RESEARCH ARTICLE

Influence of co-administration of Khat (Catha edulis Forsk) and metformin on metabolic syndrome in high fructose diet induced type 2 diabetes in rats

Nabil Ahmed Albaser1,2, Abdel-Wahab H Mohamad2, Mohammed Amood Al-Kamarany3

1Department of Pharmacology, Faculty of Medical Sciences and Pharmacy, Al Razi University, Sana’a, Yemen, 2Department of Therapeutics, Pharmacy College, National Ribat University, Burri, Khartoum, Sudan, 3Department of Pharmacy Practice, Faculty of Clinical Pharmacy, Hodeidah University, Hodeidah, Yemen

Correspondence to: Nabil Ahmed Albaser, E-mail: nabilalbaser2020@gmail.com

Received: April 30, 2021; Accepted: May 31, 2021

ABSTRACT

Background: Despite numerous reports regarding the associations of Khat chewing with serious health adverse effects, a significant number of people worldwide uses Khat daily, especially in its origin countries. The risk of co-administration of Khat and drugs that is high among these individuals, leading to increase probability of adverse Khat-drug interactions. Under these circumstances, it becomes important to evaluate the effect of concomitant administration of Khat and clinical drugs. Metformin is a drug of biguanide class used to lower blood glucose concentrations in patients with type 2 diabetes mellitus (T2DM).

Aim and Objectives: The aim of the study was to evaluate the beneficial or harmful effects of concomitant administration of Khat and metformin in a high fructose diet induced T2DM model.

Materials and Methods: Thirty-six adult male rats weighing 120–150 g were made diabetic by feeding a high fructose diet for 4 weeks. These rats while continuing to be on high fructose diet were divided into six groups and administered orally, normal control (normal saline), normal control with Khat (1500 mg/kg), non-treated diabetic group, Khat treated group (1500 mg/kg), metformin treated diabetic (300 mg/kg), and metformin (300 mg/kg) with Khat (1500 mg/kg) treated group. All the above treatments were given for 4 weeks and their effects were studied.

Results: The hyperglycemia, body weight, and lipid profile parameters were brought down significantly by Khat and metformin (P < 0.05) monotherapy; metformin being more effective than Khat. The metformin-Khat combination had a synergistic effect and resulted in much greater efficacy in ameliorating the parameters. Moreover the adverse effect of Khat like lowering of high-density lipoprotein cholesterol (HDL-C) levels was significantly mitigated by metformin-Khat combination.

Conclusion: The results suggested that metformin-Khat combination had a much greater efficacy with the added advantage of reducing the adverse effects.

KEY WORDS: Khat; Catha edulis; Metformin; Interactions; Combination

INTRODUCTION

Diabetes mellitus (DM) affects nearly 4% of the population worldwide and is expected to increase by 5.4% in 2025. It is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

Catha edulis, commonly known as “Khat”, is tree or large shrub that is endogenously found in Arab peninsula especially in Yemen, some African countries such as Ethiopia and Kenya, and in western Asia. For centuries, Khat had been used traditionally, mainly for its psychostimulant,
Khat contains more than forty alkaloids, glycosides, tannins, amino acids, vitamins, and minerals. The environmental and climate conditions determine the chemical profile of Khat leaves. In Yemen, about 44 different types of Khat exist originating from different geographic areas of the country. The phenylalkylamines and the cathedulins are the major alkaloids. The cathedulins are based on a polyhydroxylated sesquiterpene skeleton and are basically polyesters of euonyminol. Recently, 62 different cathedulins from fresh Khat leaves were characterized. The Khat phenylalkylamines comprise cathinone and the two diastereoisomers cathine and norephedrine. These compounds are structurally and pharmacologically related to amphetamine and noradrenaline. The plant contains the (–)-enantiomer of cathinone only. Thus, the naturally occurring S-(–)-cathinone has the same absolute configuration as S-(+)amphetamine. Cathinone is mainly found in the young leaves and shoots. During maturation, cathinone is metabolized to cathine ([+]norpseudoephedrine) and (-)-norephedrine. The leaves contain ([+]norpseudoephedrine) and (-)-norephedrine in a ratio of approximately 4:1. Other phenylalkylamine alkaloids found in Khat leaves are the phenylpentenylamines merucathinone, pseudomerucathine and merucathine. These compounds seem to contribute less to the stimulant effects of Khat. Almost all Khat pharmacological properties are attributed to cathinone, which is an alkaloid that decomposes rapidly in vivo by metabolism into nor-pseudoephedrine and nor-ephedrine giving amphetamine-like action. Many studies have been conducted on the effects of Khat on type 2 DM (T2DM) and the outcome showed inconsistent results and the glycemic effect of Khat remains conflicting.

Metformin, a biguanide class of oral hypoglycemic agents, is the first line drug for the treatment of T2DM. It is the most widely prescribed antidiabetic drug globally, and it is the only antidiabetic agent which has been shown to reduce overall as well as cardiovascular mortality. It is used clinically for the treatment of T2DM, and its mechanism of actions include lowering plasma glucose levels by inhibiting gluconeogenesis in liver, decreasing the intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. In addition, metformin has a variety of pleiotropic effects including improved lipid and cholesterol metabolism, decreased inflammation, and inhibition of cell growth.

Despite numerous reports regarding the associations of Khat chewing with serious health impacts, a significant number of people worldwide uses Khat daily, especially in its origin countries. The risk of co-administration of Khat and drugs that are high among these individuals, leading to increase probability of adverse Khat-drug interactions. Under these circumstances, it becomes important to evaluate the effect of concomitant administration of Khat with metformin on serum levels of fasting blood glucose (FBG) and body weight (BW), as well as plasma levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in high fructose diet induced T2DM in rats.

**MATERIALS AND METHODS**

**Animals Handling**

It is a prospective study carried out on 36 adult male rats, each weighting 120–150 g. Before experimentation, the animals were housed acclimated for 1 week to allow adaptation to the new environment and were maintained on a 12 h light/12 h dark cycle and were caged (six/cage) in fully ventilated room. They were allowed to free access to water and diet containing cereals and bread. The study lasted for 8 weeks from April to June 2020.

**Drugs and Chemicals**

Fresh Khat (leaves and stem tips) were obtained regularly from a local supplier (Ansi) and the leaves were wet, finely crushed by blender machine before being administered by gastric gavage, metformin, D-Fructose (HiMedia Laboratories Pvt. Ltd. India).

**Preparation of Khat Extract**

Fresh Khat leaves were purchased from a local market in Sana’a. The leaves were washed with distilled water and 30 g of carefully selected fresh leaves blended using an electric blender. They were soaked in 10 mL of distilled water for 2 h to make a concentration of 3000 mg/mL. Using the above method in preparation of Khat extract as a method approximately mimics human Khat chewing. The leaves are chewed to release the active constituents slowly to be ingested with saliva. Next, 0.5 mL (concentration of 1500 mg/kg BW) of the filtrate was used for gavage intragastric administration into each rat under Khat treatment for 56 days. The choice of this dose (1500 mg/kg BW) was informed by the average quantity chewers consume and dosages used in similar studies.

**Experimental Design**

At the start of the experiment, animals were divided into six groups (six rats each). All animals except Groups 1 and 2 received high fructose diet (60%) for 8 weeks as a method for induction of type 2 diabetes. For such animals, non-FBG level was measured by electronic glucose meter (Accu-Chek Active apparatus, Germany) using a drop of blood obtained from rat tail puncture. Animals which showed FBG more than 250 mg/dl were considered diabetic, enrolled in the...
experiment. Each of the six groups received various treatment modalities daily for 4 weeks as given below. The study was conducted in line with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH, 1978) and was ratified by Institutional Animal Care and use Committee (AL-Razi U-IACUC) at Al-Razi University, Sana’a, Yemen (Approval number: AL-Razi U 0014.2020).

Animal Groups

Group 1: Normal control rats they were allowed standard normal diet and water and received drug vehicle (saline) in comparable volume to administered drugs. Group 2: Khat treated normal control group: Received Khat in a dose of 1500 mg/kg for 8 weeks. The dose represents the average Khat consumption in humans according to Kennedy et al.[12] Group 3: Non treated fructose diet induced diabetic rats without any treatment. Group 4: Khat treated diabetic rats, received Khat in a dose of 1500 mg/kg for 8 weeks. Group 5: Metformin treated diabetic rats. Metformin was administered in a dose 300 mg/kg/day for 4 weeks which administered after 4 weeks of high fructose diet induced diabetes.[14] Group 6: Metformin-Khat combination treated diabetic rats. Rats were given Khat (1500 mg/kg) for 8 weeks and metformin (300 mg/kg) was administrated after 4 weeks of Khat administration and persisted for 4 weeks.

Biochemical Studies

FBG and total BW were measured at 8th week and serum TC was carried out by enzymatic colorimetric (CHOD-PAP) method.[15] Serum TGs was carried out by enzymatic colorimetric test according to GPO-PAP method.[16] Serum HDL-C was carried out by a method that depends on separation of HDL and determination of cholesterol bound to these fractions.[17] Serum LDL-C was calculated by: LDL-C = TC-(HDL-C+TGs/5) mg/dl.[18]

Statistical Analysis

The results were experienced as mean ± standard deviation (SD). The overall significance was measured by One-Way Analysis of Variance. Excel Software 2013 and Statistical Package for the Social Sciences version 25, was used. The P-values are considered significant when P < 0.05.

RESULTS

BW

The average BW of normal control was 280.83 ± 31.53 g. Experimental induction of T2DM by oral high fructose administration, resulted in significant increase in BW compared with normal control. Khat treated diabetic group showed significantly decrease in BW compared with non-treated diabetic group [Table 1]. Metformin treatment of high fructose diet induced type 2 diabetic group reduced significantly the BW comparing with non-treated diabetic group. The administration of metformin-Khat combination resulted in significant reduction of total BW compared with non-treated diabetic group and normal control [Figure 1a].

FBG

FBG levels of normal control were 89 ± 7.87 md/dl. Khat treated normal control group significantly reduce FBG levels. Fructose diet induced T2DM resulted in significant increase in FBG levels comparing with non-treated diabetic group. Such values were more than normal which are statistically significant [Table 1]. Metformin treated diabetic group showed significantly decrease in FBG levels comparing with non-treated diabetic group. Metformin-Khat combination administration resulted in significant reduction of FBG levels compared with non-treated diabetic group [Figure 1b].

Lipid Profile

Lipid profile of normal control group showed an average serum concentration of TC, LDL-C, HDL-C, and TG amounted to 89.17 ± 3.06, 48.17 ± 8.13, 26.0 ± 6.16, and 78.0 ± 3.85 mg/dl, respectively [Table 2]. Lipid profile of normal control with Khat treated group revealed significant decrease in total, LDL-C and HDL-C. On the contrary serum, TG was normal compared with normal control group. Non-treated diabetic group showed significant increase in tested parameters related to lipid profile except significant decrease in HDL-C levels compared with normal control. Khat treated diabetic group showed significantly decrease in lipid profile related parameters compared with non-treated group. Such values were significantly above normal values compared with normal control. Metformin treatment DM group reduced significantly all lipid profile parameters. Metformin showed normalization of HDL-C concentration as it was significantly more than non-treated group and insignificantly different from normal values. TC, LDL-C, and TG were significantly reduced compared with non-treated diabetic group. Such values still significantly more than normal compared with control group. The administration of metformin-Khat combination markedly elevated serum HDL-C concentration compared with both non-treated diabetic and normal control groups. Serum total, LDL-C, and TG were significantly reduced compared with non-treated diabetic group and metformin treated diabetic group [Figure 2a-d].

DISCUSSION

In this study, the treatment with fresh leaves extract of Khat, significantly decreased FBG levels and there was no significant change in the BW in normal control rats. Moreover, the study
### Table 1: Effects of metformin (300 mg/kg/day p.o) and Khat (1500 mg/kg/day p.o) either singly or in combination for 4 weeks, in fructose (60% in diet) induced T2DM in rats, on average (mean±SD) BW (g), and FBG (mg/dl) (n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal control with Khat</th>
<th>Non-treated diabetic with Khat</th>
<th>Diabetic with Khat</th>
<th>Metformin treated diabetic</th>
<th>Metformin-Khat combination treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>280.83±31.53</td>
<td>282.5±9.35</td>
<td>352.5±14.75</td>
<td>310.0±7.07</td>
<td>308.67±2.66</td>
</tr>
<tr>
<td>FBG</td>
<td>89.00±7.87</td>
<td>71.50±1.22</td>
<td>218.67±6.68</td>
<td>189.00±7.04</td>
<td>133.17±4.88</td>
</tr>
</tbody>
</table>

(*): Significant versus control P<0.05, (+): Significant versus non-treated diabetic group at P<0.05, (ε): Significant versus metformin treated diabetic group at P<0.05, BW: Body weight, FBG: Fasting blood glucose, T2DM: Type 2 diabetes mellitus

### Table 2: Effects of metformin (300 mg/kg/day p.o) and Khat (1500 mg/kg/day p.o) either singly or in combination for 4 weeks, in fructose (60% in diet) induced T2DM in rats, on average (mean±SD) serum concentration of TC, LDL-C, HDL-C, and TG (mg/dl) (n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal control with Khat</th>
<th>Non-treated diabetic with Khat</th>
<th>Diabetic with Khat</th>
<th>Metformin treated diabetic</th>
<th>Metformin-Khat combination treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>89.17±3.06</td>
<td>38.0±4.43</td>
<td>217.5±9.35</td>
<td>181.12±7.79</td>
<td>84.17±5.31</td>
</tr>
<tr>
<td>LDL-C</td>
<td>48.17±8.13</td>
<td>36.83±6.18</td>
<td>166.5±5.47</td>
<td>133.33±8.94</td>
<td>61.67±5.54</td>
</tr>
<tr>
<td>HDL-C</td>
<td>26.0±6.16</td>
<td>16.83±5.46</td>
<td>16.5±3.94</td>
<td>13.5±3.08</td>
<td>34.17±4.79</td>
</tr>
<tr>
<td>TGs</td>
<td>78.0±3.85</td>
<td>77.67±4.68</td>
<td>179.5±6.25</td>
<td>144.83±5.15</td>
<td>119.83±7.28</td>
</tr>
</tbody>
</table>

(*): Significant versus control P<0.05, (+): Significant versus non-treated diabetic group at P<0.05, (ε): Significant versus metformin treated diabetic group at P<0.05, TG: Triglycerides, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, T2DM: Type 2 diabetes mellitus

### Figure 1: Effects of metformin (300 mg/kg/day p.o) and Khat (1500 mg/kg/day p.o) either singly or in combination for 4 weeks, in high fructose diet (60%) induced type 2 diabetes mellitus in rats, on average (mean ± SD) (a) body weight (g) and (b) fasting blood glucose (mg/dl). *Significant versus control P < 0.05, + Significant versus non-treated diabetic group at P < 0.05, ε Significant versus metformin treated diabetic group at P < 0.05.
Figure 2: Effect of metformin (300 mg/kg/day p.o) and Khat (1500 mg/kg/day p.o) either singly or in combination for 4 weeks, in high fructose diet (60%) induced type 2 diabetes mellitus in rats, on average (mean ± SD) serum concentration of (a) total cholesterol (b) low-density lipoprotein cholesterol (c) high-density lipoprotein cholesterol and (d) triglycerides (mg/dl) (n = 6). *Significant versus control $P < 0.05$, + Significant versus non-treated diabetic group at $P < 0.05$, ε Significant versus metformin treated diabetic group at $P < 0.05$. 
demonstrated that Khat treatment partially reduced but did not completely relieved insulin resistance parameters. The levels of FBS and BW were significantly decreased compared to their higher values in non-treated diabetic group but still markedly higher than normal ones. Regarding the effect of Khat on lipid profile, there was a significant decrease in plasma TC, LDL-C, and TG with the adverse effects like a decrease in HDL-C. This study revealed that metformin treatment partially reduced but did not completely relieved insulin resistance parameters. The levels of FBG and BW were significantly decreased compared to their higher values in non-treated high fructose diet induced type 2 diabetic group but still markedly higher than normal compared with normal control. Metformin induced amelioration of insulin resistance may be attributed to its beneficial effects on both glycemic and lipid metabolism. Regarding the effect of metformin on lipid profile, there was a significant increase in HDL-C concentration which may be explained either by improvement of glycemic control which augments the insulin mediated effect on LCAT in HDL-C particles or serum TG, TC, and LDL-C were significantly reduced. The co-administration of metformin and Khat may offer a great advantage in providing additive effect on FBG levels BW, TG, LDL-C, and TC. The combination was also useful in reversing the reduction effects of Khat on HDL-C serum level and potentiates reduction effects on BW.

The observed effects of fresh leaves extract of Khat on some metabolic syndromes parameters T2DM rats provides additional support to the some findings of other researchers, who had previously demonstrated antidiabetic properties of Khat extract. The findings of current study are in agreement with the clinical study of Taleb et al. who showed that Khat chewing leads to mild reduction in blood glucose level (BGL) in non-diabetic patients. This effect attributed to the appetite depressant and the delayed gastric emptying time effects of Khat. The clinical study of Saif-Ali et al. showed no statistically significant difference in BGL between Khat chewer and non-chewer non-diabetic patients. In contrast, El-Sayed et al. reported that there is a strong correlation between chronic Khat consumption and T2DM developing. This is in agreement with the animal’s study of Al-Habori et al. who showed that long-term feeding of Khat leads to a significant increase in plasma HDL-C and a significant decrease in plasma glucose and TG concentrations. The FBS reduction of C. edulis in diabetic and normal rats may be mediated by the decreased rate of carbohydrate absorption into the portal hepatic circulation. The delayed gastric emptying effect of tannins and the inorganic ions presented in Khat may contribute to the delayed absorption of glucose from gastrointestinal tract. This may be explained by reduction of BGL through the mechanisms mentioned above. The results are in agreement with the known effects of metformin in the literature. However, the extent of lowering of parameters in some cases is different. This is in agreement with the known beneficial actions of metformin. The animals on metformin had a lowering of BW when compared to normal control. This effect has been attributed to the anorexic effect of metformin.

To the best of our knowledge, this study is the first study in Yemen that assessed the co-administration of Khat with metformin and their possible interaction in rats with T2DM. Thus, when Khat was combined with metformin there was greater lowering of tested parameters such as FBG, BW, and serum TG. The effects on serum glucose, TG were significant when compared to metformin group. Effects of combination therapy on BW and FBG levels were significant compared to metformin group. The additive effects seen with Khat and metformin can be explained by the different mechanisms of action of the two drugs, while Khat acts mainly by decreasing the rate of carbohydrate absorption into the portal hepatic circulation. The delayed gastric emptying effect of tannins and the inorganic ions presented in Khat may contribute to the delayed absorption of glucose from gastrointestinal tract. Metformin acts primarily by decreasing endogenous hepatic glucose production. The adverse effects such as increase in BW and lowering of lipid parameters that were seen with Khat were significantly less during therapy with the combination, such as FBG and BW. The observations were in agreement with the previous studies. Khat has a beneficial effect on BW which is mainly related to its appetite suppressant activity explained by centrally mediated elevation of plasma leptin level which suppresses hunger feeling and decreases weight and lipids. This was proven by Al-Dubai et al. that Khat chewing leads to significant reduction in plasma TG level and increase in non-esterified fatty acids with no effect on cholesterol level. TG level reduction could also be mediated through lipolysis stimulation through beta 2 adrenergic receptors.

Limitations

Due to financial constraints as a consequence of war and siege in Yemen, further investigations such as oral glucose tolerance, glycosylated hemoglobin tests, and more modern tests could not be carried out.

CONCLUSION

In view of the above-mentioned observations, it has been concluded that metformin-combination seem to be more effective than metformin in treatment of T2DM. The administration of metformin-Khat combination is much more effective in controlling the insulin resistant diabetic state. Use of metformin was associated with a mild adverse effect on HDL-C caused by Khat alone.

REFERENCES

2. American Diabetes Association. Diagnosis and classification