Full Length Research Paper

Anxiolytic action and safety of Kava: Effect on rat brain acetylcholinesterase activity and some serum biochemical parameters

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Kava is a herbal anxiolytic drug. The present study investigates the response of central cholinergic neurotransmission to kava treatment by measuring acetylcholinesterase (AChE) activity in cortex, hippocampus and striatum of adult male rats. The present study demonstrates also the effect of chronic use of kava on some liver and kidney function parameters in the sera of rats. Kava administration (75 mg/kg) induced an increase in AChE activity in the striatum after 1 week. However, significant decreases in the enzyme activity were obtained after 4 weeks of treatment in the three brain areas examined. No significant changes were observed in the enzyme activity on stopping kava administration. Kava administration for 4 weeks resulted in significant decreases in serum aspartate transaminase (AST) and alanine transaminase (ALT) activities and creatinine level, while alkaline phosphatase activity and albumin level did not show any significant changes. However, total protein and urea levels were increased significantly. In conclusion, the cholinergic system in the cortex, hippocampus and striatum may play a vital role in the anxiolytic action of kava. The present study showed no adverse effects of kava on liver and kidney function parameters.

Key words: Kava, acetylcholinesterase, cortex, hippocampus, striatum.

INTRODUCTION

Generalized anxiety disorder (GAD) is a prevalent and impairing disorder, associated with extensive psychiatric and medical comorbidity (Hidalgo et al., 2007). The use of alternative therapies has increased substantially over the last decade, particularly for more chronic conditions such as anxiety (Conner et al., 2001) . Kava is a herbal anxiolytic drug (Garrett et al., 2003; Pittler and Ernst, 2003; Shinomiya et al., 2005). It is an intoxicating beverage used by South Pacific Islanders and is traditionally prepared as an aqueous extract of the root of the kava plant (*Piper methysticum*). It has been used in Europe and North America as a mild anxiolytic (Mathews et al., 2005). It has been reported that the anxiolytic effects of kava seem to be as powerful as those of conventional anxiolytics (Lindenberg and Pitule-Schödel, 1990; Woelk et al., 1993; Boerner et al., 2003). Other randomized controlled trials suggest that kava reduces anxiety in perimenopausal women (Cagnacci et al., 2003), facilitates cognitive function and increase positive affectivity (Thompson et al., 2004), and improves sleep

quality (Emser and Bartylla, 1991). The physiological activity of kava resides in pyrone- or hydropyrone-containing components called kavalactones (Mathews et al., 2005).

Acetylcholine (ACh) is a fundamental neurotransmitter in the central nervous system (CNS), where it is critically involved in functions related to cognition and behavior, in some cases by modulating release of other neuro-transmitters, including glutamate, GABA, norepinephrine and dopamine (Kellar, 2006). There is evidence that hippocampal cholinergic systems may be particularly involved in the modulation of anxiety (File et al., 1998; Smythe et al., 1998). It has been found that kava affects the GABAergic (Jussofie et al., 1994), glutamatergic (Gleitz et al., 1996), and dopaminergic (Baum et al., 1998), transmission. However, to date, no studies have examined the effect of kava on the cholinergic transmission. 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). These reports of hepatotoxicity after centuries of apparently safe use in the South Pacific may be attributed to differences in the manner in which the commercial extract is prepared (Witton et al., 2003; Mathews et al., 2005). However, two drug monitoring studies had not found a single case of kava induced hepatotoxicity (Teschke et al., 2003). Other data suggest that kava does lead to an increase in liver enzymes (Clough et al., 2003a, b). Recently, a study of Sorrentino et al. (2006) does not back the suspicion of potential liver toxicity.

To date, no studies have examined the relation between kava anxiolytic effect and cholinergic system. In addition, studies that carried out to investigate the effect of kava, in treating anxiety, on liver functions have not produced univocal results. Therefore, the main objective of the present study was 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. Another aspect of the present study is to demonstrate the effect of chronic use of kava on some liver and kidney function parameters in the sera of adult male rats.

MATERIALS AND METHODS

Animals

The experimental animal used in this study was the adult male albino rat (*Rattus norvegicus*). Animals used for determination of 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. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt.

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 extract administered by human traditionally or medically.

Experimental design

The animals were divided into 2 main groups. The 1st main group of animals was served for determination of 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 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.

Handling of tissue samples

The animals used for the determination of 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 cortex, hippocampus and striatum (Zeman and Innes, 1963; Glowinski and Iversen, 1966). Each brain area was weighed and frozen until analyzed. 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 5ºC 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. 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.

Statistical analysis

Comparison between control and treated animals and the levels of significance were determined by using Student's t -test. Percentage difference representing the percent of variation in concentration with respect to the control was calculated.

% difference = (treated mean – control mean/control mean) x 100.

RESULTS

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 Table 1. In the cortex, kava administration induced significant decreases (P<0.05) in AChE activity after 2 and 4 weeks of treat-ment. However, hippocampal AChE activity showed a significant increase after 2 weeks followed by signi-ficant 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

Table 1. Effect of oral administration of kava extract (75 mg/kg) on AChE activity-AChBr hydrolyzed/min/gm tissue in the cortex, hippocampus and striatum of adult male albino rats.

Brain area	Time of treatment	Saline control	Treated	P-value	% difference
Cortex	1 week	1.44±0.03 (10)	1.42±0.03(6)	n.s.	-1.39
	2 weeks		1.35±0.02(6)	*	-6.25
	4 weeks		1.34±0.02(6)	*	-6.94
	During withdrawal period		1.54±0.04(6)	n.s.	6.94
Hippocampus	1 week	1.48±0.05 (6)	1.38±0.08(7)	n.s.	-6.76
	2 weeks		1.79±0.13(6)	*	20.95
	4 weeks		1.36±0.01(6)	*	-8.11
	During withdrawal period		1.44±0.07(6)	n.s.	-2.70
Striatum	1 week	2.69±0.11(8)	3.32±0.24(5)	*	23.42
	2 weeks		2.51±0.15(6)	n.s.	-6.69
	4 weeks		2.35±0.11(6)	*	-12.64
	During withdrawal period		2.89±0.08(6)	n.s.	7.34

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 2. Effect of oral administration of kava extract (75 mg/kg) for 4 weeks on some serum biochemical parameters of adult male albino rats.

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

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.

administration.

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 2. 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.

DISCUSSION

ACh is known to be rapidly hydrolyzed by AChE. The duration of action of Ach at the synaptic clefts is critically dependent on AChE activity (Cooper et al., 2003). 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 and 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 (nACh Rs) 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 nACh Rs 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 nACh R 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 and 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 and 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 and 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 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.

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