically divided into 2 classes according to their functions: (1) excitatory amino acids (EAAs) such as glutamate and aspartate (Watkins & Evans, 1981; Fonnum, 1984) on the one hand and (2) inhibitory amino acids such as Y-aminobutyric acid (GABA), glycine and taurine on the
other hand (Hussein et al., 1977; Oja et al., 1977; Huxtable, 1989).

Glutamate is the principal excitatory neurotransmitter in the brain. Glutamate neurotransmission is crucial in information intake and information processing within the brain (Lynch, 2004). Glutamate transmission is also indispensable for long-term potential (LTP) formation, the cellular correlate to memory formation (Vereker et al., 2000). The interactions of glutamate with specific membrane receptors are responsible for many neurologic functions, including cognition, memory, movement and sensation (Gasic & Hollmann, 1992). In addition, activation of glutamate receptors has been implicated in a number of neuronal processes including the generation of fast excitatory postsynaptic potentials (MacDermott & Dale, 1987), learning (Morris et al., 1986) and neurotoxicity (Choi, 1988). Moreover, it has been shown that L-glutamate and L-aspartate in the CNS are involved in
various cardiovascular regulations (Tsuda et al., 1996). It has also been suggested that glutamate plays a major widespread role in the control of neuroendocrine neurons (Van Den Pol et al., 1990). Also, glutamate has been implicated in the pathogenesis of neuronal death in a variety of neurodegenerative diseases (Engelsen, 1986; Greenamyre, 1986).

Glutamate can exert its actions through two different types of receptors: ligand-gated ion channel receptors (ionotropic) and G-protein-coupled metabotropic receptors. Ligand-gated ion channel receptors consist of N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs), and kainite receptors (KARs) (Kew & Kemp, 2005). This group mediates fast excitatory neurotransmission (Hirsch et al., 1997). On the other hand, metabotropic glutamate receptors mediate slow glutamate neurotransmission (Wierońska & Pilc, 2009; Krystal et al., 2010). Several
factors regulate the activity of ionotropic glutamate receptors. Activation of NMDAR complex needs two molecules of glutamate and two molecules of glycine, while AMPA/Kainate receptors are glycine-independent (Konradi & Heckers, 2003).

Several lines of evidence suggest that limbic glutamatergic neurotransmission plays a pivotal role in the pathogenesis of anxiety disorders (Hayashi et al., 2004; Garakani et al., 2006). Limbic and Para limbic regions (hippocampus, amygdala, orbitofrontal cortex, anterior cingulate cortex, medial prefrontal cortex and insula), which have been extensively linked to stress-response and anxiety-related disorders, are abundantly innervated by glutamatergic pyramidal cells. Glutamate pyramidal cells in the prefrontal cortex sent afferents to several limbic regions, particularly to hippocampus and amygdala (Myers et al., 2011). Glutamate not only exerts its effects through direct activation of glutamate neurotransmission but it also modulates the release of
other neurotransmitters involved in stress-response, such as serotonin (Becquet et al., 1993), dopamine (Jedema & Moghdam 1996), monoamines and GABA (Cortese & Phan, 2005).

GABA is the most important inhibitory neurotransmitter in the mammalian brain (Tunnicliff & Ngo, 1986; Santos et al., 1995). GABA can exert its action through two different types of receptors known as GABA$_A$ and GABA$_B$ receptors. GABA$_A$ receptors are ionotropic receptors mediating fast inhibitory neurotransmission in the CNS. The activity of these ionotropic receptors is modulated by a variety of clinically-used drugs, including benzodiazepines, barbiturates and general anesthetics (Rudolph & Möhler, 2004) as well as endogenously-produced neurosteroids (Hosie et al., 2007). GABA$_B$ receptors are metabotropic receptors that contribute to inhibition either postsynaptically by inducing hyperpolarization or presynaptically by modulating excitatory
neurotransmitter release (Enna 2007; Ulrich & Bettler, 2007; Ghose et al., 2010).

The GABA system plays a key role in regulating prefrontal cortex functions (Constantinidis et al., 2002). Alterations in the GABA system have been shown to underlie mental illnesses associated with prefrontal cortex dysfunction (Benes et al., 1996; Dean et al., 1999; Ohnuma et al., 1999; Lewis, 2000). Moreover, deficiencies in GABA function have been associated with such neurological disorders as Huntington’s chorea (Bird & Iversen, 1974), Parkinson’s disease (McGeer et al., 1971) and the epilepsies (Lloyd et al., 1984). Furthermore, excellent evidence exists linking the development of debilitating anxiety to the GABA system (Skolnick & Paul, 1981; Olsen, 1982; Williams, 1983).

Drugs-induced enhancement of GABA transmission (benzodiazepines, tiagabine, neurosteroids) is anxiolytic (Argyropoulos et al., 2000; Baldwin et al., 2005; Pollack et al., 2005; Durant et al., 2009; Rupprecht et al., 2009).
while a reduction of GABA transmission, as shown in man by administration of a benzodiazepine site partial inverse agonist (FG7142), precipitates anxiety (Horowski & Dorow, 2002).

Glycine is a major postsynaptic inhibitory transmitter in the spinal cord and brain stem (Aprison, 1990). In addition, glycine plays a modulatory role at NMDA receptors, one of the ionotropic receptors of excitatory amino acids in the fore-brain (Johnson & Ascher, 1987), thus serving both inhibitory and excitatory functions.

In the mammalian brain, taurine is one of the most abundant free amino acids, generally only exceeded in concentration by glutamate (Huxtable, 1989). The role of taurine as a putative neurotransmitter was reviewed by Kuriyama (1980). McBride & Frederickson (1979) proposed taurine as a possible inhibitory transmitter in the cerebellum. Taurine levels are increased during stress, hypoxia and energy deprivation (Milakofsky et
al., 1984; Böckelmann et al., 1998; Colivicchi et al., 1998) Regions that have extremely high taurine levels or are very sensitive to taurine manipulation include hippocampus (Galarreta et al., 1996), striatum (Lombardini, 1977) and corticostriatal projection (Sergeeva & Haas, 2001).

Extracellular taurine modifies the release of amino acid transmitter and modulates intracellular Ca$^{++}$ homeostasis (Foos & Wu, 2002). It has been shown that taurine affects the metabolism of transmitters such as GABA (Medina & De Robertis, 1984; Kontro & Oja, 1990; Michel & Richard, 1991; Liljequist, 1992) and serotonin (Sgaragli et al., 1981; Becquet et al., 1993).

Taurine deficiency was found to be associated with neurologic dysfunction (Sturman et al., 1985, 1986; Palackal et al., 1988). It can also be considered as a thermoregulator, a general stabilizer of excitable membranes or an endogenous antiepileptic agent (Oja & Kontro, 1983). In addition, taurine has an
antiexcitotoxic role in the mammalian brain (French et al., 1986; Trenkner, 1990). Moreover, it has been found that cell-damaging conditions induced massive taurine release in the hippocampus (Saransaari & Oja, 1996; 1997). Huxtable (1992) postulated that taurine’s actions may stabilize and conserve function in an unstable physiological system, a property termed enantiostasis. Furthermore, results of behavioral studies (Chen et al., 2004; Kong et al., 2006) suggest that taurine produces an anxiolytic-like effect in the animal models and may act as a modulator or anti-anxiety agent in the CNS.

1.3. Cholinergic System and Anxiety

Acetylcholine (ACh) is a fundamental neurotransmitter in the CNS, where it is critically involved in functions related to cognition and behavior, in some cases by modulating release of other neurotransmitters, including glutamate, GABA, norepinephrine and dopamine (Kellar, 2006). Recently, Klinkenberg et al. (2011) reviewed that ACh is still
considered as an important neurotransmitter involved in cognitive functions especially learning and memory. In addition, ACh is important in the control of locomotor activity (Day et al., 1990).

Evidence suggests that brain cholinergic neurotransmission is involved in modulation of anxiety-like behavior (Brioni et al., 1993; File et al., 2000; Newman et al., 2001). Therefore, drugs targeting neuronal nicotinic acetylcholine receptor (nAChR) could have potential for the treatment of anxiety in humans (Brioni et al., 1993; Turner et al., 2010). Recent study of Roni & Rahman (2011) demonstrated that lobeline, a nAChR antagonist has anxiolytic effects in mice. They concluded that this finding provides further evidence that nAChR ligands could have potential for the treatment of anxiety.

ACh is known to be rapidly hydrolyzed by acetylcholinesterase (AChE). The duration of action of ACh at the synaptic clefts is critically dependent on AChE
activity (Cooper et al., 2003). The response of central cholinergic neurotransmission to the chronic administration of some psychotropic drugs to rats was investigated using brain AChE activity as a neurochemical marker for cholinergic neurons (Bekpinar et al., 1994).

1.4. Synthetic Anxiolytic Drugs

Benzodiazepines (ex.: diazepam, alprazolam, midazolam and others) are well established anxiolytic drugs. Benzodiazepines, like other anesthetics, act via the postsynaptic-aminobutyric acid type A [GABA_A] receptor to potentiate the action of the major inhibitory neurotransmitter GABA (Yamamoto et al., 2007).
Chemical structure of some benzodiazepine drugs

Unfortunately, it has been shown that the use of benzodiazepines for reducing anxiety is accompanied with several side effects. Benzodiazepines have been shown to reduce long-term potentiation which could be a cellular basis for their amnesic action (Evans & Viola-McCabe, 1996). Beside the memory impairment side
effect, benzodiazepines are known to carry risks of dependence, withdrawal and cognitive effects (Stewart, 2005). Alprazolam, a benzodiazepine, has a high addictive/misuse potential (Michopoulos et al., 2006).

Therefore, the use of alternative therapies has increased substantially over the last decade, particularly for more chronic conditions such as anxiety (Conner et al., 2001).

1.5. Kava kava (*Piper methysticum*)

Kava is an intoxicating beverage made from the root extract of *Piper methysticum* that is native to the Pacific Islands where it has been used in ceremonial settings for millennia (Singh, 1992).
Kava leaves
In the late 1990s, commercial kava products gained immense popularity in Europe and North America as an effective treatment option for anxiety (Singh & Singh, 2002; O’Sullivan & Lum, 2004). Hence, kava has been
known as anxiolytic drug (Garrett et al., 2003; Pittler & Ernst, 2003; Shinomiya et al., 2005). In addition, it has been reported that the anxiolytic effects of kava seem to be as powerful as those of conventional anxiolytics (Lindenberg & Pitule-Schödel, 1990; Woelk et al., 1993; Boerner et al., 2003). Moreover, while synthetic anxiolytic drugs generally promote lethargy and mental impairment, kava has been shown to improve concentration, memory and reaction time for people suffering from anxiety (Russell et al., 1987; Johnson et al., 1991; Münte et al., 1993; Gessner et al., 1994; Heinze et al., 1994).
Kava extract in the form of drug

According to the phytotherapeutic definition, the total extract of the rhizome of *Piper methysticum* (*Piperaceae*) is regarded as the active ingredient. The
active constituents of rhizome consist of a group of structurally related lipophilic lactone derivatives with an arylethylene-alpha-pyrone skeleton. They are typically 4-methoxy-2-pyrones with phenyl or styryl substituents at the 6-position and represent 3 – 20% of the dried rhizome depending on age of the plant and specific cultivar (Shulgin, 1973). The commercial extracts (mainly of them acetone extracts containing 30 - 70% active principles) consist of a mixture of more than 18 different \( \alpha \)-pyrones, collectively known as kava pyrons, or better, kava lactones (Bilia et al., 2002).

Eighteen kava pyrones have been isolated (He et al., 1997), six of which constitute the major and pharmacologically important components: kavaine, dihydrokavaine, methysticin, dihydromethysticin, yangonin and desmethoxyyangonin. The amount of the single pyrones varies according to the origin of the drug. Chemically, the six major kava pyrones belong to two slightly different categories: kavaine, dihydrokavain,
methysticin and dihydromethysticin have only one
double bond in the pyrone ring and are therefore called
enolids, yangonin and desmethoxyyangonin have two
double bond in the pyrone ring, which makes them
dienolids. The slightly different chemical structure leads
to minor differences in pharmacodynamical effect of
kavain, dihydrokavaine, methysticin and
dihydromethysticin on the one hand, and yangonin and
desmethoxyyangonin on the other (Hänsel & Lazar,
1985; Duffield & Lidgard, 1986; Duffield et al., 1986;
Singh, 1992; Hänsel & Woelk, 1994; Hänsel et al., 1994;
Boonen et al., 1997; Häberlein et al., 1997; Lechtenberg
et al., 1999).
Kava lactones pass the blood-brain barrier and behavioral effects occur at micromolar concentrations (Keledjian et al., 1988; Lindenberg & Pitule-Schödel, 1990). Several reports indicate that kava pyrones have many psychotropic properties such as anxiolytic (Cairney
et al., 2002; Garrett et al., 2003), central muscle relaxant (Meyer & Kretzschmar, 1966; Meyer, 1979), analgesic (Jamieson & Duffield, 1990), sedative (Capasso & Calignano, 1988), anticonvulsant (Gleitz et al., 1996b), anaesthetic (Cairney et al., 2002) and neuroprotection properties (Backhauss & Krieglstein, 1992). Kava extract can also be used for the treatment of insomnia (Shinomiya et al., 2005). Other randomized controlled trials suggest that kava reduces anxiety in premenopausal women (Cagnacci et al., 2003), facilitates cognitive function and increase positive affectivity (Thompson et al., 2004) and improves sleep quality (Emser & Bartylla, 1991). It is interesting to know that kava also had great religious significance and was seen to connect the user with the ancestors and the gods (Lebot et al., 1992; Singh, 1992; Cambi & Ash, 1994).
1.6. **Mechanism of Psychotropic Action of Kava Extract**

At low dosages, kava pyrones are mainly characterized by their psychotropic action resembling that of benzodiazepines although no binding of kava pyrones to GABA and benzodiazepine receptors were detected *(Davies et al., 1992)*. In contrast to these observations, *Jussofie et al. (1994)* found that the sedative effects of kava pyrones may be mediated by an increase of GABA\textsubscript{A} receptor binding sites *in vitro* in the hippocampus and other brain regions. In addition, *Baum et al. (1998)* stated that the benzodiazepine receptors or GABAergic neurons may be the site of action of kava. *Dinh et al. (2001)* reported that GABA\textsubscript{A}, dopamine D2, opioid (mu and delta) and histamine (H1 and H2) receptors might be involved in the pharmacological action of kava extracts. On the other hand, the anticonvulsant effects of kavain (one of the kava pyrones) seem to be due to the inhibition of sodium and
calcium channels as well as effects on glutamate systems (Gleitz et al., 1996b).

1.7. Side Effects of Kava Extract

In 2002, the German health authorities banned kava extract containing products based on the suspicion of a potential liver toxicity, as derived from adverse effect reports (Schmidt et al., 2002). Further in vitro and animal studies have confirmed that water fractions of kava are less cytotoxic than organic solvent fractions (Jhoo et al., 2006; Sorrentino et al., 2006). Modern commercial products rely on alcohol or acetone extraction, a process which may extract toxic compound such as alkaloids from the plant. Therefore, some experts believe that the extraction method is the key for understanding the liver damage observed with modern kava preparations. Additionally, the traditional aqueous extract is made from the rhizome and contains glutathione, which offers protection against hepatotoxicity (Whitton et al., 2003). On the other
hand, common commercial kava extracts are made from leaves and stems as well, which contain additional alkaloids which have been associated with toxic effects (Dragull et al., 2003; Nerurkar et al., 2004). However, other data suggest that kava does lead to an increase in liver enzymes even if kava is consumed as an aqueous extract (Clough et al., 2003).

Cytochrome $P_{450}$ enzymes are responsible for metabolizing a majority of the therapeutic drugs. Kava has the potential for causing drug interactions through inhibition of the cytochrome $P_{450}$ enzymes (Mathews et al., 2002; Anke & Ramzan, 2004; Ma et al., 2004). Such interaction could either generate toxic metabolites or increase the toxicity of concomitantly administered drugs (Ernst, 2007).

Studies suggest that glutathione (GSH) is important in phase II metabolism and detoxification of kava lactones and that excess of kava lactones may rapidly
deplete intracellular GSH resulting in hepatic damage due to oxidative stress (Denham et al., 2002).  

Collectively, three possible mechanisms for kava pyrone-associated hepatotoxicity are known: inhibition of cytochrome $P_{450}$ enzymes, reduction in liver glutathione content and more remotely, inhibition of cyclooxygenase enzyme activity (Clouatre, 2004).

1.8. Experimental Studies and Case Reports

A toxicological study has been carried out in rats and dogs for the detection of chronic toxicity on application of high doses of a combination product with synthetical $D$, $L$-kavain (Hapke et al., 1971). The authors found no signs of toxicity of any kind on application of up to 400 mg/kg over 91 days, corresponding to 40 times the recommended human therapeutic dose. When the regimen was changed (after 8 weeks) to 1000 mg/kg in the group with the highest dosage (400 mg/kg), a rise
on aspartate aminotransferase (AST) activities occurred, which, however, was not mirrored in histological changes in the liver even on continuation of this extremely high dosage for a further 5 weeks. The same non-toxicity was found in mongrel dogs administered up to 200 mg/kg per day of the test substance over 3 months. There was no change in liver metabolism, histology and function tests such as alkaline phosphatase, alanine aminotransferase (ALT) and AST with the exception of one isolated case of liver damage in the highest dosage scheme (multicentric necrosis of liver parenchyma), which, according to the authors, might have been unrelated to the trial. In all events, the dosage scheme of 100 mg/kg, corresponding to 10 times the recommended therapeutic dose, was shown to be perfectly safe (Hapke et al., 1971).

Connor et al. (2001) assessed safety parameters for kava include occurrence of adverse events, withdrawal symptoms, effect on heart rate, blood pressure,
laboratory assessments and sexual function. They found no differences between kava and placebo on any of the parameters evaluated. The data support the safety of kava in treating anxiety at 280 mg kava lactones/day for 4 weeks.

In vivo study of Singh & Devkota (2003) demonstrated that the aqueous kava extracts administered to rats at a daily dose of 200 or 500 mg kavalactones/kg for 2 or 4 weeks did not affect AST, ALT, alkaline phosphatase and lactate dehydrogenase in the sera, nor malondialdehyde in the liver homogenate. Moreover, in some cases they were significantly reduced, suggesting not only a lack of toxicity but potentially a hepatoprotective effect of kava (Singh & Devkota, 2003). In addition, two drug monitoring studies had not found a single case of kava induced hepatotoxicity (Teschke et al., 2003).
Sorrentino et al. (2006) tested a commercial kava extract as used in German registered pharmaceutical preparations (Kava-Sedon, Herras Pharma, Germany) on male and female wistar rats. The dosage range and study duration applied in this study are relevant for long-term human use, and represent dosage schemes by far exceeding the normal human application. However, the results of this study do not allow the conclusion of any toxicity of kava, particularly to the liver (Sorrentino et al., 2006).

Lim et al. (2007) failed to observe any effect on serum AST and ALT activities in rats treated with pipermethystine (10 mg/kg) (abundant in leaves and stem peelings) or acetone-water extract of kava rhizome [100 mg/kg (equivalent to 63 mg total kava lactones/kg/day)] for 2 weeks. Nevertheless, serum ALT activities in kava rhizome groups were lower than in control rats.
On the other hand, the results of *in vitro* study of **Lüde et al. (2008)** indicate that kava extracts are toxic to liver mitochondria, leading to inhibition of the respiratory chain, increased reactive oxygen species (ROS) production, a decrease in the mitochondrial membrane potential and eventually to apoptosis of exposed cells.

**Strahl et al. (1998)** have reported a case of acute necrotizing hepatitis which is associated with kava consumption at standard doses. After the discontinuation of kava the liver tests quickly become normal.

Liver biopsy showed hepatocellular necrosis consistent with chemical hepatitis in a case with liver failure with a history of taking kava-containing product for 4 months (**Humberston et al., 2003**).

As indicated from the above literature, the studies that carried out to investigate the effect of kava, in
treating anxiety, on liver functions have not produced univocal results. It has been reported that the risk-to-benefit ratio of kava extracts, nevertheless, remains good in comparison with that of other drugs used to treat anxiety (Clouatre, 2004).

This study aims to investigate the effect of the daily oral administration of kava extract (75 mg/kg) for 1, 2 and 4 weeks on the concentrations of some amino acid neurotransmitters (glutamic acid, aspartic acid, GABA, glycine, taurine and glutamine) in the hippocampus, striatum and hypothalamus of adult male albino rats with an attempt to clarify the role played by these neurotransmitters in the anxiolytic action of kava as well as the withdrawal effect of kava on them. In addition, the study was extended to investigate the response of central cholinergic neurotransmission to kava treatment as well as kava withdrawal by measuring acetylcholinesterase (AChE) activity in the rat cortex, hippocampus and striatum as a neurochemical marker.
for cholinergic transmission. Studies that carried out to investigate the effect of kava, in treating anxiety, on liver functions produced discrepancy results. Therefore, another aspect of the present study is to demonstrate the effect of kava administration for 4 weeks on some liver and kidney function parameters in the sera of adult male rats.
2. MATERIALS AND METHODS

2.1. Animals

The experimental animal used in this study was the adult male albino rat (*Rattus norvegicus*). Animals used for determination of amino acid neurotransmitters and AChE activity weighing 100-160 g and those used for determination of biochemical parameters weighing 180-240 g. The animals were obtained from a fixed local supplier. They were maintained on stock diet and kept under fixed appropriate conditions of housing and handling.

2.2. Drug

Highly purified Kava (*Piper methysticum*) extract was purchased from October Pharma Co., Egypt. It was dissolved in saline solution to make a suspension and administered to the animals orally by using a gastric
tube. The whole extract was used to resemble that used by human traditionally or medically.

2.3. Experimental design

The animals were divided into 2 main groups. The 1st main group of animals was served for determination of amino acid neurotransmitter concentrations as well as AChE activity. Animals of this group were subdivided into 3 subgroups. Rats of the 1st subgroup were administered a daily oral dose of kava extract (75 mg / kg body weight, (Sorrentino et al., 2006) for 1, 2 and 4 weeks. The rats of the 2nd subgroup were served to study the withdrawal effect of kava. The animals of this subgroup were administered kava extract for 4 weeks then the drug administration was stopped for 1 week. The animals of the 3rd subgroup were administered saline solution at each of the tested time intervals which were served as controls. The 2nd main group of animals was used for determination of the biochemical parameters. Rats of this group were subdivided into 2
subgroups. Animals of the 1st subgroup were administered daily dose of kava extract (75 mg / kg) for 4 weeks and animals of the 2nd subgroup were administered saline solution for 4 weeks and were served as controls.

2.4. Handling of tissue samples

The animals used for the determination of amino acid neurotransmitter concentrations and AChE activity were killed by sudden decapitation after being fasted overnight. The brain of each animal was quickly removed and rapidly transferred to an ice cold Petri dish and dissected to obtain the right hippocampus, striatum and hypothalamus for the determination of amino acid neurotransmitter concentrations and left cortex, hippocampus and striatum for the determination of AChE activity (Zeman & Innes, 1963; Glowinski & Iversen, 1966). Each brain area was weighed and frozen until analyzed.
2.5. Determination of amino acid neurotransmitter concentrations

The amino acid concentrations of the brain areas were determined using the HPLC method employed by Márquez et al. (1986) with minor modifications (for details see Radwan et al., 2007).

2.5.1. Tissue preparation:

The brain area was homogenized in 2 ml ethyl alcohol (75%). Two other ml were used to rinse the homogenizer (Heidolph DIAX 900, Germany). The precipitated protein was removed by centrifugation at 12000 r.p.m. (21.036g) for 30 minutes at 4°C using a high speed cooling centrifuge (Type 3K-30, Sigma, Germany). The clear supernatant was evaporated to dryness and stored in a deep freezer.
2.5.2. **Dansylation reaction:**

Dansyl derivatization was carried out according to the method of Tapuhi *et al.* (1981). Dried samples of hippocampus, striatum and hypothalamus were dissolved in 0.5 ml lithium carbonate solution (40 mmole adjusted to pH 9.5 with conc. HCl) containing 2-aminobutyric acid (0.08 mg/ml) as an internal standard and then treated with 0.5 ml of Dns-Cl in acetonitrile (3 mg/ml). After stirring, the mixture was placed in an incubator at 38°C for 1 h. The samples were then removed from the incubator, transferred to an Eppendorf tube and centrifuged at 2817 g for 15min to remove the particulate matter. The supernatant was filtered through a Hamilton syringe provided with a diameter 13 mm (pore size 0.4 μm) disposable syringe filter. 10 μl of each sample were finally injected into the HPLC system.
A standard amino acid mixture (containing glutamic acid, glutamine, aspartic acid, GABA, glycine and taurine) was prepared by dissolving a known weight of each amino acid in lithium carbonate solution containing 2-aminobutyric acid to obtain a concentration of 0.01 µmole for each amino acid. This mixture was then dansylated as sample, shaken and placed in an incubator at 38°C for 1 h. After incubation, the mixture was filtered through a Hamilton syringe provided with a diameter 13 mm (pore size 0.4 µm) disposable syringe filter. 10 µL of the mixture were then injected into the HPLC system. This step was repeated 3 times and the average height ratio of each amino acid (height of amino acid / height of internal standard) was used for calculation. Amino acid concentrations were then determined by the internal standard method of quantitation.
2.5.3. Chromatography:

The HPLC system used to resolve and quantify the samples consisted of:

(i) Wellchrom Mini-star K-501 pump (Knauer, Germany) having a double piston technique which enables a high consistency in the flow rate together with a very low pulsation. The HPLC pump K-501 is provided with the new inert pump heads with ceramic inlays.

(ii) Column thermostat 5-85°C with injector equipped with a 20 µL loop (Knauer, Germany).

(iii) A luna 5 u C-18 reversed phase column (5 µm particle size, 150x4.6 mm I.D.) from phenomenex, USA.

(iv) Wellchrom spectrophotometer K-2600 with variable wavelength (Knauer, Germany) capable
of measuring up to four wavelengths simultaneously with an accuracy of ±1nm in 1nm steps. The highly sensitive detector features are extremely low noise level (≤1x10-5 AU) and baseline drift (≤1x10-5 AU/h).

(v) A chromatography workstation (Eurochrom 2000).

The mobile phase consisted of 50/50 (V/V), methanol/water containing 0.6 % glacial acetic acid and 0.008 % triethylamine. The mobile phase was degassed through an inline filter degasser supplied with a diameter 47 mm (pore size 0.2 μm) nylon membrane filter (phenomenex, USA) and operating by an oil less vacuum pump (KN.18, Germany). The flow rate of mobile phase was 1 ml/min and the wavelength was 254 nm.
2.5.4. **Data presentation:**

Eurochrom 2000 software was used to calculate height ratio of each amino acid in sample or standard (height of amino acid/ height of internal standard). Amino acid concentrations were determined by the internal standard method of quantitation. The concentrations of the amino acids studied in the different brain areas were expressed as µmole/g fresh tissue and mean ± S.E.M.

The equilibrium between the inhibitory amino acid (GABA, IAA) and excitatory amino acids (glutamic and aspartic acids, EAAs) was expressed as an equilibrium ratio % (ER %).

\[ \text{ER} \% = \frac{\text{IAA (GABA) concentration}}{\sum \text{EAAs (glutamic acid and aspartic acid) concentrations}} \times 100. \]
2.6. Determination of AChE activity

AChE activity was measured, (Simpson et al., 1964) using acetylcholine bromide as the enzyme substrate. AChBr and hydroxylamine were from Sigma Co., and all other chemicals were of high quality and purchased from commercial suppliers. Each brain area was homogenized in 1 ml of 0.1 M phosphate buffer (pH 7.00) by using a small chilled glass Teflon tissue grinder. Homogenates were centrifuged at 10000 r.p.m. for 15 min. at 5°C in a refrigerated centrifuge (GS-6r, Beckman, USA). The deposits were discarded and the supernatant used for enzyme activity determination which carried out in 3-4 replicates, and the optical densities were measured against blank at 540 nm, using a spectrophotometer (Spectronic 1201, Milton Roy Co., USA). The results were calculated by constructing a standard curve and the enzyme activity was expressed as μmoles AChBr hydrolyzed/min./gm tissue.
2.7. Determination of biochemical parameters

Animals served for biochemical analysis were euthanized and blood samples were collected in tubes and centrifuged at 3000 r.p.m. for 10 min. to obtain clear sera. Aminotransferase enzyme, AST and ALT activities; (Breuer, 1996) alkaline phosphatase, ALP activity; (Moss, 1982) total protein; (Young, 1995) albumin; (Doumas et al., 1971) urea; (Tabacco et al., 1979) and creatinine (Glick et al., 1986) were determined by using reagent kits.

2.8. statistical analysis

Comparison between control and treated animals and the levels of significance were determined by using Student’s t-test using Statgraphics version 2.6, copyright 1985 (Daniel, 2000). Percentage difference representing the percent of variation in concentration with respect to the control was calculated.
\[
\% \text{ difference} = \frac{(\text{treated mean} - \text{control mean})}{\text{control mean}} \times 100.
\]
3. RESULTS

3.1. Effect of kava on amino acid neurotransmitter concentrations

The effect of the daily oral administration of kava extract (75 mg/kg) on the concentrations of amino acids [glutamic acid, aspartic acid, glutamine, GABA, glycine and taurine (μmole/g fresh tissue)] in the hippocampus of adult male albino rats are presented in Tables 1-6.

Kava administration evoked early significant and highly significant decreases in hippocampal aspartate and glycine levels after 1 week, being -16.56% and -16.75%, respectively. Glutamic acid and its amide glutamine showed highly significant decreases after 2 weeks of kava administration, recording -11.42% and -13.13%, respectively. However, GABA and taurine levels showed significant and highly significant increases after 4 weeks (+8.75%) and 1 week (+9.98%) of drug administration, respectively. One week after stopping
kava administration, a highly significant increase in glutamine and GABA levels was observed, being +13.13% and +13.88% above the control levels, respectively.

The effect of the daily oral administration of kava extract (75 mg/kg) on the concentrations of amino acids [glutamic acid, aspartic acid, glutamine, GABA, glycine and taurine (μmole/g fresh tissue)] in the striatum of adult male albino rats are presented in Tables 7-12.

In the striatum, kava administration resulted in a significant and highly significant decreases in glutamic acid and GABA levels after 1 week, recording -9.09% and -6.88%, respectively below the control levels. This decrease was also observed after 4 weeks of kava administration in case of aspartic acid, being highly significantly (-24.74%) below the control level. However, a significant increase was noticed in glycine
concentration after 1 week, being +8.49% above the control level. General nonsignificant changes were observed in the striatal amino acid levels on stopping kava administration.

The effect of the daily oral administration of kava extract (75 mg/kg) on the concentrations of amino acids [glutamic acid, aspartic acid, glutamine, GABA, glycine and taurine (µmole/g fresh tissue)] in the hypothalamus of adult male albino rats are presented in Tables 13-18.

In the hypothalamus, the daily oral administration of kava for one week induced highly significant increases in the levels of glutamic acid (+13.36%), aspartic acid (+19.12%), glutamine (+79.62%), and glycine (+17.68%) but significant increase in GABA (+9.04%). The highly significant increases in glutamine and glycine concentrations continued after 2 weeks of kava administration, being +46.04% and +42.07% above the control levels, respectively. Taurine levels showed a highly significant decrease after 4 weeks of daily kava
administration, recording -8.95% below the control level. One week after stopping kava administration, GABA level showed a significant increase (+11.78%) as compared with the control level.

Table 19 shows the effect of kava administration on the equilibrium ratio percent (ER%) between the concentrations of the inhibitory amino acid (GABA) and the excitatory amino acids (glutamic acid and aspartic acid).

Slight changes were observed in the ER% throughout the experimental periods in the 3 brain areas. However, marked increases in the ER% were recorded in the three brain areas after 1 week of stopping kava administration, being +10.10% in the hippocampus, +13.75% in the striatum and +15.26 in the hypothalamus.
3.2. Effect of Kava on AChE activity

The effect of daily oral administration of kava extract on AChE activity in the cortex, hippocampus and striatum of adult male rats are demonstrated in Tables 20, 21 and 22, respectively.

In the cortex, kava administration induced significant decreases (P<0.05) in AChE activity after 2 and 4 weeks of treatment. However, hippocampal AChE activity showed a significant increase after 2 weeks followed by significant decrease after 4 weeks of kava administration. In the striatum, AChE activity showed early significant increase after 1 week and delayed significant decrease after 4 weeks of treatment. However, no significant changes were observed in the enzyme activity on stopping kava administration.
3.3. **Effect of Kava on biochemical parameters**

Data concerning the effect of daily kava administration for 4 weeks on some serum biochemical parameters of adult male albino rats are shown in Table 23. Serum AST and ALT activities showed significant and highly significant (P<0.01) decreases after 4 weeks of daily administration of kava extract, being -13.17 and -23.63% below the control level, respectively. However, serum ALP activity showed no significant change due to kava administration. Serum total protein and urea levels increased significantly after 4 weeks of drug treatment, whereas, serum creatinine showed highly significant decrease. Serum albumin level showed a nearly control like value.
Table (1): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glutamic acid (µmol/g fresh tissue) in the hippocampus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>1 week</td>
<td>7.97±0.17 (8)</td>
<td>8.42±0.11 (5)</td>
<td>n.s.</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>7.06±0.18 (6)</td>
<td>**</td>
<td>-11.42</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>8.14±0.21 (5)</td>
<td>n.s.</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>During withdrawal</td>
<td></td>
<td>7.91±0.13 (6)</td>
<td>n.s.</td>
<td>-0.75</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p > 0.05 nonsignificant.

**: p < 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (2): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of aspartic acid (μmol/g fresh tissue) in the hippocampus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated value</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1 week</td>
<td>4.59±0.29 (6)</td>
<td>3.83±0.12 (6)</td>
<td>*</td>
<td>-16.56</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>4.74±0.20 (6)</td>
<td>n.s.</td>
<td>3.27</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>4.62±0.08 (5)</td>
<td>n.s.</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>5.08±0.20 (5)</td>
<td>n.s.</td>
<td>10.68</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

* : p< 0.05 significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (3): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glutamine (μmol/g fresh tissue) in the hippocampus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>1 week</td>
<td>2.59±0.04</td>
<td>2.71±0.11</td>
<td>n.s.</td>
<td>4.63</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>2.25±0.08</td>
<td>**</td>
<td>-13.13</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>2.66±0.08</td>
<td>2.66±0.08</td>
<td>n.s.</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>During withdrawal</td>
<td></td>
<td>2.93±0.07</td>
<td>**</td>
<td>13.13</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

** : p< 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (4): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of GABA (μmol/g fresh tissue) in the hippocampus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>1 week</td>
<td></td>
<td>5.09±0.17</td>
<td>n.s.</td>
<td>-3.23</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>5.09±0.11</td>
<td>n.s.</td>
<td>-3.23</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>5.26±0.17</td>
<td>5.72±0.10</td>
<td>*</td>
<td>8.75</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>5.99±0.09</td>
<td>**</td>
<td>13.88</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p > 0.05 nonsignificant.

* : p < 0.05 significant versus saline control values.

** : p < 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (5): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glycine (μmol/g fresh tissue) in the hippocampus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>1 week</td>
<td></td>
<td>1.59±0.05 (6)</td>
<td>**</td>
<td>-16.75</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>1.91±0.06 (8)</td>
<td>1.91±0.11 (6)</td>
<td>n.s.</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>1.92±0.02 (6)</td>
<td>n.s.</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>1.79±0.04 (6)</td>
<td>n.s</td>
<td>-6.28</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

** : p< 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (6): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of taurine (μmol/g fresh tissue) in the hippocampus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated value</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine</td>
<td>1 week</td>
<td>5.81±0.06</td>
<td>6.39±0.11</td>
<td>**</td>
<td>9.98</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>5.70±0.19</td>
<td>n.s.</td>
<td>-1.89</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>5.54±0.11</td>
<td>n.s.</td>
<td>-4.65</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>5.62±0.08</td>
<td>n.s.</td>
<td>-3.27</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

** : p< 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (7): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glutamic acid (μmol/g fresh tissue) in the striatum of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>1 week</td>
<td></td>
<td>5.70±0.20</td>
<td>*</td>
<td>-9.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>6.27±0.11</td>
<td>6.27±0.09</td>
<td>n.s.</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8)</td>
<td>(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>6.24±0.17</td>
<td>n.s.</td>
<td>-0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>During withdrawal</td>
<td></td>
<td>5.90±0.21</td>
<td>n.s.</td>
<td>-5.90</td>
</tr>
<tr>
<td></td>
<td>period</td>
<td></td>
<td>(6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p > 0.05 nonsignificant.

* : p < 0.05 significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (8): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of aspartic acid (µmol/g fresh tissue) in the striatum of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1 week</td>
<td>1.90±0.05 (8)</td>
<td>1.81±0.11 (5)</td>
<td>n.s.</td>
<td>-4.74</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>1.93±0.10 (7)</td>
<td>n.s.</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>1.43±0.03 (6)</td>
<td>**</td>
<td>-24.74</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>1.71±0.09 (6)</td>
<td>n.s.</td>
<td>-10.00</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

** : p< 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (9): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glutamine (µmol/g fresh tissue) in the striatum of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>1 week</td>
<td>3.05±0.07 (9)</td>
<td>3.06±0.11 (7)</td>
<td>n.s.</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>3.10±0.10 (6)</td>
<td>n.s.</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>2.84±0.09 (6)</td>
<td>n.s.</td>
<td>-6.89</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>3.06±0.09 (6)</td>
<td>n.s.</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p > 0.05 nonsignificant.

% Difference represents a comparison between saline control and treated values.
Table (10): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of GABA (μmol/g fresh tissue) in the striatum of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>1 week</td>
<td>4.36±0.05</td>
<td>4.06±0.05</td>
<td>**</td>
<td>-6.88</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>4.23±0.06</td>
<td>n.s.</td>
<td>-2.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>4.22±0.08</td>
<td>n.s.</td>
<td>-3.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td>4.62±0.12</td>
<td>n.s.</td>
<td>5.96</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.  
 n.s.: p> 0.05 nonsignificant.  
 ** : p< 0.01 highly significant versus saline control values.  
 % Difference represents a comparison between saline control and treated values.
Table (11): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glycine (μmol/g fresh tissue) in the striatum of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>1 week</td>
<td>1.06±0.02 (7)</td>
<td>1.15±0.03 (7)</td>
<td>*</td>
<td>8.49</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>1.06±0.03 (8)</td>
<td>n.s.</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>1.12±0.03 (7)</td>
<td>n.s.</td>
<td>5.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td>1.07±0.02 (7)</td>
<td>n.s</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

* : p< 0.05 significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (12): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of taurine (μmol/g fresh tissue) in the striatum of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine</td>
<td>1 week</td>
<td>6.67±0.09 (6)</td>
<td>6.72±0.14 (5)</td>
<td>n.s.</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>6.59±0.13 (7)</td>
<td>n.s.</td>
<td>-1.20</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>6.33±0.12 (6)</td>
<td>n.s.</td>
<td>-5.10</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>6.26±0.16 (6)</td>
<td>n.s.</td>
<td>-6.15</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses. n.s.: p> 0.05 nonsignificant. % Difference represents a comparison between saline control and treated values.
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>1 week</td>
<td>8.53±0.19 (8)</td>
<td>9.67±0.29 (5)</td>
<td>**</td>
<td>13.36</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>8.15±0.38 (6)</td>
<td>n.s.</td>
<td>-4.45</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>8.49±0.34 (6)</td>
<td>n.s.</td>
<td>-0.47</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>8.09±0.27 (6)</td>
<td>n.s.</td>
<td>-5.16</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

** : p< 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (14): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of aspartic acid (μmol/g fresh tissue) in the hypothalamus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1 week</td>
<td></td>
<td>5.67±0.22</td>
<td>**</td>
<td>19.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.76±0.12</td>
<td>(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>5.16±0.27</td>
<td>n.s.</td>
<td>8.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>4.82±0.19</td>
<td>n.s.</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>4.80±0.18</td>
<td>n.s.</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

** : p< 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (15): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glutamine (µmol/g fresh tissue) in the hypothalamus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>1 week</td>
<td></td>
<td>7.49±0.43</td>
<td>**</td>
<td>79.62</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>4.17±0.33</td>
<td>6.09±0.28</td>
<td>**</td>
<td>46.04</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>(9)</td>
<td>5.08±0.25</td>
<td>n.s.</td>
<td>21.82</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td>(9)</td>
<td>3.69±0.32</td>
<td>n.s.</td>
<td>-11.51</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p > 0.05 nonsignificant.

** : p < 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (16): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of GABA (μmol/g fresh tissue) in the hypothalamus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>1 week</td>
<td>3.65±0.10 (9)</td>
<td>3.98±0.10 (6)</td>
<td>*</td>
<td>9.04</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>3.83±0.18 (6)</td>
<td>n.s.</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>3.86±0.21 (7)</td>
<td>n.s.</td>
<td>5.75</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>4.08±0.05 (5)</td>
<td>*</td>
<td>11.78</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.
n.s.: p> 0.05 nonsignificant.
* : p< 0.05 significant versus saline control values.
% Difference represents a comparison between saline control and treated values.
Table (17): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of glycine (μmol/g fresh tissue) in the hypothalamus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>1 week</td>
<td>1.64±0.04 (7)</td>
<td>1.93±0.06 (6)</td>
<td>**</td>
<td>17.68</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>2.33±0.07 (7)</td>
<td>**</td>
<td>42.07</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>1.53±0.06 (6)</td>
<td>n.s.</td>
<td>-6.71</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>1.62±0.06 (6)</td>
<td>n.s</td>
<td>-1.22</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.
 n.s.: p> 0.05 nonsignificant.
 **: p< 0.01 highly significant versus saline control values.
 % Difference represents a comparison between saline control and treated values.
Table (18): Effect of daily oral administration of kava extract (75 mg/kg) on the concentration of taurine (μmol/g fresh tissue) in the hypothalamus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P. value</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine</td>
<td>1 week</td>
<td>12.18±0.16 (8)</td>
<td>12.75±0.25 (6)</td>
<td>n.s.</td>
<td>4.68</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>11.59±0.44 (6)</td>
<td>n.s.</td>
<td></td>
<td>-4.84</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>11.09±0.22 (6)</td>
<td>**</td>
<td></td>
<td>-8.95</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td>12.15±0.24 (6)</td>
<td>n.s.</td>
<td></td>
<td>-0.25</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: p> 0.05 nonsignificant.

** : p< 0.01 highly significant versus saline control values.

% Difference represents a comparison between saline control and treated values.
Table (19): Effect of oral administration of kava extract on the equilibrium ratio percent (ER %) between the concentration of inhibitory amino acid (GABA) and excitatory amino acids (glutamic acid and aspartic acid) in the hippocampus, striatum & hypothalamus of adult male albino rats with reference to saline control.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>% differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>1 week</td>
<td>41.88</td>
<td>41.55</td>
<td>-0.79</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>43.14</td>
<td>3.01</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>44.83</td>
<td>7.04</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>46.11</td>
<td>10.10</td>
</tr>
<tr>
<td>Striatum</td>
<td>1 week</td>
<td>53.37</td>
<td>54.06</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>51.59</td>
<td>-3.34</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>55.02</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>60.71</td>
<td>13.75</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1 week</td>
<td>27.46</td>
<td>25.95</td>
<td>-5.50</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>28.78</td>
<td>4.81</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>29.00</td>
<td>5.61</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>31.65</td>
<td>15.26</td>
</tr>
</tbody>
</table>

% difference represents a comparison between saline control and treated values.
Table (20): Effect of oral administration of kava extract (75 mg/kg) on AChE activity AchBr hydrolyzed/min/gm tissue in the cortex of adult male albino rats.

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated value</th>
<th>P-value</th>
<th>%D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>1 week</td>
<td>1.44±0.03(10)</td>
<td>1.42±0.03(6)</td>
<td>n.s.</td>
<td>-1.39</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>1.35±0.02(6)</td>
<td>*</td>
<td>-6.25</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>1.34±0.02(6)</td>
<td>*</td>
<td>-6.94</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>1.54±0.04(6)</td>
<td>n.s.</td>
<td>6.94</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: P>0.05 nonsignificant. *:P<0.05 significant versus saline control values.

% difference represents a comparison between saline control and treated values.
Table (21): Effect of oral administration of kava extract (75 mg/kg) on AChE activity AChBr hydrolyzed/min/gm tissue in the hippocampus of adult male albino rats.

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P-value</th>
<th>%D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>1 week</td>
<td>1.48±0.05(6)</td>
<td>1.38±0.08(7)</td>
<td>n.s.</td>
<td>-6.76</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>(6)</td>
<td>1.79±0.13(6)</td>
<td>*</td>
<td>20.95</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>1.36±0.01(6)</td>
<td>*</td>
<td>-8.11</td>
</tr>
<tr>
<td></td>
<td>During withdrawal</td>
<td></td>
<td>1.44±0.07(6)</td>
<td>n.s.</td>
<td>-2.70</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: P>0.05 nonsignificant. *:P<0.05 significant versus saline control values.

% difference represents a comparison between saline control and treated values.
Table (22): Effect of oral administration of kava extract (75 mg/kg) on AChE activity AChBr hydrolyzed/min/gm tissue in the striatum of adult male albino rats.

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Time of treatment</th>
<th>Saline control</th>
<th>Treated</th>
<th>P-value</th>
<th>%D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striatum</td>
<td>1 week</td>
<td>2.69±0.11(8)</td>
<td>3.32±0.24(5)</td>
<td>*</td>
<td>23.42</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td></td>
<td>2.51±0.15(6)</td>
<td>n.s.</td>
<td>-6.69</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td></td>
<td>2.35±0.11(6)</td>
<td>*</td>
<td>-12.64</td>
</tr>
<tr>
<td></td>
<td>During withdrawal period</td>
<td></td>
<td>2.89±0.08(6)</td>
<td>n.s.</td>
<td>7.34</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: P>0.05 nonsignificant. *:P<0.05 significant versus saline control values.

% difference represents a comparison between saline control and treated values.
Table (23): Effect of oral administration of kava extract (75 mg/kg) for 4 weeks on some serum biochemical parameters of adult male albino rats.

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Saline control</th>
<th>Treated</th>
<th>P - value</th>
<th>% D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST U/L</td>
<td>179.67±7.65 (6)</td>
<td>156.00±3.17 (6)</td>
<td>*</td>
<td>-13.17</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>45.83±1.33 (6)</td>
<td>35.00±0.76 (7)</td>
<td>**</td>
<td>-23.63</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>107.00±2.89 (6)</td>
<td>113.80±3.07 (6)</td>
<td>n.s.</td>
<td>6.36</td>
</tr>
<tr>
<td>Total protein g/dL</td>
<td>6.45±0.08 (8)</td>
<td>6.79±0.09 (8)</td>
<td>*</td>
<td>5.27</td>
</tr>
<tr>
<td>Albumin g/dL</td>
<td>3.15±0.07 (8)</td>
<td>3.08±0.06 (8)</td>
<td>n.s.</td>
<td>-2.22</td>
</tr>
<tr>
<td>Urea mg/dL</td>
<td>26.86±0.67 (7)</td>
<td>31.40±1.69 (6)</td>
<td>*</td>
<td>16.90</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>0.93±0.04 (9)</td>
<td>0.78±0.02 (9)</td>
<td>**</td>
<td>-16.13</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M with the number of animals between parentheses.

n.s.: P>0.05 nonsignificant.

*: P<0.05 significant versus saline control values.

**: P<0.01 highly significant versus saline control values.

% D: % difference represents a comparison between saline control and treated values.
4. DISCUSSION

The hippocampus is known to be one of the brain structures involved in anxiety (Gray, 1982; 1987; Pratt, 1992). Recent evidence supports the involvement of dorsal hippocampus in the modulation of anxiety-like behavior (Nasehi et al., 2011). It has been suggested that the inhibition of hippocampal activity can be an important mechanism of antianxiety action of anxiolytic drugs (Plaźnik et al., 1994; Wisłowska-Stanek et al., 2005, 2006). In addition, several studies have indicated that, the hippocampal formation is also involved in the modulation of anxiety-related behaviors which in turn are mediated through glutamate NMDA receptors (Rezvanfard et al., 2009; Sun et al., 2009; Barkus et al., 2010).

In the present study, kava administration induced a significant decrease in hippocampal aspartate level after one week and glutamate level after 2 weeks. Several
findings showed that the substances which decrease glutamatergic neurotransmission, such as antagonists of ionotropic glutamate receptors, exert anxiolytic-like effects in animals (Chojnacka-Wójcik et al., 2001). In addition, recent study of Catches et al. (2012) demonstrates a clear anxiolytic and antidepressant phenotype associated with Genetic ablation of the mice GluK4 kainate receptor subunit and a parallel disruption in hippocampal plasticity, providing support for the importance of this receptor subunit in mood disorders. It has been suggested that glutamatergic synaptic transmission appears to be diminished in the presence of kavain due to the reduction of excitatory post synaptic potential (EPSP) in intracellular experiments and the decrease of the population of spike amplitude in the extracellular experiments (Grunze et al., 2001).

Therefore, the decrease in hippocampal glutamate and aspartate levels due to kava treatment, in the present study, and the previously suggested inhibition in
glutamatergic synaptic transmission in the presence of kavain may mediate the anxiolytic action of kava and explain its reported tolerability (Neto, 1999; Malsch & Kieser, 2001; Bonavita et al., 2003; Khan & Haleem, 2007).

In the present study, GABA levels showed a delayed significant increase after 4 weeks in the hippocampus which persisted one week after stopping drug intake. There is an increasing evidence for the anxiolytic action of GABA (Skolnick & Paul, 1981; Olsen, 1982; Williams, 1983). In addition, Olkkola & Ahonen (2008) reported that the actions of benzodiazepines are due to the potentiation of the neural inhibition that is mediated by GABA. Moreover, in the recent review of Trincavelli et al. (2012) the authors present current knowledge about the role of the GABAA/benzodiazepine-receptor complex in anxiety disorders, new insights into the molecular biology of the receptor complex, and the importance of this target in the development of new
therapeutic agents in anxiety. Kava extract shows a similar activity profile as the benzodiazepines (Jussofie et al., 1994; Baum et al., 1998).

Thus, it could be suggested that the increase in GABA level in the hippocampus may play an important role in the anxiolytic action of kava after chronic treatment (4 weeks).

It is noteworthy to mention that the inhibitory effect of kava was initiated by a decrease in hippocampal aspartate level (after 1 week), extended by a decrease in glutamate level (after 2 weeks) and by an increase in GABA level after 4 weeks.

It has been reported that taurine reduces the release of aspartate, glutamate and GABA from cortical synaptosomes (Kamisaki et al., 1993). In the present experiment, there was a significant increase in taurine level after the administration of kava for 1 week in the hippocampus which was accompanied by a significant
decrease in aspartate level. Thus, the decrease in aspartate level might be due to the decrease in its synthesis and not due to its release enhancement. On the other hand, taurine is believed to be an inhibitory neurotransmitter acting as an agonist on glycine receptors (Betz, 1992). Moreover, glycine receptors may be the primary receptor for taurine in the developing neocortex (Flint et al., 1998) and postnatal hippocampus (Mori et al., 2002). It could be suggested that the increase in taurine level after 1 week from the administration of kava in the hippocampus may act to compensate the decrease in glycine level recorded at the same time period.

In the present investigation, there has been observed significant decreases in the levels of the two striatal excitatory amino acids, glutamate and aspartate after 1 week and 4 weeks, respectively. N-methyl-D-aspartate (NMDA) receptors in brain are regulated both by glutamate, which serves as the primary agonist, and
glycine, which binds to an allosteric modulatory site (Dingledine et al., 1990; Reynolds & Miller, 1990). Thus, it could be suggested that the increase in glycine level in the striatum after 1 week may have a compensatory role for NMDA receptors during the decreased level of glutamate.

The significant decrease in striatal aspartate level after 4 weeks of treatment could reflect that this amino acid may be involved in the mechanism of the anxiolytic action of chronic treatment of kava.

The hippocampus is a brain region associated with learning and memory (Maubach, 2003). Albouy et al. (2008) have presented results which stress on the importance of both hippocampus and striatum in procedural memory consolidation.

Several studies reported no change in cognitive function, immediately following kava ingestion (Prescott et al., 1993; Foo & Lemon, 1997) while others reported
subtle disruption to attentional and memory processes (Münte et al., 1993; Heinze et al., 1994). In addition, the use of kava has been associated with hallucinations (Brunton, 1988).

Many investigations reported an enhancement of memory after intradorsal injection of glutamate (Packard, 1999; Packard & Teather, 1999). The present results indicated a significant decrease in glutamate level after 1 week in the striatum and 2 weeks in the hippocampus as well as a significant decrease in aspartate level after 1 week in the hippocampus and 4 weeks in the striatum. It could be concluded that the negative impact of kava on memory processing may be mediated by these induced decreases in the levels of both aspartate and glutamate.

On the other hand, GABA_\text{A} receptors are known to down regulate memory consolidation processes: picrotoxin and bicuculline enhance memory, while benzodiazepines and muscimol depress it (Izquierdo &
The results of Evans & Viola-McCabe (1996) suggested that benzodiazepines reduced long-term potentiation primarily through reduction of excitatory post-synaptic potentiation, and that this effect occurred through modulation of GABA<sub>A</sub> receptor function which could in part account for the ability of benzodiazepines to disturb new memory formation. Therefore, the increase in GABA level (after 4 weeks) in the hippocampus, in the present study, may suggest another tool for memory impairment as a side effect due to chronic treatment with kava, which resembles that of benzodiazepines.

The data of the present investigation showed also that the daily oral administration of kava for one week induced a general significant increase in both excitatory (glutamic and aspartic) and inhibitory (GABA and glycine) amino acid levels as well as glutamine in the hypothalamus.
Kavain was reported to inhibit the stimulated glutamate release in cerebrocortical synaptosomes (Gleitz et al., 1996a, b) and freely moving rats (Ferger et al., 1998).

Thus, the increase in the hypothalamic glutamate level after 1 week may result from the inhibition of its release induced by kavain which is an active ingredient in kava extract, and the increase in glutamine level may be a result of the increase in glutamate level.

The excitability of the central nervous system reflects a balance between excitatory and inhibitory influences that is normally maintained within relatively narrow limits. Drugs can increase excitability either by blocking inhibition or by enhancing excitation (Ahmed et al., 1992a, b; Radwan et al., 1999). Moreover, Wisłowska-Stanek et al. (2008) reported that glutamate/GABA ratio offers a hypothetical index of excitatory processes in the brain. Therefore, the increase in hypothalamic GABA level after 1 week may
be a result of enhanced synthesis of GABA from excess glutamate which might be considered as an attempt of brain to counteract the increase in glutamate level to maintain the homeostasis between the major excitatory and inhibitory neurotransmitters. This opinion is supported by the calculated ER% which reflects a nearly normal-like state (-5.50%).

In the present investigation, it was noticed also that the glycine level showed a significant increase after 1 week of kava administration in the hypothalamus. Meanwhile, the chronic (4 weeks) administration of kava had no significant effect on glycine level. These results agree with the findings of Aburawi et al. (2001) who reported that the acute triazolam (benzodiazepine) administration produced an increase in glycine levels in almost all brain areas studied in the rat while the chronic administration of a single daily dose of triazolam produced normal glycine levels in most of the brain areas. Thus, it is clear that the effect of kava on glycine
level is similar to that of benzodiazepines, therefore it may be possible to suggest that glycine may play a role in the anxiolytic effect of kava during the sub chronic treatment.

One week after stopping kava administration revealed general non-significant changes except for significant increase in the glutamine level in the hippocampus and GABA level in the hippocampus and hypothalamus. These changes were accompanied by an increase in ER% in favor of neurochemical inhibition state.

Shekhar et al. (1990) suggested that endogenous GABA acts on GABA\textsubscript{A} receptors in a discrete area of the posterior hypothalamus to regulate the level of experimental anxiety in rats. Therefore, the increase in GABA levels in the hypothalamus and hippocampus one week after stopping kava administration may reflect a long-lasting effect of anxiolytic action of kava.
It has been cited that the active ingredients in kava have been found to bind to many sites in the brain that are associated with addiction and craving (Steiner, 2001). The author reported that the preliminary findings suggest that kava may reduce the craving associated with addiction.

In a study of Geier & Konstantinowicz (2004), 50 patients with non-psychotic anxiety were treated with a daily dose of 3 x 50 mg (kava special extract) during a 4-week treatment period followed by a 2-week safety observation phase. They found that kava was well tolerated and showed a safety profile with no drug-related adverse events or post-study withdrawal symptoms. In clinical settings, kava has been associated with better tolerability and lack of physiological dependence and withdrawal (Connor et al., 2001).
The increase in hippocampal glutamine one week after stopping kava intake in the present study may serve to keep the normal range of glutamic and aspartic acid levels.

In a number of studies, kava extracts were compared favorably to prescribed medications such as benzodiazepenes and tricyclic antidepressants and without the side effects commonly seen with those drugs (Woelk et al., 1993; Volz & Kieser, 1997). Benzodiazepines are now known to carry risks of dependence, withdrawal, and negative side effects (Stewart, 2005). Alprazolam and diazepam, the two most prescribed benzodiazepine anxiolytics have potential for addictive use (Juergens, 1991).

Glutamate is a key candidate for changes in excitatory transmission mechanisms and benzodiazepine dependence (Izzo et al., 2001; Allison & Pratt, 2003; Van Sickle et al., 2004; Souza-Pinto et al., 2007).
There is a close inter-relationship between GABA and glutamate transmission in the hippocampus, such that if GABAergic inhibition is removed, glutamatergic polysynaptic responses mediated by AMPA and NMDA receptors are evoked (Crepel et al., 1997).

From the present results, there are no significant changes in the excitatory amino acids one week after stopping kava administration. Meanwhile, there are significant increases in GABA levels in the hypothalamus and hippocampus. The ER% indicates a state of inhibition in the brain areas under investigation.

There is evidence that hippocampal cholinergic systems may be particularly involved in the modulation of anxiety. Intrahippocampal infusions of cholinergic antagonists increase anxiety (File et al., 1998; Smythe et al., 1998). In addition, cholinergic agonists such as nicotine induced anxiolytic effects under certain test conditions (Ouagazzal et al., 1999) and reduced stress-induced anxiety in humans (Pomerleau et al., 1984;
Jarvik et al., 1989). Furthermore, Degroot et al. (2001) found that infusions of physostigmine in the dorsal hippocampus decreased anxiety as measured in plus-maze and shock-probe tests. From the present data and the above mentioned studies, it may be suggested that the observed decrease in AChE activity after 4 weeks may mediate the anxiolytic effect of kava extract through increasing the cholinergic transmission in the brain areas under investigation.

Benzodiazepines are established anxiolytic drugs (for example: midazolam; diazepam; triazolam). Olkkola & Ahonen (2008) reported that the actions of benzodiazepines are due to the potentiation of the neural inhibition that is mediated by GABA. It is thought to act mainly via the post synaptic GABAA receptor to potentiate the action of GABA (Yamamoto et al., 2007). Nicotinic ACh receptors (nAChRs) exist on GABAergic interneurons within the neocortex (Xiang et al., 1998; Alkondon et al., 2000). Results of Yamamoto et al.
(2007) provided evidence that the nAChRs on GABAergic synaptic boutons within the neocortex do indeed interact with midazolam, allowing the endogenous ACh to increase the release of GABA. On the other hand, Schetinger et al. (2000) showed that diazepam presented an inhibitory effect on AChE activity in the cerebral cortex of the adult rat. In light of the present data, the potentiating effect of kava extract to GABAergic transmission may be originally mediated by inhibition of AChE activity, leading to increase of cholinergic transmission that can affect nAChR on GABAergic neurons to increase the release of GABA.

The present data also showed that the decrease in AChE activity was delayed till after 4 weeks of kava administration in the hippocampus and striatum, whereas, the inhibitory effect of kava extract on AChE activity in the cortex was observed after 2 weeks of kava administration. Therefore, it could be suggested that the cortex may be, more likely, the target area for early
anxiolytic effect of kava mediated mediated by cholinergic transmission. As can be noticed from the present data, stopping kava administration for 1 week after 4 weeks of treatment revealed non-significant changes in the enzyme activity in the three brain areas studied. In clinical settings, kava has been associated with better tolerability and lack of physiological dependence and withdrawal (Connor et al., 2001; Geier & Konstantinowicz, 2004). In addition, Bilia et al. (2002) found that kava was well tolerated and non-addictive at therapeutic dosage. Therefore, the present nonsignificant change in AChE activity after stopping kava treatment for one week may provide an additional evidence for the reported safety of kava.

Although, kava extract shows a similar activity profile as the benzodiazepines (Baum et al., 1998), and without the side effects commonly seen with those drugs (Woelk et al., 1993; Volz & Kieser, 1997), the sales of kava extracts were either severely restricted or
prohibited in Europe due to reports of hepatotoxicity attributed to kava consumption (Schmidt et al., 2002). Liver biopsy showed hepatocellular necrosis consistent with chemical hepatitis in a case with liver failure with a history of taking kava containing product for 4 months (Humberston et al., 2003). More recently, the in vitro study of Lüde et al. (2008) indicated that the kava extracts are toxic to liver mitochondria leading to apoptosis of exposed cells. In contrast, Connor et al. (2001) assessed safety parameters for kava. The data support the safety of kava in treating anxiety at 280 mg kava lactones/day for 4 weeks. In addition, in vivo study of Singh & Devkota (2003), demonstrated that the aqueous kava extracts administrated to rats at a daily dose of 200 or 500 mg kavalactones/kg for 2 or 4 weeks did not affect AST, ALT, alkaline phosphatase and lactate dehydrogenase in the sera nor malondialdehyde in the liver homogenate and in some cases they were significantly reduced. The authors suggesting not only a
lack of toxicity but potentially a hepatoprotective effect of kava. Furthermore, in a study sample comprising data from three controlled trials of kava in generalized anxiety disorder, no changes in liver function were found (Connor et al., 2006). As can be noticed from the present study, daily kava administration for 4 weeks resulted in significant decreases in serum AST and ALT activities and creatinine level, while ALP activity and albumin level did not show any significant changes. However, total protein and urea levels were increased significantly.

The present results support the previous findings indicating the safety of kava to the liver (Sorrentino et al., 2006; Lim et al., 2007). The increase in serum urea level, in the present results, was expected due to the increase in total protein level. Creatinine is a chemical waste molecule that is generated from muscle metabolism. It is transported through the blood stream to the kidneys, where they filter most of the Creatinine
and dispose it in the urine. As the kidneys become impaired, the Creatinine level in the blood will rise. Thus the measurement of serum Creatinine level has been found to be a fairly reliable indicator of kidney function. Therefore, the concomitant highly significant decrease in Creatinine level, in the present data, suggests that there may be no adverse effect on kidney function.

In conclusion, the anxiolytic effect of kava may be mediated on one hand by the decrease in excitatory amino acid levels in the hippocampus and striatum which may be accompanied by a risk of memory impairment. On the other hand, the increase in GABA levels may play a role in the effect of kava in the hypothalamus and hippocampus. The results of amino acids indicate that the hippocampus is more likely to be a target area for kava than striatum. Although the anxiolytic effect of kava treatment is similar to that of benzodiazepines, the withdrawal of kava may be safe and lack the adverse effects of benzodiazepines
withdrawal. The cholinergic system in the cortex, hippocampus and striatum may play a vital role in the anxiolytic action of kava which started after 2 weeks in the cortex and delayed in the hippocampus and striatum till 4 weeks of treatment. The present study showed no adverse effects of kava on liver and kidney function parameters. Hence, the use of kava in treating anxiety may be preferred to the use of conventional anxiolytics due to the lack of withdrawal and addictive properties. Nevertheless, it is recommended to follow up the liver and kidney functions in case of long term use of kava.
5. References


Crepel V, Khazipov R, Ben-Ari Y (1997). Blocking GABA(A) inhibition reveals AMPA-and NMDA-receptor mediated polysynaptic responses in the CA1


methysticum cultivars with CNS receptors in vitro.


Duffield AM, Lidgard RO, Low GK-C (1986). Analysis of the constituents of Piper methysticum by Gas Chromatography Methane chemical ionization


File SE, Gonzales LE, Andrews N (1998). Endogenous acetylcholine in the dorsal hippocampus reduces anxiety through actions on nicotinic and


Foos TM, Wu JY (2002). The role of taurine in the central nervous system and the modulation of


Gleitz J, Beile A, Peters T (1996a). (±)-Kavain inhibits the veratridine and KCl-induced increase in intracellular Ca\textsuperscript{2+} and glutamate-release of rat
cerebrocortical synaptosomes.

Neuropharmacology, 35: 179-186.


Drugs" (Efron D H, Holmstedt B, Kline N S, eds.),

Meyer HJ, Kretzschmar R (1966). Kawa-Pyrone, eine
euartige Substanzgruppe zentraler
Muskelrelaxantien vom Typ des Mephenesins. Klin
Wochenschr, 44: 902-903.

subclass of GABA\textsubscript{A} receptors in mammalian brain

Michopoulos I, Douzenis A, Christodoulou C, Lykouras L

plasma levels of amino acids and released
Cited from: Kong WX, Chen SW, Li YL, Zhang YJ,
Wang R, Min L, Mi X (2006). Effects of taurine on


Münte TF, Heinze HJ, Matzke M, Steitz J (1993). Effects of oxazepam and an extract of kava roots (Piper


Ohnuma T, Augood SJ, Arai H, McKenna PJ, Emson PC (1999). Measurement of GABAergic parameters in the prefrontal cortex in schizophrenia: focus on GABA content, GABA(A) receptor alpha-1 subunit messenger RNA and human GABA transporter-1


Ouagazzal AM, Kenny PJ, File SE (1999). Modulation of behavior on trials 1 and 2 in the elevated plus-maze test of anxiety after systemic and
hippocampal administration of nicotine. Psychopharmacology, 144: 54-60.


Rezvanfard M, Zarrindast MR, Bina P (2009). Role of ventral hippocampal GABA(A) and NMDA


Sergeeva OA, Haas HL (2001). Expression and function of glycine receptors in striatal cholinergic
interneurons from rat and mouse. Neuroscience, 104: 1043-1055.


Trenkner E (1990). The role of taurine and glutamate during early postnatal cerebellar development of


Turner JR, Castellano LM, Blendy JA (2010). Nicotinic partial agonists varenicline and sazetidine-A have


Vereker E, O'Donnell E, Lynch MA (2000). The inhibitory effect of interleukin-1beta on long-term potentiation is coupled with increased activity of


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