Decreased antioxidants in the saliva of Khat chewers

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Abstract  Objective: Khat is a plant of the Celastraceae family that is chewed for several hours/day in Yemen and most of the East African countries. Cathinone and cathine are the main Khat components and are structurally and functionally related to amphetamine. The present study has been designed to assess levels of antioxidants in the saliva of Khat chewers.

Methods: Saliva samples of 50 volunteers were collected from Khat-chewers and non Khat-chewers, 25 samples each. Saliva samples were collected and used for measurements of salivary antioxidant system including; catalase, total and protein thiols, glutathione and uric acid (UA). Moreover, activity of α-amylase and lactate dehydrogenase (LDH) and levels of total protein, glucose, and cholesterol were also measured.

Abbreviations: CAT, catalase; GSH, reduced glutathione; P-SH, protein thiol; ROS, reactive oxygen species; Total-SH, total thiols; UA, uric acid

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Results: The activities and levels of antioxidants were significantly decreased in Khat-chewing group as compared to controls ($p < 0.001$), except the level of UA which was significantly increased. Khat has also been found to have a lowering effect on the activity of salivary amylase and glucose level ($p < 0.001$). However, the levels of salivary LDH, total protein, and cholesterol were significantly increased in the saliva of Khat chewers ($p < 0.001$).

Conclusion: Present data suggest that Khat chewing generates free radicals and reactive oxygen species to a level that antioxidants cannot cope with, thus overwhelming the antioxidant system capacity.

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1. Introduction

People in Yemen and East African countries spend between 4–6 h a day in chewing the leaves of the Khat (Catha edulis) plant, however, this habit spread to some other countries where Yemeni and other East African communities are living. General health and socioeconomic impacts have been reported due to Khat chewing in Yemen. The common adverse effects of Khat use which include insomnia, anorexia, hyperthermia, mydriasis and endocrinological disturbances are due to the release of the psychoactive agents such as cathinone. Minor antioxidant components have been found in the plant which cannot overwhelm the effects of free radicals and oxidants implicated in Khat toxicity, although the decreased activity of antioxidant enzymes due to reactive oxygen species (ROS) and oxidative stress has been reported in rats and humans following Khat chewing. Antioxidants represent one of the defense mechanisms against oxidative stress which are present in all body fluids and tissues including the saliva. Recently, the relationship between Khat and free radicals has been reviewed. The present study has been designed to assess the levels of antioxidants in the saliva of male Khat chewers of the Thamar city, Yemen.

2. Materials and methods

2.1. Chemicals

Ellman reagent (DTNB) was purchased from HiMedia, India. Kits of the biochemical tests were purchased from Spinreact, Spain. All other chemicals used were of highest grade commercial products.

2.2. Study design, population and grouping

Fifty healthy individuals aged 20–30 years selected from the Thamar city were divided into two groups with $n = 25$ individuals each:

1. Non-Khat chewers (control) group: local males never chew Khat.
2. Khat chewers group: local males with the habit of chewing Khat.

Those included in the present study fulfill the following criteria: healthy, non diabetic and sex and aged match volunteers and those excluded are suffering from periodontitis, carcinoma and diabetes. The study was performed in accordance with the Helsinki Declarations and approved by the Ethics Committee, Thamar University, Yemen.

2.3. Sample collection

Saliva samples of 50 individuals (25 each group) were collected between 8–10 AM on fasting and 12 h after the Khat chewing session. The samples were centrifuged immediately; the supernatant of each sample was used for biochemical analyses.

2.4. Biochemical assays

2.4.1. Total thiols (Total-SH)

Total thiol groups were quantified in the saliva according to the method of Ellman as modified by Sedlak and Lindsay and described by Masoud et al..

2.4.2. Low molecular weight thiols

Low molecular weight thiols, LMW-SH [primarily GSH] were measured in the saliva according to the method of Ellman and described by Masoud et al..

2.4.3. Catalase [CAT] activity

CAT activity was assayed in the saliva following the method of Luck and described by Masoud et al..

2.4.4. Cholesterol, total protein, glucose and uric acid [UA] level

Cholesterol, total protein, glucose and UA levels were measured in the salivary supernatant according to the protocol provided by commercial kits, Spinreact, Spain (CV% ranges between 0.21–0.71). Results were expressed as mg/dl.

2.4.5. Lactate dehydrogenase [LDH] and amylase activities assay

LDH and amylase activities were measured in the salivary supernatant according to the protocol provided by commercial kit, Spinreact, Spain (CV% 1.13 and 1.64 for LDH and amylase respectively). Results were expressed as U/dl.

2.4.6. Statistical analysis

Data were expressed as mean ± S.D. and were analyzed by student’s $t$-test. Differences between groups were considered significant when $p < 0.001$. All analyses were performed using the sigma-stat software [version 3.5].
3. Results

The antioxidant capacity of saliva has been significantly altered following Khat chewing. A significant decrease in the activity of CAT by 34.85% \((p < 0.001)\) was observed in the saliva of Khat chewers as compared to controls (Fig. 1). Salivary thiols including Total-SH, glutathione (GSH), and protein thiol (P-SH) have been shown to be reduced in the saliva of Khat chewers by 43.37%, 34.85% and 35.17% \((p < 0.001)\) for Total-SH, GSH and P-SH respectively which is represented in Table 1. Moreover, a significant increase in the level of UA in Khat-chewers by 23.38% \((p < 0.001)\) as compared to controls has been observed (Fig. 2). The activity of amylase has been shown in Fig. 3 where it was significantly decreased in the Khat-chewing group by 21.63% \((p < 0.001)\) as compared to the control group. Concomitantly, a significant decrease of glucose by 9.91% \((p < 0.001)\) was seen in the saliva of Khat chewers as compared to controls (Fig. 4). However, Khat chewing resulted in significant increases in the activity of LDH by 64.44% (Fig. 5), total protein level by 17.9% (Fig. 6) and cholesterol content by 3.9 fold (Fig. 7) in the saliva of the Khat chewing group as compared to the control group \((p < 0.001)\).

4. Discussion

Khat is now widely chewed for 4–6 h daily in Yemen and East African countries, however, only the immigrant communities from these countries are Khat chewers in Western countries\(^{2,15}\). In the present study we report for the first time that the salivary antioxidants CAT, GSH and Total-SH were decreased concomitantly with an increase in UA, LDH, total protein and cholesterol levels. However, reductions in the activity of amylase and glucose level have been observed in the saliva of Khat chewer group. Many researchers have reported increased oxidative stress, therefore, reduction in antioxidants following Khat chewing in the serum of male Khat chewers\(^{9}\), plasma of female Khat chewers\(^{8}\), in rats\(^{6}\), in keratinocytes and fibroblasts\(^{16}\) and in hepatic and renal tissues have been reported\(^{17}\). Increased generation of ROS may cause toxic effects by oxidative damage of proteins, lipids and DNA where the oxidative damage of these biomolecules contributes to disease development. The antioxidant defense system helps cells to detoxify free radicals, these antioxidants include non-enzymatics like GSH and enzymes; superoxide dismutase,

### Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Non-Khat chewers</th>
<th>Khat chewers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total thiols</td>
<td>0.634</td>
<td>0.359*</td>
</tr>
<tr>
<td>Glutathione</td>
<td>0.344</td>
<td>0.172*</td>
</tr>
<tr>
<td>Protein thiol</td>
<td>0.29</td>
<td>0.188*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.D.; \(n = 25\). * \(p < 0.001\) significantly different from controls.

![Figure 1](image1.png)  
**Figure 1** Activity of CAT in the saliva of non-Khat chewers and Khat chewers. Results are expressed as mean ± S.D.; \(n = 20\). Data were analyzed by student’s \(t\)-test. * \(p < 0.001\) was considered significant from the control group.

![Figure 2](image2.png)  
**Figure 2** Activity of UA in the saliva of non-Khat chewers and Khat chewers. Results are expressed as mean ± S.D.; \(n = 20\). Data were analyzed by student’s \(t\)-test. * \(p < 0.001\) was considered significant from the control group.

![Figure 3](image3.png)  
**Figure 3** Activity of amylase in the saliva of non-Khat chewers and Khat chewers. Results are expressed as mean ± S.D.; \(n = 20\). Data were analyzed by student’s \(t\)-test. * \(p < 0.001\) was considered significant from the control group.
CAT, glutathione peroxidase and glutathione reductase.\textsuperscript{18} Hence, lower salivary CAT levels in Khat chewers can reflect increased generation of ROS that leads to oxidation and antioxidant imbalance, that they overwhelm the capability of CAT of scavenging these radicals. Thiol groups (including GSH, Total-SH) play a crucial role as antioxidants and also in other reactions including catalysis, regulation, electron transport and those preserving the correct structure of proteins.\textsuperscript{19} The redox status of salivary thiols can be a diagnostic indicator of different pathological states including carcinoma.\textsuperscript{20}

The present finding indicates that Khat chewing decreases the capacity of thiols as antioxidants in the saliva of Khat chewers. Our findings are in agreement with those of Lukandu et al.,\textsuperscript{16} who have reported that an extract of Khat induced cell death by apoptosis in primary human oral cells in vitro. They also observed an increase in intracellular ROS and depletion of GSH suggesting that Khat induces cell death. UA can be both beneficial, as an antioxidant and free radical scavenger, and deleterious if present at an elevated level. UA is considered to be a catabolic end product of purine nucleotides or in another way from flavonoid metabolism.\textsuperscript{21} The decreased and elevated UA levels may contribute to the development and progression of a number of disease states, significant alterations in UA levels should be minimized.\textsuperscript{22} UA however, significantly contributes to the reduction of oxidative stress.\textsuperscript{23} This increase in the present study is indicative of increased production of ROS in the saliva of Khat chewers, as a natural body response of scavenging excessive free radicals or due to the enhanced Xanthine oxidase observed during oxidative stress.\textsuperscript{24} This finding is in agreement with those of Al-Habori and Al-Mamary who reported an increase in UA in the plasma of rabbits in a time dependent manner following the administration of Khat.\textsuperscript{24} Moreover, Khat has an amphetamine like effect, which in turn was reported to increase the extracellular levels of UA.\textsuperscript{25} Non enzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. The autooxidation of glucose can generate hydroxyl radicals. Moreover, advanced glycation end products can be also formed as glucose reacts with proteins in a nonenzymatic manner.\textsuperscript{26,27} This might explain the decrease in the glucose levels of the present study, meanwhile, production of ROS following Khat chewing is responsible for low activity of amylase as seen in the present study where ROS production can cause damage to the proteins including enzymes, which might also affect the levels of glucose present in the saliva. LDH is found in the saliva only following leakage through the oral mucosa.\textsuperscript{28,29} Increased extracellular activity of LDH, which is an intracellular enzyme, is the result of disrupted cell membrane integrity, which occurs during the lipid peroxidation and oxidative stress\textsuperscript{30} hence, the increase in the present study can be attributed to the generation of ROS.

Levels of certain proteins in the plasma and saliva increase during acute inflammatory states or are secondary to certain types of tissue damage. Khat chewing, stimulates the sympathetic nervous system thus leading to the production of little saliva but with high protein concentration. Salivary protein...
concentration increases in older adults than in the young and in adults. The salivary levels of total protein are significantly higher in oral cancer patients compared to those of normal healthy control. Therefore, chewing Khat is responsible for this increase in the total protein by the generation of ROS.

Salivary lipids are mostly of glandular origin, but some (such as cholesterol) are believed to diffuse directly from the serum. Cholesterol is thought to derive from the plasma and the clearance of compounds from the plasma into the saliva may involve several processes such as ultrafiltration through gap junctions between cells of secretory units and low molecular weight lipids such as cholesterol are involved. Cholesterol concentration in the saliva is correlated with the concentration in serum. In healthy individuals, this positive correlation can be used for the selection of individuals with high levels of serum cholesterol. Biochemically, Khat leaves decreased plasma cholesterol and triglycerides in rabbits.

Khat, or the mixture of substances contained in the leaves, also has interesting cholesterol-lowering effects. These are opposed to our findings which might be attributed to the oxidative damage of epithelial cells in the mouth. Alterations in cholesterol contents might alter the activity of various membrane proteins. Inhibition of critical antioxidant enzymes and scavenger proteins may be the mechanism by which free radicals reduce antioxidant capacity. For optimal enzymes and protein functions, the pH of the saliva is an important factor; however, the significant decrease of pH has been reported among Khat chewers.

It is therefore, concluded from these findings that chewing Khat is responsible for production of ROS in the saliva of Khat chewers which in turn reduces the capacity of antioxidants.

Conflict of interest
No financial, personal or other conflict of interest.

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References