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Antihyperglycaemic effect of kava kava *(piper methysticum)* in streptozotocin-induced diabetic rats

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ABSTRACT

The present study aimed to investigate the possible effects of kava kava (KK) (Piper methysticum) on the hyperglycaemia induced in rats by streptozotocin (STZ; 55 mg/kg; i.p.). Blood samples, were used to determine serum levels of glucose, insulin, total cholesterol (TC) and triglycerides (TG). Some relevant markers for oxidative stress viz., serum lipid peroxides level (measured as malondialdehyde; MDA) and total antioxidant capacity as well as serum nitric oxide (NO) level measured as nitrate/nitrite were determined. Hyperglycaemic animals received orally KK (200 mg/kg) or (500 mg/kg) on daily basis for 28 consecutive days and their effects were determined 24 h after the administration of the last dose. In addition to another group received gliclazide (5 mg/kg; p.o.) as a reference drug. Results of the present study revealed that STZ-induced hyperglycaemia is associated with decreased serum insulin level with increased level of TC and TG. Hyperglycaemia was also associated increased level of plasma MDA together with decreased total antioxidant capacity and level of plasma NO. KK was able to improve the hyperglycaemia in STZ injected rats with an increase in the serum insulin level and a decrease in the level of TG. The higher dose of KK showed a restoration of the increased serum level of TC and MDA and of the suppressed insulin and total antioxidant capacity as well as the decreased plasma level of NO. From our results it can be conclude that KK has an antihyperglycaemic effect and has the ability to attenuate some of its associated abnormalities.

Keywords: Kava kava, antihyperglycaemia, streptocotocin, diabetes, rats

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INTRODUCTION

Diabetes mellitus (DM) a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. DM is known to be associated with neurological complications in both the peripheral nervous system (PNS) and the central nervous system (CNS) [2]. Even though insulin secretion is mainly regulated by changes in circulating concentrations of glucose and other metabolic fuels, stimuli such as neurotransmitters and gastrointestinal hormones makes an important contribution to the overall regulation of pancreatic beta cell function. Controlling blood sugar is essential for avoiding long-term complications of DM like learning and memory deficit.

Anxiety disorders and DM are one of the most common psychiatric and endocrine disorders in general populations, respectively [3, 4]. The incidence and prevalence of anxiety disorders are very common in diabetic patients than in the general population. In diabetic patients, recognition and control of anxiety symptoms are of utmost importance to increase compliance with treatment and to improve quality of life [5]. The progression of DM is associated with an impaired ability of the neurons in the CNS to release neurotransmitters resulting in behavioral changes [6].

Kava Kava (KK) is a traditional beverage prepared from the roots and rhizome of the KK plant, *Piper methysticum*, which has been widely used for centuries throughout the South Pacific, without any apparent side effects except dermopathy. In the late 1990s, commercial KK products gained immense popularity in Europe and North America as an effective treatment option for anxiety [7]. The organic-solvent extracts of the KK root are promoted for relaxation to relieve stress, anxiety, and tension along with relief of sleeplessness and symptoms of menopause. These psychological effects are thought to be the result of the non specific, complex and multifaceted effects of several kavalactones produced by *Piper methysticum* on the human CNS. These effects include alterations in the activity of voltage gated ion channels and the modulation of several neurotransmitter systems [7-9]. Recently, it has been advised consumers of possible liver injury to discontinue the use of KK-containing supplements [10].

The present study was devoted to investigate the influence of KK, an anxiolytic, on the hyperglycemia induced in streptozotocin (STZ)-treated rats which was induced by a single intraperitoneal (i.p.) injection of streptozotocin. The drug was administered orally once per day for 28 consecutive days and their effects were evaluated 24 h after the administration of the last dose. The serum levels of glucose, insulin, total cholesterol (TC) and triglycerides (TG) were determined. Moreover, the blood levels of some relevant biomarkers for oxidative stress and NO were determined. Plasma lipid peroxides level (measured as malondialdehyde; MDA) and total antioxidant capacity activity were taken as *in vivo* reliable indices for the contribution of free radical generation and in turn, oxidative stress in STZ-induced hyperglycaemia. Plasma nitrate/nitrite level was used as a convenient marker for NO formation. The effects produced by this treatment are compared with standard drug gliclazide.

MATERIALS AND METHODS

2.1. Drugs and chemicals

Kava kava (October Pharma, Egypt) and gliclazide (Servier, France) was used in the present investigation. The drugs were freshly prepared in distilled water and given orally. The concentration of the drugs were adjusted so that each 100 g animal body weight received 0.5 ml, containing the required dose. Streptozotocin, N-(1-Naphthyl) ethylene-diamine dihydrochloride (NEDD) were purchased from Sigma-Aldrich, USA. Thiobarbituric acid (TBA) and Sulphanilamide were purchased from Fluka (Italy) and Merck (Germany), respectively. All other chemicals were of the highest commercially available grade.

2.2. Animals

Adult male albino rats, weighing 180-250 g, were used in this study. They were obtained from the Animal House Colony of the National Research Center (Dokki, Giza, Egypt), and were housed under conventional laboratory conditions throughout the period of experimentation. The animals were fed a standard rat pellet diet and allowed free access to water. The study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of National Research Center.

2.3. Induction of hyperglycaemia

Hyperglycaemia was induced by a single i.p. injection of STZ (55 mg/kg) [11]. Briefly, rats were weighed and injected with STZ dissolved in a citrate buffer (0.1 M, pH 4.5). After 48 h blood samples were withdrawn from the retro-orbital venous plexus under light ether anaesthesia and the plasma was separated by centrifugation for the determination of glucose level. Only rats with plasma glucose levels more than 250 mg/dl were selected and considered as hyperglycaemic animals that have been subjected to further experimentation.

2.4. Experimental design

Hyperglycaemic rats were weighed and randomly allocated into 3 groups (8-10 rats each). One group served as hyperglycaemic control, while the other 2 groups were treated orally with KK (200 mg/kg/day) and (500 mg/kg/day) [12] for 28 consecutive days, respectively. Drugs treatment were started 48 h after STZ injection after hyperglycaemia was confirmed. In addition, a universal normal group which received only the citrate buffer (8-10 rats) was used. Twenty-four hours after the last dose of either drug treatment, serum was collected and prepared for the further biochemical determination.

Biochemical parameters assessment

Blood sample was withdrawn from the retro-orbital venous plexus using heparinized capillary tubes from each 18 h food-deprived rat. The blood samples were centrifuged at 3000 rpm for 10 min and the sera were obtained. An

aliquot of the separated serum was then used for the determination of glucose level, TC and TG while the rest was stored at -70ºC for the subsequent determination of insulin, MDA, NO levels and the total antioxidant capacity.

2.4.1. Determination of serum glucose level
Glucose level was determined as quinine amine using a test reagent kit (Stanbio, USA) according to the method of Tinder (1969) [13]. The absorbance was measured at 510 nm and the results were expressed as mg/dl.

2.4.2. Determination of serum insulin level
Serum insulin concentration was determined by enzyme-linked immunosorbent assay by using a diagnostic kit (Crystalchem, USA) according to the method of Judzewitsch *et al.* (1982) [14]. The absorbance was measured at 450 nm using ELISA reader and the results were expressed as µIU/ml.

2.4.3. Determination of serum triglycerides level
Triglycerides was estimated by enzymatic methods by using diagnostic kit (Biodiagnostic, Egypt) according to the method of Fossati and Prencipe (1982) [15]. The absorbance was measured at 510 nm and the results were expressed as mg/dl.

2.4.4. Determination of serum total cholesterol level
Total cholesterol was estimated by enzymatic methods by using diagnostic kit (Biodiagnostic, Egypt) according to the method of Allain *et al*. (1974) [16]. The absorbance was measured at 500 nm and the results were expressed as mg/dl.

2.4.5. Determination of serum lipid peroxides level
Lipid peroxides level was determined as thiobarbituric acid (TBA)-reactive substances according to the method of Mihara and Uchiyama (1978) [17]. The absorbance was measured at 534 nm and the results were expressed as nmol/ml.

2.4.6. Determination of serum nitric oxide level
The total amount of NO was indirectly estimated in terms of its main metabolites, nitrate and nitrite by the Griess reaction using NEDD and sulphanilamide as described by Miranda *et al*. (2001) [18]. The absorbance was measured at 540 nm and the results were expressed as nmol/ml.

2.4.7. Determination of total antioxidant capacity
Total antioxidant capacity was determined by ability to eliminate a certain amount of the provided H_2O_2 using a test reagent kit (Biodiagnostic, Egypt) according to the method of Koracevic *et al.* (2001) [19]. The absorbance was measured at 510 nm and the results were expressed as mM/l.

Statistical analysis
Data are expressed as means ± SEM. Statistical significance was taken as $P<0.05$ for all experiments, using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test to judge the difference between various groups for the parametric parameters.

RESULTS

3.1. Effect of KK on serum glucose level
A single i.p. injection of STZ (50 mg/kg) produced an elevation of serum glucose level which was evidenced 48 h after administration. The elevation was found to be persistent during the period of investigation and increased by an average value reaching about 370% of the normal values. Oral treatment of hyperglycaemic rats with gliclazide for 14 consecutive days decreased the elevated serum glucose level reaching about 210% of the normal value. Similar treatment with KK (200 mg/kg/day) and (500 mg/kg/day) decreased the elevated serum glucose level reaching about 147% and 140% of the normal values, respectively. Oral treatment of hyperglycaemic rats with gliclazide for 28 consecutive days succeeded to cause a decrease in the elevated serum glucose level reaching nearly the normal value. Similar treatment with KK (200 mg/kg/day) and (500 mg/kg/day) succeeded to cause a decrease in the elevated serum glucose level reaching about 133% and 117% of the normal values, respectively (Figure 1).

3.2. Effect of KK on serum insulin level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was accompanied with a decreased in the serum insulin level reaching about 6% of the normal value. Oral treatment of hyperglycaemic rats with gliclazide for 28 consecutive days caused a decrease in the serum insulin level reaching about 40% of the normal values. Similar treatment with KK (200 mg/kg/day) and (500 mg/kg/day) caused an increase in the serum insulin level reaching about 21% and 35% of the normal values, respectively (Figure 2).

3.3. Effect of KK on serum TC level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was associated with an increase in the serum TC level reaching about 170%. Oral treatment of hyperglycaemic rats with gliclazide for 28 consecutive days succeeded to cause a decrease in the elevated serum TC level reaching nearly the normal value. Similar treatment with KK (200 mg/kg/day) did not show any change in the serum TC level, however treatment with KK (500 mg/kg/day) succeeded to reach the normal value of the serum TC level (Table 1).

3.4. Effect of KK on serum TG level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was associated with an increase in the serum TG level by about 260% of the normal value. Oral treatment of hyperglycaemic rats with gliclazide for 28 consecutive days succeeded to cause a decrease in the elevated serum TC level reaching nearly the normal value. Similar treatment with KK (200 mg/kg/day) showed a decrease in the serum TG level reaching about 115% of the normal value, however treatment with KK (500 mg/kg/day) succeeded to reach the normal value of the serum TG level (Table1).

3.5. Effect KK on serum MDA level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was encountered with an elevated plasma MDA level by about 3 fold rise of the normal value. Oral treatment of hyperglycaemic rats with gliclazide for 28 consecutive days succeeded to cause a decrease in the elevated serum MDA level reaching nearly the normal value. Similar treatment with KK (200 mg/kg/day) did not show any change in the serum MDA level, however, treatment with KK (500 mg/kg/day) showed a decrease in the serum MDA level reaching about 193% of the normal value (Table 1).

3.6. Effect of KK on serum NO level

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was accompanied with a decreased plasma NO level reaching about 44% of the normal value. Oral treatment of hyperglycaemic rats with gliclazide for 28 consecutive days succeeded to cause an increase in the decreased serum NO level reaching about 81% of the normal value. Similar treatment with KK (200 mg/kg/day) and (500 mg/kg/day) showed an increase in the serum NO level by about 70% and 80%, respectively (Table 1).

3.7. Effect of KK on serum total antioxidant capacity

Induction of hyperglycaemia with a single i.p. injection of STZ (50 mg/kg) was accompanied with a decreased plasma NO level reaching about 39% of the normal value. Oral treatment of hyperglycaemic rats with gliclazide for 28 consecutive days succeeded to cause an increase in the elevated serum NO level reaching about 88%. Similar treatment with KK (200 mg/kg/day) did not show any change in the serum total antioxidant capacity, however, treatment with KK (500 mg/kg/day) showed an increase in the serum total antioxidant capacity reaching about 67% (Table 1).

DISCUSSION

Streptozotocin-induced hyperglycaemia in animals is considered to be a potent model for the preliminary screening of compounds active against diabetes and is widely used [20]. Results of the present study revealed that STZ-induced hyperglycaemia is associated with some biochemical changes. These changes were manifested as an elevated serum level of glucose which is a direct consequence from the decreased serum level of insulin and the destruction of the beta cells of the pancreas. STZ selectively destroys the islets of Langerhans by oxidant production and producing inappropriate NO response [21]. Elevation of the TC and TG is also one of the characteristics of hyperglycaemia together with increase in the oxidative stress [22].

Persistent hyperglycaemia, the common characteristic of DM, is the cause of the most diabetic complications which can be normalized by the action of insulin [23]. Blood glucose level is strictly controlled by insulin secretion from pancreatic cells and insulin action on liver, muscle and other target tissues [24]. Since, insulin has important regulatory effects on lipid as well as glucose metabolism, and therefore it is expected that DM is associated with significant abnormalities in lipid metabolism. Hyperlipidemia in DM results from altered lipoprotein metabolism due to increased production, decreased clearance, and/or changes in insulin, glucose and free fatty acid levels associated with DM [25]. These abnormalities can usually be reversed with glycemic control [26].

The present study also provides a further evidence that STZ-induced hyperglycaemia in rats is accompanied by increased levels of MDA together with depleted levels of NO in the serum concomitantly with suppression in the total antioxidant capacity. It has been proven that induction of experimental hyperglycaemia with STZ results in enhanced lipid peroxidation. Hyperglycaemia is considered one of the factors responsible for the development of oxidative stress that results from enhanced formation of ROS by glucose autooxidation. Oxidative stress in DM coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of the free radicals [27].

Oral treatment of hyperglycaemic rats with KK (200 mg/kg and 500 mg/kg; p.o.) for 28 consecutive days succeeded to elevate the insulin serum level with a subsequent reduction in the serum glucose level. Moreover, similar treatment with KK (200 mg/kg and 500 mg/kg; p.o.) showed a decrease in the serum TG level and elevation in the serum NO level.

In the current study, it has been also observed that the higher dose of KK succeeded to reduce the serum levels of TC and MDA i.e. attenuated the lipid peroxidation and the total antioxidant capacity which suggested that KK have an antioxidant property. This result was in accordance with Singh and Devkota (2003)[12] who found that the level of MDA in the liver was reduced upon the administration of KK suggesting the lack of its toxic effect. On the other hand, other studies had proposed an increase in the formation of MDA and correlated this elevation with increase in the liver enzymes [10]. However, Singh and Devkota (2003)[12] documented an absence of a direct link between the kavalactones and hepatotoxicity.

Kava kava *(Piper methysticum)* has an anxiolytic activity. Clinical studies have shown that kavalactones are effective in the treatment of anxiety at subclinical and clinical levels, anxiety associated with menopause and anxiety due to various medical conditions. This effect is achieved due to pharmacological properties of KK which 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 norepinephrine, reversible inhibition of monoamine oxidase B thus, increasing dopamine levels [7, 28]. Dopamine in the CNS is involved in the control of both motor and emotional behaviour [29] and peripherally modulates insulin secretion in the pancreatic islets [30].

Kava kava also suppresses the synthesis of the eicosanoid thromboxane A(2), which antagonises GABA(A) receptor function [7, 28] which exerts protective and regenerative effects on islet beta cells and reverses DM through restoration of the β-cell mass and reverses the disease. Furthermore, it has been suggested that GABA suppresses insulitis and systemic inflammatory cytokine production. The β-cell regenerative and immunoinhibitory effects of GABA provide insights into the role of GABA in regulating islet cell function and glucose homeostasis, which may find clinical application [31].

CONCLUSION

From the experimental findings in this study, we can conclude that KK supplementation has beneficial effect in improving hyperglycaemia in STZ-treated rats and has beneficial effect in restoring some of its associated abnormalities. These effects might be related to its anxiolytic activity.

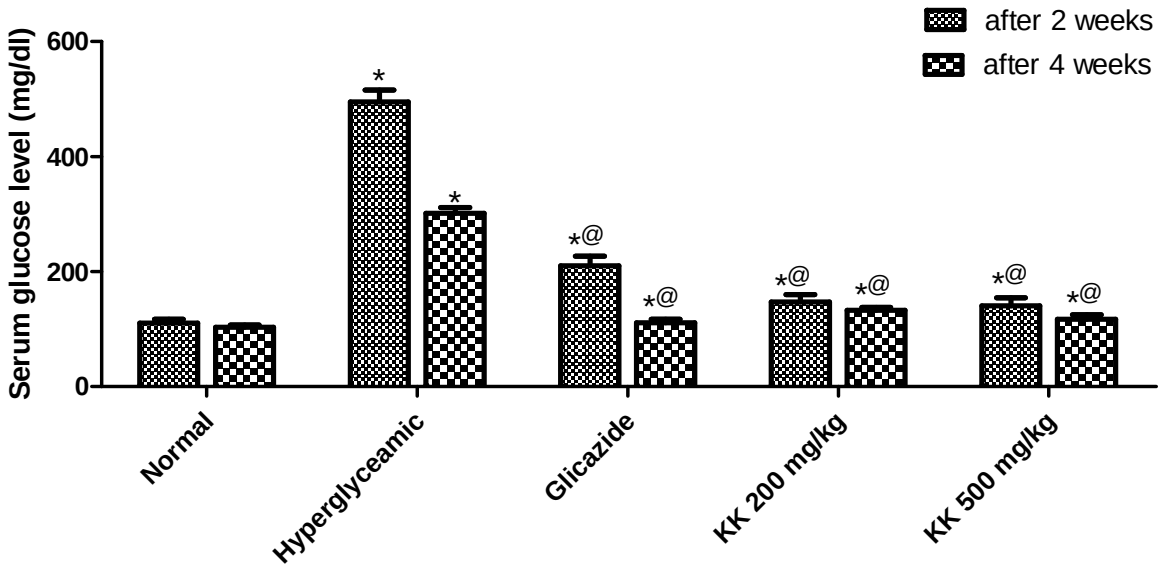

Figure (1): Effect of kava kava on serum glucose level in STZ-treated rats.

Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 50 mg/kg). Kava kava (KK; 200 mg/kg and 500 mg/kg; p.o.) was administered for 28 consecutive days. Treatment with either drug was started 48 h after STZ injection. Blood samples from 18 h food deprived animals were withdrawn using heparinized capillary tubes and serum was used for glucose determination 48 h after STZ injection, after 14 consecutive days of treatment and twenty-four hours after the last dose. Results are expressed as means ± SEM (n =6–10). *Significant difference from normal rats $P < 0.05$. @ Significant difference from hyperglycaemic rats $P < 0.05$.

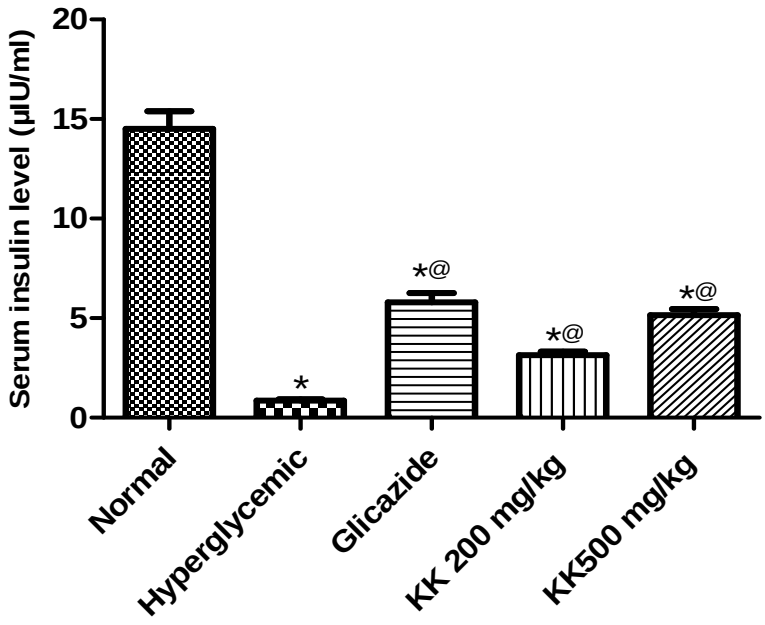

Figure (2): Effect of kava kava on serum insulin level in STZ-treated rats.

Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 50 mg/kg). Kava kava (KK; 200 mg/kg and 500 mg/kg; p.o.) were administered for 28 consecutive days weeks. Treatment with either drug was started 48 h after STZ injection. Blood samples from 18 h food deprived animals were withdrawn using heparinized

capillary tubes and serum was used for insulin determination. Results are expressed as means ± SEM (n=6–10). *Significant difference from normal rats P <0.05. @ Significant difference from hyperglycaemic rats P < 0.05.

Table (1): Effect of kava kava on serum levels of triglycerides, total cholesterol, nitric oxide, malondialdehyde and total antioxidant capacity in STZ-treated rats

	Normal	Diabetic	Glicazide	KK 200 mg/kg	KK 500 mg/kg
TC (mg/dl)	72.67±3.57	123.49±10.59*	67.21±2.10*@	116.93±8.57*	81.42±7.87*@
TG (mg/dl)	51.16±2.40	133.11±11.29*	49.92±1.84*@	58.83±3.27@	52.55±2.61@
NO (µM)	89.33±4.9	38.94±2.11*	72.01±2.35*@	62.33±5.38*	71.45±5.89*@
MDA (nmol/ml)	74.03±3.26	216.98±1.19*	86.64±2.40*@	189.74±4.53	143.16±5.07*@
Total antioxidant capacity (mM/l)	0.404±0.022	0.157±0.006*	0.357±0.024*@	0.160±0.013*	0.272±0.019*@

Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 50 mg/kg). Kava kava (KK; 200 mg/kg and 500 mg/kg; p.o.) were administered for 28 consecutive days weeks. Treatment with either drug was started 48 h after STZ injection. Blood samples from 18 h food deprived animals were withdrawn using heparinized capillary tubes and serum was used for determination of TG, TC, NO, MDA and total antioxidant capacity. Results are expressed as means ± SEM (n=6-10). * Significant difference from normal rats P < 0.05. @ Significant difference from hyperglycaemic rats P < 0.05.

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