

ORIGINAL ARTICLE

# Enhanced efficacy and reduced side effects of diazepam by kava combination



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## ABSTRACT

The long term use of antiepileptic drugs possesses many unwanted effects; thus, new safe combinations are urgently mandated. Hence, the present study aimed to investigate the anticonvulsant effect of kava alone or in combination with a synthetic anticonvulsant drug, diazepam (DZ). To this end, female Wistar rats were divided into two subsets, each comprising 6 groups as follows: group (i) received 1% Tween 80 p.o. and served as control, while groups (ii) and (iii) received kava at two dose levels (100 and 200 mg/kg, p.o.). The remaining three groups received (iv) DZ alone (10 mg/kg p.o.) or kava in combination with DZ (v) (5 mg/kg, p.o.) or (vi) (10 mg/kg, p.o.). Results of the present study revealed that kava increased the maximal electroshock seizure threshold (MEST) and enhanced the anticonvulsant effect of diazepam following both acute and chronic treatment. Moreover, neither kava nor its combination with DZ impaired motor co-ordination either acutely or chronically. Furthermore, kava ameliorated both the reduction in locomotor activity as well as changes in liver function tests induced by chronic administration of DZ. Moreover, no elevation was shown in the creatinine concentration vs. control group following chronic administration of kava or DZ either alone or in combination with kava. In conclusion, the present study suggests the possibility of combining a low dose

**Abbreviations:** AED, antiepileptic drug; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BDZ, benzodiazepine; DZ, diazepam; ECT, electroconvulsive treatment; FDA, Food and Drug Administration; GABA,  $\gamma$ -aminobutyric acid; GABA<sub>A</sub>,  $\gamma$ -aminobutyric acid type A; MEST, maximal electroshock threshold; OTC, over the counter; WHO, World Health Organization.

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DZ with kava to reduce harmful effects and might be recommended for clinical use in patients chronically treated with this synthetic anticonvulsant drug.

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## Introduction

Conventionally, treatment of epilepsy is symptomatic using available synthetic antiepileptic drugs (AEDs) [1]. However, the incidence of a plethora of side effects with orthodox AEDs possesses major compliance limitations to epileptic therapies [2]. Many herbal sedatives, *viz* kava were thought to enhance the effects of antiepileptic drugs [3]. Kava extract (*Piper methysticum*), that is popularly used as an over the counter (OTC) anxiolytic drug, retains anticonvulsant action without impairing alertness or cognitive functioning [4]. Kavalactones, which constitute the major active components of kava extract, are thought to possess anticonvulsant efficacy [3,5].

A report by Gomes et al. [5] provided compelling evidence for the alternative use of kava in lieu of benzodiazepines (BDZs) in treating mild anxiety. Indeed, BDZs present an important class in treatment of epilepsy [6]; however, the incidence of side effects with the use of this class limits their utility [7]. Hence, it became the objective of the current investigation to study the effect of using either kava alone or its combination with diazepam (DZ), a BDZ compound, in an animal model of epilepsy. Moreover, the study investigated the reduction in incidence of side effects induced by DZ, thus providing evidence for the safe use in combination with kava.

## Material and methods

### Animals

Adult female Wistar rats, weighing 120–200 g (6–9 weeks) [8], obtained from the animal house of the National Research Center (Dokki, Giza, Egypt) were kept at a constant humidity ( $60 \pm 10\%$ ), temperature ( $23 \pm 2^\circ\text{C}$ ) and a light/dark (12 h) cycle. Animals were allowed standard laboratory chow (20% proteins, 5% fats, and 1% multivitamins) and water *ad lib*. *Animal care and experimental protocol complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by Research Ethical Committee of Faculty of Pharmacy Cairo University (Cairo, Egypt)*. All experiments were performed during the light phase of the light/dark cycle after at least a 60 min period of acclimatization to the experimental room. Female rats were used owing to their ability to eliminate drugs less rapidly than males [9]. Worthy of note, testing, and chronic treatments were performed at random times during the estrus cycle to minimize the effects of any cyclic changes in endogenous neurosteroids [10].

### Experimental design

Animals were divided into 2 major sets; each subset comprised 6 groups ( $n = 8\text{--}10$ ; each). The first subset of groups was utilized for testing behavioral (locomotor activity and muscle co-ordination), while the other subset was used for the anticonvulsant effect by inducing electroconvulsive shock. However,

biochemical assays were carried out on both subsets. Rats were allocated in the following groups (i) received 1% Tween 80 *p.o.* and served as control group, while groups (ii) and (iii) received lyophilized aqueous extract of kava (Atos Pharma, Sharqeya, Egypt) at two dose levels. The first dose (100 mg/kg, *p.o.*) was utilized according to previous work by Bilia et al. [11], while the second dose regimen (200 mg/kg, *p.o.*) was based on a previous pilot study. The remaining three groups received (iv) (DZ) alone (El-Nile Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt) at a dose of (10 mg/kg) [12] or kava in combination with DZ (v) (5 mg/kg, *p.o.*) or (vi) (10 mg/kg, *p.o.*). Treatments were carried out either acutely (1 h prior to testing) or on chronic basis (once daily for seven consecutive days) [13].

### *Induction of electroconvulsive shock by electroconvulsive treatment device (ECT)*

The anticonvulsant effect was evaluated by using electroconvulsive treatment for small mammals (ECT Unit, Ugo Basil, 57,800, Comerio, Italy). Briefly, rats were subjected to ascending alternating electric current via the ear electrodes of the ECT unit. Electric shock of duration 0.2 s, frequency 50 pulse/s and with pulse width 0.5 ms was generated by ECT unit starting from 1 mA till the end point (occurrence of tonic convulsions evidenced by hind limb extension) [14].

### *Locomotor activity test*

The locomotor activity of rats was assessed using microprocessor controlled activity cage (Ugo-Basile, Model No. 7430, Comerio, Italy). Before each exposure, animals were acclimatized for 1 h to the test room. Locomotor activity was measured as the total number of horizontal and vertical activity of each rat. This measurement is defined as the number of beam interruptions throughout a 5-min observation period [15]. Each drug was injected *p.o.* 60 min prior to locomotor activity testing [16].

### *Motor co-ordination assay*

The motor co-ordination or performance was investigated using accelerating rotarod (Ugo-Basile, Model No. 7750, Comerio, Italy, acceleration: 4–40 rpm in 5 min). The time between placing the subject on the rotating drum and that of its falling-off was recorded as the retention time. Twenty-four hours prior to the test execution, rats were subjected to 3–5 training sessions separated by at least 30 min in order to maintain their equilibrium on the test apparatus. A maximum of 300 s period was allowed for the animals to stay on the rod, with an accelerating velocity starting from 4 rpm and accelerated linearly to 40 rpm at the end of the session (5 min long) [17]. Only rats that succeeded to maintain their equilibrium on the rotarod for more than 120 s were selected for drug testing [13]. This selection step at the beginning was to ensure that all rats showed normal motor co-ordination.

### Biochemical tests

Animals used for biochemical analysis were euthanized and blood samples were collected and centrifuged at 4000 rpm (ependorff, Hamburg, Germany) for 10 min to obtain clear sera. Aspartate transaminase (AST; Quimica Clinica Aplicada S.A., Spain) [18]; ALT alanine transaminase (ALT; Quimica Clinica Aplicada S.A., Spain) [18]; alkaline phosphatase (ALP; biodiagnostics, Giza, Egypt) activities [19] and creatinine concentration (Stanbio laboratory, Boerne, TX) [20] were assessed according to the manufacturer's instructions.

### Statistical analysis

Data are expressed as mean of 8 experiments  $\pm$  S.E.M., and statistical comparisons were carried out using one way analysis of variance (ANOVA) followed by Tukey Kramer Multiple Comparisons Test. All analyses utilized *GraphPad Prism version 5*<sup>®</sup>. The minimal level of significance was identified at  $P < 0.05$ .

## Results

### Effect of kava, diazepam, or combination of kava with diazepam acutely and chronically on maximal electroconvulsive shock

Following acute treatment with kava (100 mg/kg), the seizure threshold was prolonged by 42% of control group. On the other hand, kava (200 mg/kg) prolonged seizure threshold by 83% from control, hence showing significant protection versus

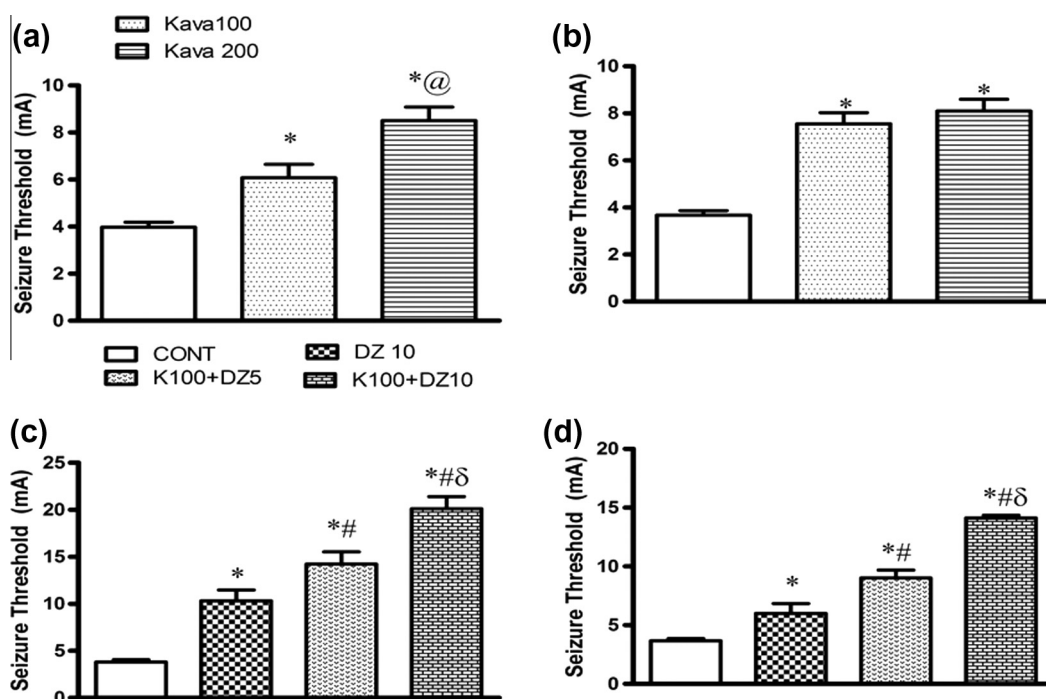
the lower dose. Chronically, kava (100 mg/kg) showed significant seizure threshold prolongation, by 48% from control group while kava (200 mg/kg) prolonged the seizure onset by 58% (Fig. 1a and b).

Following both acute and chronic DZ (10 mg/kg), seizure threshold was prolonged by 115% and 52% from control, respectively. Nevertheless, adding kava (100 mg/kg) to DZ (10 mg/kg) showed surprising increment in the seizure threshold, by 3 and 2 folds following both acute and chronic treatment, respectively. These results were significant from DZ (10 mg/kg) alone. By the same token, adding kava (100 mg/kg) to DZ (5 mg/kg), a significant increase in seizure threshold was observed by 2 folds and 108% from control values following both acute and chronic treatment, respectively (Fig. 1c and d).

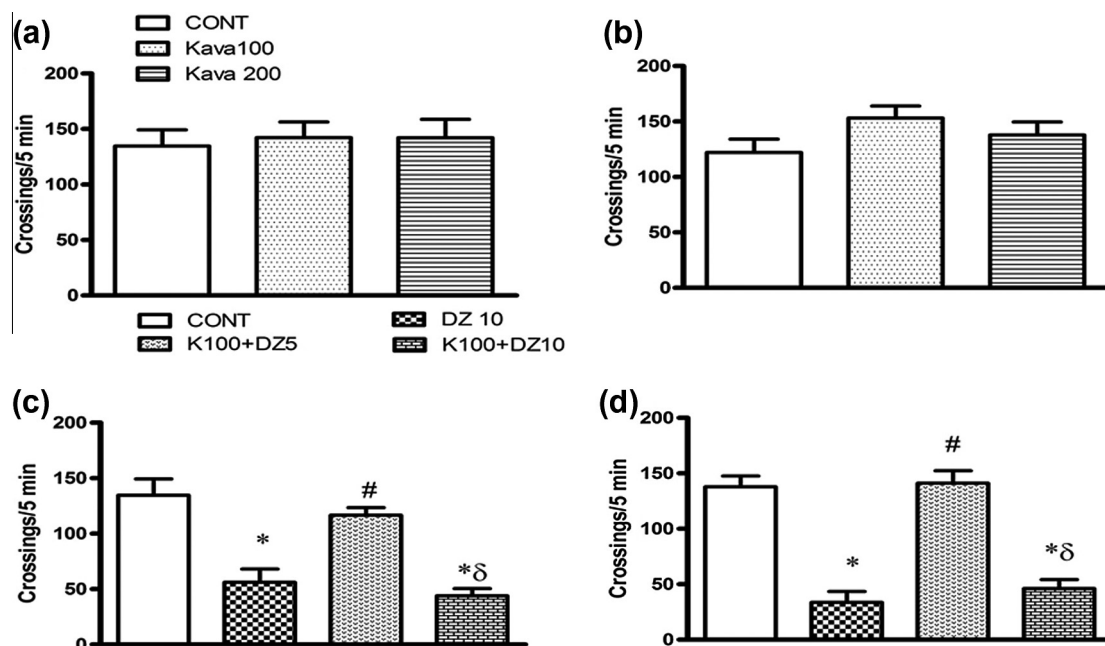
### Locomotor activity of kava, diazepam, or diazepam combination with kava

Following acute and chronic treatment with kava (100; 200 mg/kg, p.o.), there was no change in the locomotor activity vs. control (Fig. 2a and b). Conversely, acute and chronic treatment with either DZ (10 mg/kg) alone or in combination with kava reduced the locomotor activity of rats significantly vs control value (Fig. 2c and d).

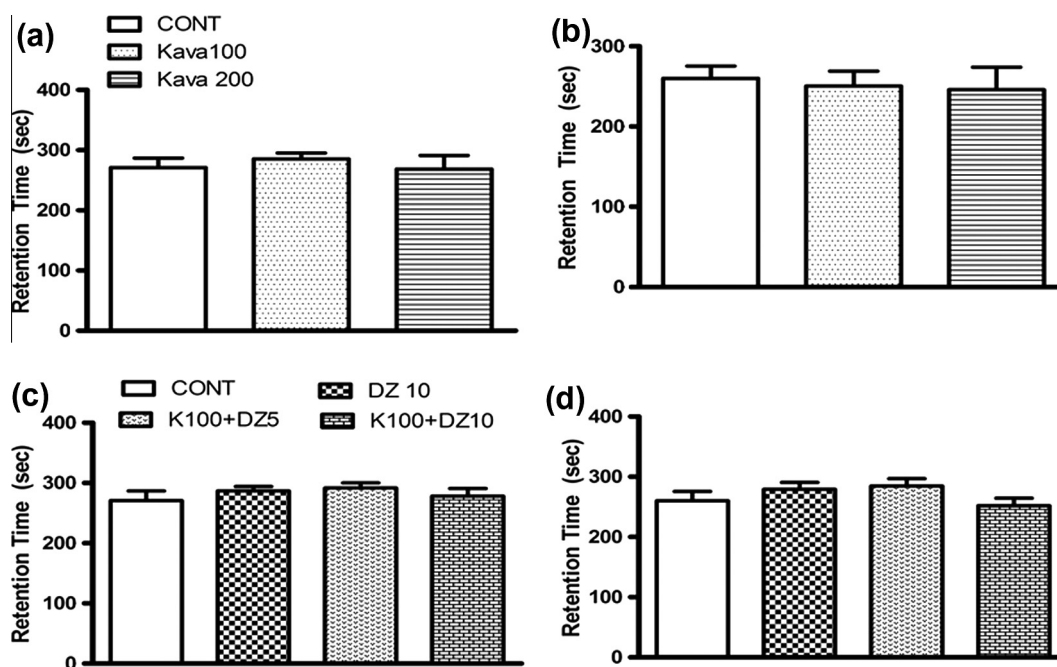
However, on combining kava (100 mg/kg) with DZ (5 mg/kg), rats showed no significant difference vs. control either acutely or chronically, i.e., the noticeable reduction in locomotor activity occurred with DZ individual treatment disappeared; moreover, locomotor activity became significantly higher than that of DZ alone (Fig. 2c and d).



**Fig. 1** Effect of kava alone (top panels) or in combinations with DZ (lower panels) under acute (a and c) or chronic (b and d) administration. Values are means ( $n = 8-10$ , each group)  $\pm$  S.E.M. \*, @, #,  $\delta$  different from control, Kava (100 mg/kg), DZ (10 mg/kg), or Kava(100 mg/kg)+DZ (5 mg/kg) respectively at  $P < 0.05$ . Statistical comparisons were carried out using one way ANOVA followed by Turkey-Kramer Multiple Comparison Test.



**Fig. 2** Effect of kava alone (top panels) or in combinations with DZ (lower panels) under acute (a and c) or chronic (b and d) administration. Values are means ( $n = 8-10$ , each group)  $\pm$  S.E.M. \*, @, #,  $\delta$  different from control, Kava (100 mg/kg), DZ (10 mg/kg), or Kava(100 mg/kg)+DZ (5 mg/kg) respectively at  $P < 0.05$ . Statistical comparisons were carried out using one-way ANOVA followed by Turkey–Kramer Multiple Comparison Test.



**Fig. 3** Effect of kava alone (top panels) or in combinations with DZ (lower panels) under acute (a and c) or chronic (b and d) administration. Values are means ( $n = 8-10$ , each group)  $\pm$  S.E.M.  $P < 0.05$ . Statistical comparisons were carried out using one-way ANOVA followed by Turkey–Kramer Multiple Comparison Test.

#### Motor co-ordination after treatment with the tested drugs

In the current study, neither kava nor DZ altered the motor co-ordination of rats either after acute or after chronic

treatment. Moreover, on adding kava to DZ at the two dose levels, 5 or 10 mg/kg, the motor co-ordination of rats did not change following the acute or chronic treatment (Fig. 3).



**Table 1** Liver function tests following kava chronic treatment at two dose levels.

Groups	AST activity (U/ml)	ALT activity (U/ml)	ALP activity (IU/L)
Control	42.4 ± 6.1	31.8 ± 3.9	149 ± 14.8
<i>Kava</i>			
100 mg/kg	51.8 ± 4.2	40.4 ± 5.3	174 ± 21.1
200 mg/kg	33.8 ± 1.7	38.7 ± 6.6	146 ± 13.5

Each value represents the mean (8–10 rats) ± S.E.M. Statistical comparisons ( $P < 0.05$ ) were carried out using one-way ANOVA followed by Turkey–Kramer Multiple Comparison Test.

#### *Changes in liver function tests following chronic treatment with kava, DZ or their combination*

Following chronic treatment with kava (100; 200 mg/kg, p.o.), there was no change in AST, ALT, and ALP vs. control (Table 1). Chronic treatment with DZ (10 mg/kg) alone or its combination with kava (100 mg/kg, p.o.) caused significant increase in the ALT activity from control value. Conversely, the combination of kava (100 mg/kg, p.o.) with DZ (5 mg/kg, p.o.) showed significant decrease in the ALT activity vs. DZ but not control (Fig. 4b). Meanwhile, chronic DZ (10 mg/kg) caused significant reduction in the ALP activity (Fig. 4c). However, the combination of kava (100 mg/kg) with DZ (5; 10 mg/kg) induced no change in ALP activity.

#### *Serum creatinine level following chronic treatment with kava, DZ, or combination of kava with DZ at two dose levels*

On chronic treatment with either kava (100; 200 mg/kg, p.o.) or DZ (10 mg/kg), no alteration in serum creatinine level vs. control was elicited (Table 2). Paradoxically, chronic combinations of kava (100 mg/kg, p.o.) with DZ (5; 10 mg/kg, p.o.) decreased serum creatinine level from control value (Table 2). Moreover, kava (100 mg/kg, p.o.), DZ (10 mg/kg, p.o.), and combinations of kava (100 mg/kg, p.o.) with DZ (5; 10 mg/kg, p.o.) did not alter the ratio of kidney weight/body weight (Table 2).

## Discussion

Antiepileptic therapy with limited side effects remains unattainable for many patients. The present study highlights the following major findings (i) kava, a drug of natural origin, increased the maximal electroconvulsive shock threshold (MEST), thus offering an anticonvulsive potential in rats. This protection was evident with both acute and chronic kava administration; (ii) combination of kava with diazepam (DZ) improved the protective effect of the latter on MEST; (iii) kava alone at the indicated dose levels did not alter normal locomotor activity nor motor co-ordination; in addition, (iv) kava in combination with DZ did not cause any change in motor co-ordination; (v) combining kava with DZ ameliorates the reduction in locomotor activity induced by chronic administration of DZ; (vi) safety of kava on both liver and kidney functions.

Kava, at both tested doses, (100; 200 mg/kg, p.o.), showed significant increase in the MEST compared to control group (1% Tween 80). These findings provide experimental evidence for an anticonvulsant effect either for acute or chronic administration (Fig. 1a and b), which is in line with findings by Bilia et al. [11]. The exact mechanism responsible for kava's action is

still undefined [5]; however, evidence supports that enhanced ligand binding to  $\gamma$ -aminobutyric acid (GABA) type A receptors plays a marked role in mediating the anticonvulsant effects of kava [4]. Jussofie et al. [21] studied kava extract using GABA<sub>A</sub> receptor agonist (muscimol), which showed enhancement of the binding to GABA<sub>A</sub> receptor in a concentration-dependent manner. This effect was independent of binding of kavalactones to GABA<sub>A</sub> and benzodiazepines receptors [11]. However, other mechanisms have been proposed for the anticonvulsant effect of kava. These mechanisms encompass blockade of voltage-gated sodium and calcium ion channels, positive modulation of the early K<sup>+</sup> outward current, diminished excitatory neurotransmitter release consequent to calcium ion channel blockade through a non-NMDA antigitamatergic action [4,11]. During acute exposure, kava dose dependently modulated MEST (Fig. 1a). Although kava at both dose levels modulated MEST (Fig. 1b), the (200 mg/kg, p.o.) showed no significant difference from the (100 mg/kg, p.o.) following chronic treatment. A plausible explanation for this observation resides in the fact that kavalactones lead to rapid up-regulation of GABA<sub>A</sub> receptor in the hippocampus and frontal cortex of rats which on chronic treatment suppresses the direct agonist effect of kavalactones on these receptors [21].

DZ acutely (10 mg/kg, p.o.) increased the seizure threshold of rats significantly from control (Fig. 1c) which is in line with Meierkord et al. [22]. Diazepam, a benzodiazepine, acts as an allosteric agonistic modulator of the post- and pre-synaptic GABA<sub>A</sub> receptor chloride channel complex [11]. Although following acute DZ administration (10 mg/kg, p.o.), seizure threshold increased by 115%; however, in chronic paradigm, the threshold increased only by 52%, a finding that points toward development of tolerance to DZ anticonvulsant action; such observation corroborates previous reports [23–25].

In fact, subsensitivity to GABA action has been demonstrated following chronic benzodiazepine treatment [7].

In the present study, the co-administration of kava (100 mg/kg, p.o.) whether acutely or chronically with either DZ (10 mg/kg, p.o.) or DZ (5 mg/kg, p.o.) improved their anticonvulsant activity against electro-induced convulsions. Surprisingly, co-administration of kava (100 mg/kg, p.o.) with DZ (5 mg/kg, p.o.) elicited enhanced protection than DZ (10 mg/kg) alone (Fig. 1c and d). Noteworthy, on acute or chronic combination of kava (100 mg/kg, p.o.) with DZ (5 mg; 10 mg/kg), the seizure threshold increased significantly vs. DZ (10 mg/kg, p.o.) alone. These results are suggestive for enhancement effect of kava on diazepam after acute and chronic administration.

Although Wafford [7] stated that kavalactones are centrally acting skeletal muscle relaxants, the current study provided

evidence that kava at the two designated dose levels did not alter motor co-ordination activity whether acutely or chronically (Fig. 3a and b). By the same token, the previous finding holds true for DZ at the designated dose (Fig. 3c and d), which is in line with a report by Felipe et al. [26]. It was also noted that following acute and chronic combinations of kava with DZ, no motor impairment was observed compared to control.

Moreover, kava at the two dose levels (100; 200 mg/kg, p.o.) did not alter the locomotor activity vs. control group following both acute and chronic treatment (Fig. 2a and b). Nevertheless, Garrett et al. [27] provided evidence that kava dose-dependently decreased locomotor activity; however, one might argue that the discrepancy in the current finding and the reported literature might be attributed to species difference. In addition, in a prolonged study, ataxia, lethargy, and abnormal breathing were evident in female rats at 1 and 2.0 g/kg following 2 weeks of treatment with Kava [28]. Based on the behavioral findings, one might deduce that kava may exert its anticonvulsant action directly on the central nervous system and not through sedation.

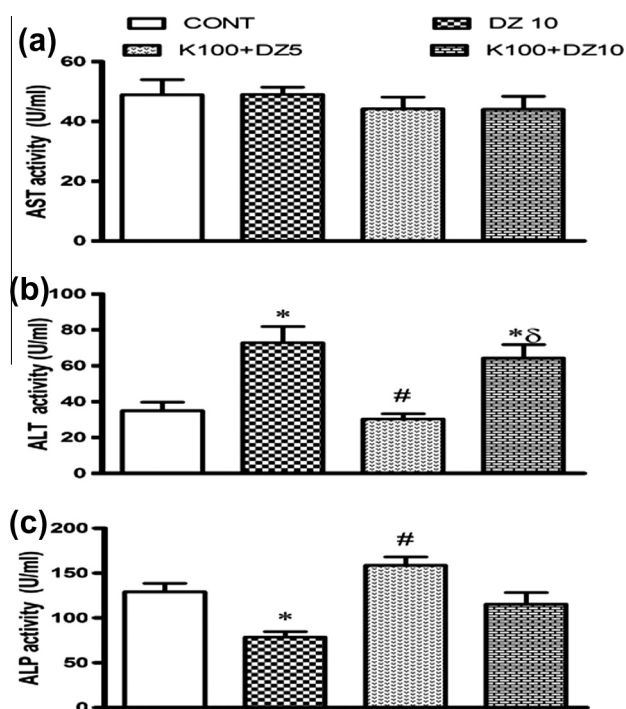
Noteworthy, DZ (10 mg/kg, p.o.) reduced locomotor activity in both acute [12,27] and chronic [29] exposures compared to control counterparts (Fig. 2c and d). This diminution in locomotor activity of rats following to DZ administration is in line with findings by Himmel et al. [12]. By the same token, the combination of kava and DZ (10 mg/kg) caused significant reduction in locomotor activity of rats following acute as well as chronic treatment (Fig. 2c and d). A plausible explanation for this phenomenon might be attributed to the ability of kava

to enhance the binding of GABA<sub>A</sub> ligands to its receptor in a concentration-dependent manner [21]. This notion is further supported by the finding that combination of kava with DZ (5 mg/kg) showed no diminution in locomotor activity. Furthermore, Garrett et al. [27] stated that DZ caused decrease in locomotor activity in a dose-dependent manner.

Since kava-containing products were reported to develop liver failure requiring liver transplantation that occurred in some patients, the Food and Drug Administration (FDA) issued a consumer advisory in 2002 about the potential risk associated with the use of these products [30]. However, the data presented in Table 1 support the potential safety of kava on liver enzymes activity where chronic kava administration elicited no elevation of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) at the two dose levels. Noteworthy, it was previously reported that kava, at similar doses to those employed in the current study, did not affect neither liver enzymes nor cytochrome-P450 isoforms [31]. By the same token, Singh and Devkota [32] demonstrated that daily dose of 200 or 500 mg of kava did not alter liver functions manifest as alkaline phosphatase, lactate dehydrogenase nor AST and ALT. Moreover, Noor (2010) showed not only no change in liver functions but also showed significant reduction in AST and ALT, suggesting not only a lack of toxicity but potentially a hepatoprotective effect of kava [33]. However, one cannot rule out that different extraction methods and the solvents employed in the preparation of kava-containing products might be account for the reported hepatotoxicity associated with kava. Nevertheless, in the current study, the employed kava extract was the aqueous one, which has been shown to exert hepatoprotection [34].

In the current study, chronic administration of DZ (10 mg/kg) did not alter the activity of AST (Fig. 4a) but increased the activity of ALT (Fig. 4b). However, adding kava (100 mg/kg, p.o.) to DZ (10 mg/kg, p.o.) decreased the percent elevation occurring with DZ (10 mg/kg, p.o.) alone. On the contrary, this elevation disappeared completely following administration of kava (100 mg/kg) with low dose of DZ (5 mg/kg, p.o.) chronically. However, the activity of ALP on chronic treatment with the combination of kava (100 mg/kg) with DZ (5 or 10 mg/kg) showed no significant change in ALP activity vs. control, although DZ individual treatment showed significant decrease vs. control. This may be explained in view of enzyme induction property of the combination, thus inducing metabolism of DZ.

On testing serum creatinine as an indicator to kidney function, neither of the two doses tested for kava following chronic administration caused any change in the creatinine level vs. control (Table 2). Accordingly, the present study showed no adverse effects of kava on liver and kidney function parameters, the matter which is consistent with Noor [33]. Following creatinine assaying, data showed no change in the creatinine concentration of DZ (10 mg/kg, p.o.), after chronic administration (Table 2). Only the combinations of DZ (5 and 10 mg/kg) with kava (100 mg/kg) decreased the creatinine blood level significantly vs. control group. This low creatinine level may be due to decrease in muscle mass, but that is not the case in the herein study since there was no significant lose in body weight (Fig. 5). That may be attributed to the metabolic property of DZ that causes diazepam to increase renal clearance of creatinine, hence decreasing its serum concentration [35].



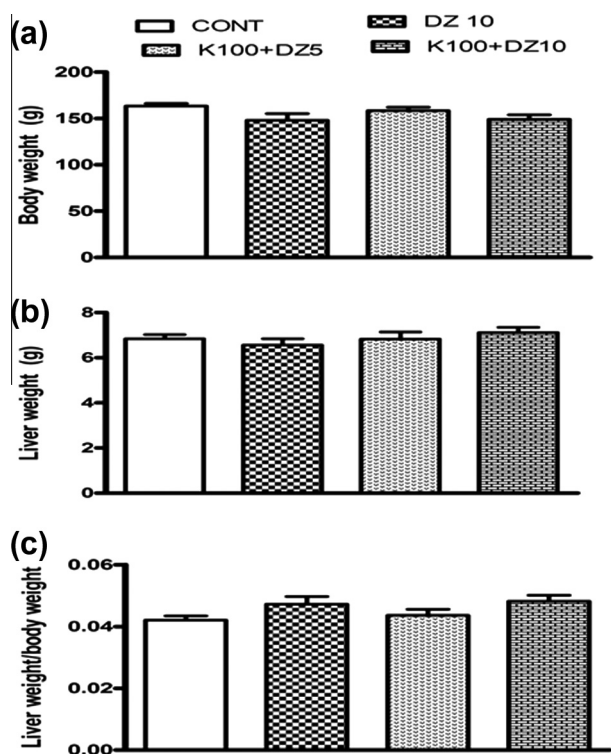
**Fig. 4** Effect of chronic administration of kava in combination with DZ on (a) AST, (b) ALT and (c) ALP activities. Values are means ( $n = 8-10$ , each group)  $\pm$  S.E.M. \*, @, #,  $\delta$  different from control, DZ (10 mg/kg), or Kava (100 mg/kg)+DZ (5 mg/kg) respectively at  $P < 0.05$ . Statistical comparisons were carried out using one-way ANOVA followed by Turkey-Kramer Multiple Comparison Test.

**Table 2** Effect of chronic treatment with kava on serum creatinine level and relative kidney weight following chronic treatments.

Groups		Serum creatinine level (mg/dL)	Relative kidney weight
Control		1.66 ± 0.12	0.0071 ± 0.0001
Kava	100 mg/kg	1.21 ± 0.08	0.0067 ± 0.0003
	200 mg/kg	1.39 ± 0.05	0.0065 ± 0.0002
DZ	10 mg/kg	1.33 ± 0.06	0.0071 ± 0.0002
Kava (100 mg/kg) +	DZ (5 mg/kg)	1.15 ± 0.1 <sup>a</sup>	0.0071 ± 0.0005
	DZ (10 mg/kg)	1.19 ± 0.07 <sup>a</sup>	0.0072 ± 0.0002

Each value represents the mean (8–10 rats) ± S.E.M. Statistical comparisons ( $P < 0.05$ ) were carried out using one-way ANOVA followed by Tukey–Kramer Multiple Comparison Test.

<sup>a</sup> Vs control.



**Fig. 5** Effect of chronic administration of kava in combination with DZ on (a) body weight, (b) liver weight, and (c) liver weight/body weight. Values are means ( $n = 8$ –10, each group) ± S.E.M. Statistical comparisons were carried out using one-way ANOVA followed by Turkey–Kramer Multiple Comparison Test.

## Conclusions

Collectively, data presented in this study point toward the feasibility of combining kava (100 mg/kg) together with a lower dose DZ than conventional therapy to treat epilepsy, particularly, chronically. This is evident by the ability of such a combination to increase MEST threshold without affecting either motor co-ordination or locomotor activity, i.e., conserving normal lifestyle. Moreover, this combination protected against incidence of liver or kidney functional changes that render this combination a safe alternative for synthetic anticonvulsants without loss of therapeutic efficacy. It is recommended for

future researchers to carry out histopathological investigation on liver and kidney tissues to ensure the safety of the herein combination on body organs. Moreover, it would be interesting to further assess the mechanism behind the improving effect of kava in combination with DZ and the effect of kava and its combination on brain mediators as well.

## Conflict of interest

The authors have declared no conflict of interest.

## Acknowledgement

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