Kava Extract Drug: What is the Actual Risk of Hepatotoxicity in Adult Male Albino Rats?

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Abstract
There is increasing widespread use of complementary and alternative medicine in the treatment of psychiatric symptoms and disorders worldwide. Kava extract is a useful herbal medicine for treatment of insomnia and anxiety. However, a number of case reports have raised serious concerns about kava’s safety regarding liver toxicity. On the basis of these reports, regulatory agencies have banned or restricted sale of kava products in many countries, but not Egypt. Because herbal products are marketed as dietary supplements as well as drugs, they are not subjected to rigorous experimental testing for safety and efficacy. Hence, the present experimental study was conducted to examine the putative hepatotoxic effect of prolonged administration of commercially kava extract drug on albino rats for 60 days through evaluations of liver function tests as well as hepatic structural and ultrastructural morphological changes. Thirty adult male rats were randomly divided into 3 equal groups; 10 rats each. Group 1 (negative control group) rats were kept in the same environment without handling. Group 2 (vehicle group) rats were gavaged with 1.5 ml of corn oil. Group 3 (kava group) rats received 2 g/kg (600 mg kavalactones) of commercial kava powder inside the capsule in 1.5 ml corn oil orally. The anesthetized animals were sacrificed after blood collection and dissected, livers were prepared for light and electron microscopic examinations. Results from kava treated rats showed significant reduction in their final body weights accompanied with significant increase in both absolute and relative liver weight. Analysis of sera from kava group depicted significant elevations in gamma-glutamyl transferase, aspartate transaminase, alanin transaminase, alkaline phosphatase, total bilirubin, and calculated globulin levels associated with significant decrease in total protein, albumin, and Albumin/Globulin ratio in comparison to control values. These biochemical alterations were confirmed by the presence of different morphological and ultrastructural changes in examined liver sections. These potential hepatotoxic effects of kava should be taken into considerations with close monitoring of liver function tests at frequent intervals during drug use for long time.

Introduction
Kava is a member of the family Piperaceae and is named Piper methysticum G. Forster (Lebot et al., 1997). The term kava refers not only to the plant native to the South Pacific islands but also to both traditional beverages and herbal medicinal products derived from its rhizome and roots (Teschke et al., 2008). The traditional kava extracts are commonly water based and for medicinal purposes, solvents such as ethanol and acetone have been used for anxiolytic kava extracts (Schmidt et al., 2005).
The main active constituents believed to be responsible for the pharmacological (and perhaps toxicological) effects of Piper methysticum are the styryl α-pyrones (called kavapyrones or kavalactones). Several kavalactones have been identified, among these, six major kavalactones constitute approximately 95% of the lipid extract derived from the dried roots and rhizomes; these are kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin (Ramzan and Tran, 2004).

Various preparations of kava have been marketed since the 1980s, especially in Europe and North America to manage mild anxiety, tension, and restlessness. Some reports also indicate that kava preparations may have analgesic, spasmyloytic, neuroprotective, and
antimitotic activities (Connor and Davidson, 2002).

Most of the mechanisms proposed to explain the observed benefits of kava appear to be quite remote from physiological interaction with the liver. The anxiolytic actions of the drug are centrally mediated and are speculated to involve a reduction in the excitability of the limbic system, particularly the amygdala complex. The pharmacological properties of kava are postulated to include blockade of voltage-gated sodium ion channels, enhanced ligand binding to gamma-aminobutyric acid (GABA) type A receptors, diminished excitatory neurotransmitter release due to calcium ion channel blockade, reduced neuronal reuptake of noradrenaline (norepinephrine), reversible inhibition of monoamine oxidase B and suppression of the synthesis of the eicosanoid thromboxane A₂, which antagonises GABA (A) receptor function (Singh and Singh, 2002).

Kavalactones are usually metabolized in the liver by CYP450 enzymes (Whitton et al., 2003) particularly CYP3A and 1A (Guo et al., 2009). Several kavalactone reactive metabolites have been implicated in kava hepatotoxicity. Amongst those, two electrophilic quinoid metabolites have been identified in vitro (Zou et al., 2004) and another reactive metabolite 6-phenoxy-3-hexen-2-one (6-PHO) has been identified as a mercapturic acid derivative in human urine (Zou et al., 2005).

Kava extracts show good efficacy in the treatment of anxiety. In comparison with prescribed anxiolytics and even many over the counter products, moreover, kava extracts continue to demonstrate a far better risk-to-benefit ratio (Clouatre, 2004). However, a number of case reports have raised serious concerns about kava’s safety. These reports suggest that, occasionally, even normal doses (200-300 mg/d standardized to contain 70% kavalactones) of kava can cause severe liver injury. On the basis of these reports, regulatory agencies have banned or restricted sale of kava and kava products in many countries (Russmann et al., 2001).

Reports of kava hepatotoxicity first emerged in Germany in 1998. By 2005, the World Health Organization had received 55 reports of kava hepatotoxicity, which included three cases of hepatic failure and two cases of hepatic comas (Barnes et al., 2007). This led to a total ban of kava-containing products in some European countries, and warnings and voluntary product recalls in the USA and Australia (Gow et al., 2003). Despite this there is a myth about kava induces potential liver dysfunction especially with lacking of adequate experimental reproducibility and animal models for assessing the pathogenesis of kava hepatotoxicity (Teschke et al., 2008). Thus, relevant hepatotoxic effects of kava still need to be proven.

The present experiment was conducted to examine the putative hepatotoxic effect of prolonged administration of commercially kava extract drug on adult male albino rats for 60 days through evaluations of liver function tests in addition to the histological study of hepatic structural and ultrastructural changes.

Material and methods

A) Drugs:

Commercially available kava-kava extract capsules were used (Manufactured by October Pharma S.A.E., 6th October City, Giza, Egypt). Each 150 mg/capsule contains 30% kavalactones (45 mg/capsule). The powdered extract inside the capsule was gavaged to each animal after being dissolved in commercially available corn oil (Crystal, ARMA Oils Company, 10th of Ramadan City).

B) Animal Grouping:

Thirty adult male albino rats with body weights ranged between 170-190 gm were used throughout the experiment and received balanced diet with free access to water. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals (Cuschieri and Backer, 1977).

Animals were randomly divided into 3 equal groups; 10 rats each. After 10 days of acclimatization, they were subjected to treatment regimen as follows:

Group 1 (negative control group): The
animals in this group were kept in the same environment to demonstrate the normal basic parameters of the animals under the study for 60 consecutive days.

Group 2 (vehicle or Corn Oil group): Each fasted animal was gavaged with 1.5 ml of corn oil for 60 consecutive days.

Group 3 (kava group): Each fasted rat received 2 g/kg (600 mg kavalactones) of commercial kava powder dissolved in 1.5 ml corn oil, orally for 60 consecutive days. The dose was chosen according to Clayton et al. (2007).

C) Experimental parameters:

Twenty-four hours after the end of the experimental period and under appropriate ether inhalation anesthesia the animals were weighted, subjected to thoraco-abdominal incision to collect blood samples from descending aorta into a clean test tubes, then animals sacrificed by cervical dislocation, and finally livers were removed. Absolute and relative liver weights [(Liver weight/Body weight) x 100]) were recorded.

1. Biochemical assay: The serum was extracted from each blood sample for spectrophotometric determination levels of gamma-glutamyl transferase (GGT) (Tietz, 1995), aspartate transaminase (AST) and alanin transaminase (ALT) (Frankel and Gradwohl, 1970), alkaline phosphatase (ALP) (Donald and Ralph, 1993), total bilirubin (Walter and Gerade, 1970), total protein (Fall and Woollen, 1984), and albumin (Doumas et al., 1971). Also, the globulin values and Albumin/globulin ratios were calculated.

2. Histopathological Examination: Each excised liver was divided into two parts. One part was fixed in formalin 10%, prepared for paraffin sections of 4-6 µm thickness and stained with hematoxylin and eosin for light microscopic examination (Drury and Wallington, 1980). The second part (1mm x 1mm thickness) was fixed in glutaraldehyde for 20 hours then washed with phosphate buffer, fixed in 1% tetraoxide. Ultrathin sections were cut by an ultramicrotome then stained with uranyl acetate and carried on copper grids (Reynolds, 1963). Grids were examined by transmission electron microscope (TEM, SEO, 100kV) at Military Veterinary Hospital of Armed Forces, Nasr City.

Statistical Analysis:

Experimental data were analyzed by one-way ANOVA using SPSS version 16.0 software (SPSS, Chicago, IL). Significant differences among the treatments were compared by LSD test and level of 0.05 considered significant.

Results

In the present work, no deaths were recorded throughout the experimental period.

As shown in table (1), administration of vehicle did not affect the growth of rats as evidenced by non-significant decrease in their final body weights' mean values (percentage changes; 0.74%). In addition, the mean values of absolute and relative livers weights of vehicle treated rats (percentage changes; 0.21% increase and 0.49% decrease, respectively) depicted statistically non significant changes when compared with those of control animals. On the other hand, kava treated animals showed a significant decrease in their mean final body weights' values by 9.12%. Additionally, the mean values of absolute and relative livers weights from kava gavaged rats exhibited significant increases (percentage changes; 30.13% and 27%, respectively) as compared to control animals.

As appears in table (2), liver function indices of vehicle treated rats clarified non significant elevations of AST, ALT, ALP, TBL, and globulin levels (percentage changes; 10.19%, 4.97%, 4.10%, 2.78%, and 1.08%, respectively) as well as non significant depletion of GGT, total protein, albumin, and albumin/globulin ratio levels (percentage changes; 2.47%, 0.95%, 2.29%, and 0.63%, respectively) when compared to the control animal indices. While, liver biochemical parameters of kava treated rats displayed statistical significant rises in GGT, AST, ALT, ALP, TBL, and globulin values (percentage changes; 149.72%, 123.73%, 132.13%, 95.56%, 165.28%, and 56.47%, respectively), in addition to, significant diminishes in total protein, albumin, and albumin
globulin ratio values (percentage changes; 6.77%, 44.3%, and 36.88%, respectively) as compared to those values of control group.

Light microscopic evaluation of control liver sections stained with H&E (Figure 1) showed normal hepatic lobules consist of branching and anastomosing hepatocytes cords separated by blood sinusoids and radiating from central vein. Liver cells appeared polyhedral in shape with acidophilic cytoplasm and large round central nucleus. Portal tracts contain the portal triad (branch of portal vein, branch of hepatic artery, and bile ducts). The same histological findings were observed in the livers of vehicle treated rats. However, examination of H&E stained liver sections from kava gavaged animals (Figures 2-5) revealed different histopathological changes in their hepatic architectures such as dilated congested central vein, cloudy swelling and vacuolar degeneration of hepatocytes, focal lobular necrosis with portal and lobular inflammatory cell infiltrations.

Ultrastructural analyses from control (Figure 6) and vehicle livers illustrated normal morphological appearance of the hepatic cells. Hepatocytes exhibited large round nucleus with normal distribution of heterochromatine, round mitochondria, intact endoplasmic reticulum as well as Golgi apparatus, and glycogen particles.

On the other hand, TEM examination of the liver tissues from kava treated rats (Figures 7-15) displayed vacuolization of the hepatocytes cytoplasm, sinusoidal endothelial damage, irregular nucleus with disturbed heterochromatine and electrolucent granular nucleoplasm, large lipid droplets, dense degenerated mitochondria, mitochondrial swelling and loss of crystae, collagen fibers, swollen rough endoplasmic reticulum, intense glycogen accumulation or rosettes, degenerated Kupffer cell, damaged stellate or Ito cells, deformed Disse's space with large gape formation, blood cells accumulated inside dilated veins, and necrotic cells.
Table (1): Changes in body as well as absolute and relative liver weights of vehicle and kava treated rats as compared to control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Mean±SEM)</th>
<th>Vehicle (Mean±SEM)</th>
<th>Changes %</th>
<th>Kava (Mean±SEM)</th>
<th>Changes %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body Weight (g)</td>
<td>182.8±2.11</td>
<td>181.3±2.07&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.82% ↓</td>
<td>184±2.39&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.66% ↑</td>
</tr>
<tr>
<td>Final Body Weight (g)</td>
<td>257.8±3.23</td>
<td>255.9±4.35&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.74% ↓</td>
<td>234.3±2.01&lt;sup&gt;***&lt;/sup&gt;</td>
<td>9.12% ↓</td>
</tr>
<tr>
<td>Absolute Liver Weight (g)</td>
<td>9.39±0.02</td>
<td>9.41±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.21% ↑</td>
<td>12.22±0.56&lt;sup&gt;***&lt;/sup&gt;</td>
<td>30.13% ↑</td>
</tr>
<tr>
<td>Relative liver weight (g)</td>
<td>4.11±0.05</td>
<td>4.09±0.06&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.49% ↓</td>
<td>5.22±0.25&lt;sup&gt;***&lt;/sup&gt;</td>
<td>27% ↑</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM for group of 10 animals each; NS = non-significant difference compared to control; * = significant difference compared to control; changes % compared to control.

Table (2): Changes in liver function tests of vehicle and kava treated rats as compared to control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Mean±SEM)</th>
<th>Vehicle (Mean±SEM)</th>
<th>Changes %</th>
<th>Kava (Mean±SEM)</th>
<th>Changes %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT (U/L)</td>
<td>27.13±0.44</td>
<td>26.46±0.53&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.47% ↓</td>
<td>67.75±2.85&lt;sup&gt;***&lt;/sup&gt;</td>
<td>149.72% ↑</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>28.66±1.32</td>
<td>31.58±1.29&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>10.19% ↑</td>
<td>64.12±2.79&lt;sup&gt;***&lt;/sup&gt;</td>
<td>123.73% ↑</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>31.81±0.78</td>
<td>33.39±0.83&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.97% ↑</td>
<td>73.84±2.52&lt;sup&gt;***&lt;/sup&gt;</td>
<td>132.13% ↑</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>87.23±1.51</td>
<td>90.81±1.95&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.10% ↑</td>
<td>170.59±6.22&lt;sup&gt;***&lt;/sup&gt;</td>
<td>95.56% ↑</td>
</tr>
<tr>
<td>TBL (mg/dl)</td>
<td>0.72±0.03</td>
<td>0.74±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.78% ↑</td>
<td>1.91±0.13&lt;sup&gt;***&lt;/sup&gt;</td>
<td>165.28% ↑</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>7.35±0.03</td>
<td>7.28±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.95% ↓</td>
<td>6.89±0.05&lt;sup&gt;***&lt;/sup&gt;</td>
<td>6.77% ↓</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.57±0.05</td>
<td>4.47±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.29% ↓</td>
<td>2.54±0.10&lt;sup&gt;***&lt;/sup&gt;</td>
<td>44.3% ↓</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2.78±0.04</td>
<td>2.81±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.08% ↑</td>
<td>4.35±0.11&lt;sup&gt;***&lt;/sup&gt;</td>
<td>56.47% ↑</td>
</tr>
<tr>
<td>Albumin/Globulin Ratio</td>
<td>1.60±0.04</td>
<td>1.59±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.63% ↓</td>
<td>0.59±0.04&lt;sup&gt;***&lt;/sup&gt;</td>
<td>36.88% ↓</td>
</tr>
</tbody>
</table>

GGT= gamma-glutamyl transferase; AST = aspartate transaminase; ALT = alanin transaminase; ALP = alkaline phosphatase; TBL = total bilirubin; Values are given as mean±SEM for group of 10 animals each; NS = non-significant difference compared to control; * = significant difference compared to control; changes % compared to control.

Figure (1): A photomicrograph of a section of liver tissue from control rat showing hepatocyte cords (HC) of cells with rounded central basophilic nucleoli radiating from central vein (CV) and separated from each others by blood sinusoids (S). Original Magnification (OM) x 100. Small window illustrates normal portal tract triad of portal vein (PV), portal artery (PA), and bile ducts (BD). OM x 200.
Figure (2): A photomicrograph of a section of liver tissue from kava treated rat showing degenerated hepatocyte cells with extensive vacuolization (V), pyknotic nucleoli (P), hydropic changes (H), and dilated sinusoids (S). OM x 200.

Figure (3): A photomicrograph of a section of liver tissue from kava treated rat showing moderate central venous congestion (CV), extensive hydropic degeneration (H) of hepatocyte cells with pale area surrounding the central round nucleoli, and dilated sinusoids (S) with some exudate. OM x 200.

Figure (4): A photomicrograph of a section of liver tissue from kava treated rat showing necrotic areas (N) with sloughed materials, interstitial inflammatory cells infiltrations (IC), and pyknotic nucleoli (P). OM x 200.

Figure (5): A photomicrograph of a section of liver tissue from kava treated rat showing alteration of portal tract with portal vein (PV) dilatation, congestion, and exudates, dilated portal artery (PA), necrotic areas (N), and interstitial inflammatory cells infiltrations (IC). OM x 400.
Figure (6): An electron micrograph of ultrathin section from control rat liver showing normal appearance of hepatocyte with vesicular nucleus (N) with well developed nucleolar membrane (NM), mitochondria (M), rough endoplasmic reticulum (rER), and glycogen particles (G) distributed in well formed cytoplasm. OM x 4000.

Figure (7): An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing degenerated stellate cell (SC) with vacuolated cytoplasm (V), more glycogen granules (G), distorted and vacuolated (V) nucleus (N), peripheral clumping of abnormally condensed heterochromatin (HC), electronlucent granular nucleoplasm (NP), swollen damaged (D) mitochondria (M), swollen rough endoplasmic reticulum (rER), and loss of
Figure (8): An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing degraded hepatocytes with extensive intercytoplasmic vacuolation (V) and abnormal clumping of its contents (C), small size nucleus (N) with abnormal heterochromatin (HC) condensation in the central part, slight alteration of Kupffer cell (KC), central venous congestion (CV) with accumulated red blood cells (RBC), and increased lipid droplets (L). OM x 2000.

Figure (9): An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing normal organelles. OM x 15,000.
necrotic hepatocyte (NHC) with extensive intercytoplasmic vacuolization, damaged mitochondria (M), and absent nucleus lies adjacent to minimally damaged liver cells with nearly normal nucleus (N) and heterochromatin distribution (HC) as well as part of dilated central vein (CV) with red blood cells (RBC). OM x 3000.

**Figure (10):** An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing extensive cellular fibrosis (F), necrotic area (NA) with degenerated material, cytoplasmic vacuolization (V), and dilated rough endoplasmic reticulum (rER). OM x 8000.

**Figure (11):** An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract
showing part of hepatocyte with markedly swollen mitochondria (M), extensive glycogen rosettes (G), and part of nucleus (N) with electrolucent nucleoplasm. OM x 10,000.

**Figure (12):** An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing part of hepatocyte with dilated rough endoplasmic reticulum (rER), damaged mitochondria (M), rarefaction of the cytoplasm, and part of altered nucleus (N). OM x 15,000.
**Figure (13):** An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing devastated sinusoidal endothelial cells (E) with markedly vacuolated (V) nucleus (N), deformed Disse’s space (DS), hepatocyte with vacuolated (V) nucleus (N) and rarefied cytoplasm, as well as red blood cell (RBC). OM x 4000.

**Figure (14):** An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing necrotic changes in Disse’s space (DS) with large gap (LG) formation, sinusoidal endothelial cells (E), sinusoidal space (SS), and part of red blood cell (RBC). OM x 3000.
Figure (15): An electron micrograph of ultrathin section from rat liver after prolonged exposure to kava extract showing damaged Kupffer cell (KC) with altered heterochromatine (HC), intracytoplasmic vacuolization (V), large amounts of electron dense phagocytosed material (PM), large amount of lipid droplets (L), and deformed Disse's space (DS). OM x 6000.
Discussion

Over the past several decades, interest in complementary and alternative medications continues to grow and has increasingly become a part of everyday treatment. With the rising cost of prescription medications and their production of unwanted side effects, patients are exploring herbal and other natural remedies to improve their own lives. Because widespread use of herbal remedies in the treatment of physical and psychiatric symptoms and disorders is increasing, a risk–benefit profile of commonly used herbs is needed (van der Watt et al., 2008).

One of these drugs, kava-kava, an extract of the roots of the Piper methysticum plant, is used both ritually and socially in the South Pacific, and is sold as herbal medicine to treat insomnia and anxiety (Gyllenhaal et al. 1999). The effect of this drug on liver function and histopathology is questionable. However, there is increasing reports and serious concern that kava may cause liver damage. Kava still used in Egypt and not banded. Hence, the present study was designated to document or rule out the toxic effects of prolonged administration of the pharmaceutical preparation kava extract on the rats’ livers through studying liver function tests as well as structural and ultrastructural morphological changes of this organ.

The present work showed a significant decrease in the final body weights in kava group compared to control group, which is in agreement with the result of Clayton et al. (2007) who documented reduction in the mean body weights of male rats after prolonged gavage of 2.0 g/kg kava extract for 14 weeks by 17.2 % than those of the vehicle controls. However, chronic administration of 73 mg/kg body weight ethanolic kava extract to rats for 3 months induced no relevant difference in body weight development (Sorrentino et al., 2006). This reduction in body weight is due to loss of appetite, malnutrition, and loss of body fat after chronic kava usage (Matthews et al., 1988).

The significant increase of the absolute and relative weights of the livers in kava treated rats as noticed in the present work are in accordance with the findings reported by Clayton et al. (2007) who recorded an increase in the absolute and relative livers weights of both male and female rats gavaged with different doses of kava extract (up to 2 g/kg body weight) for 14 weeks. Also, administration of a high dose of kavalactones (380 mg/kg/day) to rats for 8 days markedly increased their absolute and relative liver weights (Yamazaki et al., 2008).

The most concerning potential toxicity of kava is its effect on the liver (Ulbricht et al., 2005). At least 78 case reports exist of hepatotoxicity associated with the use of kava supplements (Clouatre, 2004). Liver related problems range from increased liver enzyme tests, hepatitis, cirrhosis, liver failure, liver transplant, and death. As a result, several countries have banned the sale of kava (Ulbricht et al., 2005). Almost all reported cases of kava-associated hepatotoxicity involve the use of kava dietary supplements (either acetone or ethanol extracts packaged in capsules) rather than the traditional root beverage (filters of aqueous extracts from the dried ground kava rhizome and root powder) (Brown et al., 2007).

Abnormalities of liver function indices reported in the current study are in line with other several experimental and clinical studies. Male and female rats treated with 1 and 2 g/kg/day (0.96 and 1.92 g/kg/day kavalactones, respectively) kava extracts for 14 weeks demonstrated multiple-fold increases in serum GGT activity (Clayton et al., 2007). Abnormal laboratory results may also be encountered owing to altered pathophysiology or enzyme induction secondary to kava associated liver toxicity with subsequent elevation of ALT, AST, and bilirubin concentrations may be observed in a healthy individual taking such herbal products (Dasgupta and Bernard, 2006). Additionally, Kraft et al. (2001) and Campo et al. (2002) recorded progressive fulminant hepatic failure in an old woman and young girl, respectively, after prolonged ingesting a kava preparation associated with marked elevations of liver function tests plus mixed inflammatory infiltrates that necessitates liver transplantation.

Moreover, other clinical studies in Australia and Tongan islands displayed higher
GGT and ALP levels in aqueous kava extracts drinkers than non-users, suggestive of hepatobiliary injury (Currie and Clough, 2003; Sarris and Kavanagh, 2009). Traditional aqueous kava extracts were the most probable cause of hepatitis in two patients presenting with markedly elevated transaminases and hyperbilirubinemia. A consequent survey of heavy kava drinkers showed elevated GGT, AST, and ALT. The authors concluded that not only commercially available, but also traditionally prepared kava extracts may cause liver injury. The increased activity of GGT in heavy kava consumers reflects an induction of CYP450 enzymes (Russmann et al., 2003). Also, chronic heavy users taking 300 to 400 g per week suffer from a variety of metabolic abnormalities such as increased liver enzymes, decreased albumin and plasma protein, and increased cholesterol level (Matthews et al., 1988).

On the contrary, potential liver toxicity was not found in Wistar rats of both sexes after chronic (3 and 6 months) oral application of an ethanolic kava extract, as anxiolytic drugs, at dosage of 3 or 73 mg/kg body weight (Sorrentino et al., 2006). Additionally, male rats administered aqueous kava extracts in daily dosages of 200 or 500 mg of the active kavalactones/kg for two or four weeks showed no changes in their tested sera hepatic enzymes levels nor malondialdehyde level (indicator of lipid peroxidation), suggesting the lack of a toxic effect by kava on the liver (Singh and Devkota, 2003). This disagreement can be explained by either less experimental duration or lower dosage regimens than used in the current study.

Hepatic necrosis or cholestatic hepatitis with subsequent development of fulminant liver failure that necessitates liver transplantation due to both alcoholic and acetonin Kava extracts were noticed in several patients (Stickel et al., 2003).

Animals gavaged with kava extract, in the current work, developed obvious structural and ultrastructural alterations in the liver histomorphology as compared with the control group. Kava induced hepatic histopathological changes were documented in several experimental and clinical works. Necropsies of male and female rats that received kava extract in Clayton et al. (2007) study showed increases in the incidence and severity of hepatocellular damage.

Microscopically, cellular hypertrophy consisted of an irregular increase in the size of hepatocytes, usually in a centrilocular distribution. Centrilobular fatty change consisted of poorly demarcated areas of hepatocytes with large, clear cytoplasmic vacuoles, usually in the centrilocular and midzonal regions. Cystic degeneration consisted of multilocular cystic areas containing a finely granular or flocculent eosinophilic material, apparently resulting from the distention and occasional rupture of adjacent hepatocytes. Also, repeated peroral administration of kava extract (320 mg/kg body weight) to Spraque-Dawley rats induced histopathological changes in liver tissues (Hoelzl et al., 1994).

In addition, isolated rat livers perfused with the kavalactone kavain showed loss of overall liver architecture, disrupted endothelium, vacuolization of the parenchymal cells of hepatocytes which lying close to the sinusoidal vasculature, and constriction as well as narrowing of the vascular bed (Fu et al., 2008). Gebhardt (2001) reported that kavapyrones, not ethanolic and acetonin extracts, induced cytotoxicity in rat hepatocytes, in vitro. Moreover, histopathological results from human clinical cases revealed hepatic symptoms following use of kava supplements showed extensive, severe hepatocellular necrosis and infiltration with lymphocytes, eosinophils and activated macrophages (Escher et al., 2001; Russmann et al., 2001).

The electron microscopy results of the in vitro study done by Fu et al., (2008) displayed the existence of gaps which indicate disruption of the normal liver architecture, strong vacuolized hepatocytes surround the blood vessels, narrowing of blood vessels, constriction of sinusoidal blood vessels and retraction of the endothelium. Noteworthy is that the severe changes in sinusoidal microvasculature and overall loss of the integrity of the liver parenchymal cell architecture was accompanied with striking structural alterations of the liver macrophage population (Kupffer cells) in the sinusoids of kava-treated livers. Close
examination by TEM revealed that Kupffer cells appear to be swollen and the presence of large cytoplasmic vacuoles and phagocytosed material is apparent. Furthermore, the cells have lost their typical ruffled cell membrane aspect and concurrently they appear to disengage from the sinusoidal endothelial lining.

In contrast, other experimental studies failed to show potential liver morphological changes (Hänsel and Woelk, 1994; Sorrentino et al., 2006). This disagreement is due to lower dosage used in the previous studies than the current work.

The adverse effects of kava were often unapparent and rare, though prolonged and/or overdose kava usage was a reportedly potential rare but life-threatening risk factor for hepatotoxicity (Stickel et al., 2003). Adverse hepatic effects have occurred in patients using either commercial kava extracts or traditional aqueous extracts, suggesting that toxicity is independent of extraction solvent and linked to the kava plant itself (Teschke et al., 2009).

The pathophysiology of kava hepatotoxicity remains inconclusive. Liver injury associated with kava could have immunological and idiosyncratic origins (Teschke et al., 2003). The obvious lack of dose-dependency and the marked eosinophilic infiltrate in some liver biopsies, as well as rapid improvement of clinical picture after prednisolone substitution support this assumption (Stickel et al., 2003). It is generally accepted that the increased release of pro-inflammatory mediators and reactive oxygen species by stimulated liver macrophages causes severe and acute liver damage (Tsukamoto and Lin, 1997). Inflammatory mediators, growth factors and reactive oxygen species released following direct or indirect activation of Kupffer cells by toxic agents are associated with acute and chronic liver disease (Roberts et al., 2007). Hence hepatic inflammation is present in cases of kava consumption and toxicity. It is plausible that kava preparations could cause inflammation directly, since more than 40 compounds have been isolated from kava, including the pharmacologically active kavalactones, the highly toxic chalcone, flavokavain B (Jhoo et al., 2006) and the toxic alkaloid pipermethysticine, which is present in kava leaves and stem peelings (Dragull et al., 2003). Alternatively, inflammation may be caused indirectly by mechanisms such as the effects of toxic metabolites, inhibition of cyclooxygenase enzymes, and the depletion of liver glutathione (Zou et al., 2005).

Oxidation of glutathione during phase II metabolism, where glutathione is conjugated to kavalactones to produce water soluble products for excretion was documented. This demand on hepatic glutathione may leave the liver susceptible to reactive oxygen species such as hydrogen peroxide, which is normally detoxified in a reaction catalysed by the enzyme glutathione peroxidase (Whitton et al., 2003). This mechanism is more feasible, since kava-treated HepG2 cells (a hepatocellular carcinoma cell line) exhibit an increased ratio of oxidized to reduced glutathione (Lüde et al., 2008). Reactive kavalactone metabolites may bind to cellular DNA and hepatic proteins, disrupting enzymatic activity or alkylating DNA (Johnson et al., 2003). Portal and central pressure perturbations associated with kavain solution may affect normal physiological liver function (Fu et al., 2008).

Also, increased levels of GGT and cholesterol may be related to hepatocellular hypertrophy caused by induction of microsomal enzymes, similar to the action of anti-epileptic drugs (Clayton et al., 2007). In general, serum GGT is inducible in hepatobiliary diseases, such as alcoholic hepatitis and cholestasis by microsomal enzymes of the liver (Ohta and Toda, 2001). Increased levels of GGT correlated with ALP indicate a hepatobiliary origin when cholestasis occurs in the liver (Giannini et al., 2005).

Yamazaki et al. (2008) suggested that commercial kava products might exert their potencies to induce CYP1A1 in human and its consequence have possibly related to hepatotoxicities with or without the effects on other CYP isoforms. Another possible mechanisms of kava hepatotoxicity involved mitochondrial toxicity. The mitochondrial membrane potential was decreased, the respiratory chain inhibited and uncoupled and
mitochondrial β-oxidation was inhibited by kava extracts. The ratio of oxidized to reduced glutathione was increased. These results indicate that the kava extracts are toxic to mitochondria, leading to inhibition of the respiratory chain, increased reactive oxygen species production, a decrease in the mitochondrial membrane potential and eventually to apoptosis of exposed hepatic cells (Lüde et al., 2008).

In summary, prolonged administration of commercially available kava drug in high doses was associated with hepatic dysfunction of experimental adult male rats. These findings were confirmed by marked disturbances in liver function tests as well as histopathological and ultrastructural alterations of liver morphology as demonstrated by light and electron microscopical examinations of liver sections. These potential kava effects on the liver should be taken into account with close monitoring of liver function tests at frequent intervals when this drug prescribed to patients for long time.

References


استخلص عقار الكافا: ما هو الخطر الفعلي من التسمم الكبدي في ذكور الفئران البيضاء البالغة؟

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المملوک العربي

هناك زيادة كبيرة لاستخدام الطب التكميلي والبدائل في علاج الأعراض والاضطرابات النفسية في جميع أنحاء العالم. مستخلص الكافا هو دواء عشبي مفيد لعلاج الأرق والقلق. ومع ذلك، أثارت عددا من التقارير حالة اهتمام بالغ بشأن سلامة الكافا فيما يتعلق بصحة الكبد. على أساس هذه التقارير، تم اكتشاف بعض المنتجات الكافية من قبل الوكالات التنظيمية في كثير من البلدان، ولكن ليس في مصر. لأن المنتجات العشبية يتم تسويقها في هيئة مكملات غذائية، فضلا عن أدوية، لا تتعرض هذه المنتجات لاختبار تجريبي صارم لسلامتها وفعاليتها. لذلك، أجريت هذه الدراسة التجريبية لتقييم التأثير السماني المفترض لمستخلص عقار الكافا لفترة طويلة على كبد الفئران البيضاء لمدة 60 يومًا من خلال تقييم وظائف الكبد وكذلك التغييرات الشكلية الهيكلية والتركيبية الدقيقة للكبد. شملت الدراسة ثلاثون فارا من الذكور البالغة التي قسمت عشوائيا إلى 3 مجموعات متساوية: 10 فئران في كل مجموعة.

المجموعة 1 (مجموعة الضبابية السلبية) حيث تم الاحتفاظ بالفئران في نفس البيئة دون معالجة.

المجموعة 2 (مجموعة المذيب) تم إعطاء الفئران 1,5 مللي زيت الذق في الفم.

المجموعة 3 (مجموعة الكافا) تم إعطاء الفئران 2 جرام/كيلوجرام (200 ملجرام الكافالاكتونز) من مسحوق الكافا التجاري في 1,5 مللي زيت الذقة في الفم. تم تخدير الفئران وذبحها لجمع عينات الدم وتشريح الكبد واعداد للفحص المجهرى الضوئي وال الإلكتروني.

أظهرت نتائج الفئران المعالجة بالكافا انخفاضًا كبيرًا في دلالة أحيائية في أوزان جسمها النهائي يتوافق مع زيادة كبيرة ذات دلالة إحصائية في كل من أوزان الكبد المطلقة والنسبية. كما أظهر تحليل الأنسلاك من مجموعة الكافا ارتفاعات كبيرة ذات دلالة إحصائية في مستويات جاما جلوتاميل ترانسفراز، إسبارتات ترانسامينوز، ألانين ترانسامينوز، الفوسفاتاز القلوية، والبيبرين الكولي، والجلوبولين الذي وجد مصابين بانخفاض كبير ذو دلالة إحصائية في مستويات البروتين الكولي والألبومين، ونسبة الألبومين/الجلوبولين مقارنة بقيم المجموعة الضبائية. وقد تأكدت هذه التغييرات البيوكيميائية بوجود تغييرات شكلية مختلفة هستولوجيا وتركيبية دقيقة في شرائح الكبد التي تم فحصها. هذا التأثير السمي على الكبد من الكافا ينبغي أن يؤخذ في الاعتبار مع متابعة لوظائف الكبد على فترات متكررة عند اعطاء هذا الدواء للمرضى لفترة طويلة.