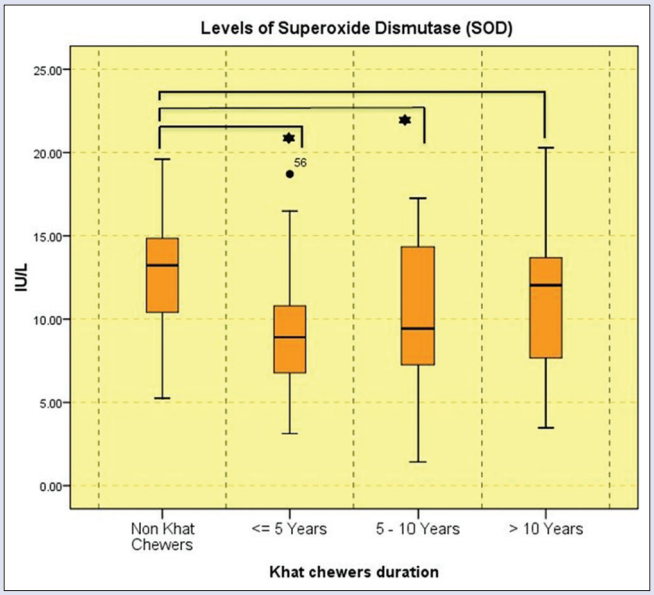

Figure 1: Box plot graph for the levels of superoxide dismutase, representing the highest, lowest, and median values in the non-khat chewers group and khat groups according to short- and long-term chewing. The symbol "*" indicates that the mentioned differences is statistically significant according to the adjusted Bonferroni, $P < (0.05/3 = 0.017)$

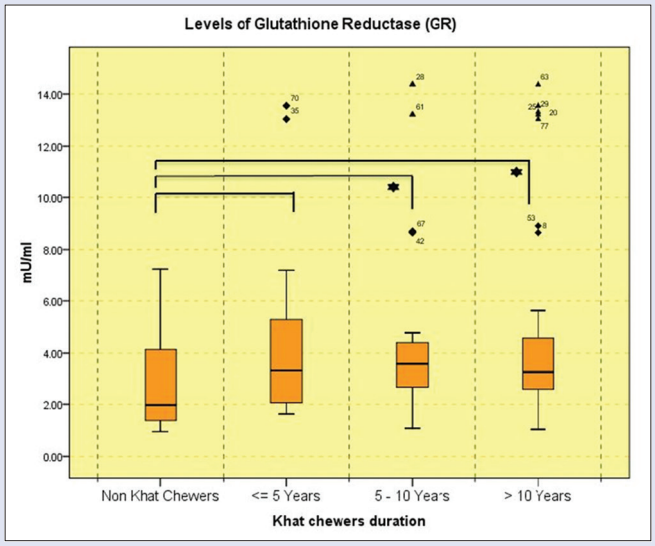

Figure 2: Box plot graph for the levels of glutathione reductase, representing the highest, lowest, and median values in the non-khat chewers group and khat groups according to the short- and long-term chewing. The symbol "*" indicates that the mentioned differences are statistically significant according to the U-test using adjusted Bonferroni, $P < (0.05/3 = 0.017)$

There was no significant effect of fruit/vegetable consumption, fast food dependent diet, energy drink consumption, and soft drinks consumption on the level of any of the investigated enzymes in the studied group.

DISCUSSION

In the current study, we investigated the possible influence of CKC on the antioxidant status by assessing the impact of this habit on antioxidant enzymes SOD and GR. In addition to this, the potential oxidative destruction of the DNA has been explored by measuring a surrogate biomarker, 8-OHdG. The results had showed that CKC has significantly reduced the levels of SOD in comparison to the corresponding control group. Meanwhile, the level of GR was significantly elevated, and a marginal, yet, a significant elevation in the level of 8-OHdG was observed in the khat chewers group. In this context, a recent study from Yemen reported a similar reduction, in the SOD and catalase activities, which agrees with our findings. This was explained as an indicative of cellular protein damage caused by an increased ROS production. The authors have also found an increase of GST, claiming that this could be explained by the leakage of cellular GST to the bloodstream.[19]

However, the drop in SOD activity may be due to an increase in the production of ROS, which could be resulting from the increase in lipid hydroperoxides. GR is the enzyme facilitating the reduction of GSSG to the reduced form of glutathione (GSH). The link between GR and oxidative stress was proven by its role in ROS-releasing macrophage in oxidative response. It was observed that higher persistent response was present in cells with no GR.[20]

The significant elevation in GR activity reflects the presence of highly toxic compounds. These compounds would be either exogenous as pesticides that are commonly sprayed in large amounts on khat trees or endogenous as a result of increased production of ROS. In a previous study, we were able to detect eight pesticide multiresidues in khat leaves consumed in the Jazan Region.[21]

GSH, which is present in most organs and cellular systems, is significantly protecting cells from oxidative injury, that is, why the decline in the GSH levels is associated with some pathological conditions, for example, chromosomal DNA fragmentation, cataract, and cystic fibrosis.[22] A recent report has even described free radical formation and GSH depletion as a possible early cause of khat cytotoxicity.[22,23] In addition, it was reported that there is a significant drop in the levels of erythrocyte SOD and catalase in khat chewers; on the other hand, an elevation in serum GST was observed. Moreover, there were depletions of GSH and Vitamin C along with an elevation of malondialdehyde (MDA) in khat chewers in comparison to controls. This might reflect an obvious oxidative status.[19]

Earlier studies have showed that rats treated with alkaloids fraction of khat extract significantly reduced the capacity of free radical metabolizing/scavenging enzymes and led to increased free radical concentration and followed by higher oxidative stress.[11] The possible effects of the sustained oxidative stress induced by khat consumption may lead to the development of several pathologies, including liver toxicity, cardiovascular toxicity, neurodegenerative disorders, and cancer.[24] On the other hand, another study has also reported that CKCs had higher levels of free radicals observed in their serum samples.[10] This increase in the levels of free radicals could either be due to khat consumption or the pesticide contamination of khat. In this regard, we have already reported the contamination of khat leaves with many pesticides as mentioned previously.[21]

Usually, the oxidative stress encompasses DNA damage, which is associated with the release of derivatives of nucleoside oxidation. 8-OHdG is one of the oxidized nucleosides that are excreted in body fluids during DNA repair. Its levels have been positively correlated with neurodegenerative disorders, cancers, and chronic inflammatory conditions.[25] Other literature showed that continuous reparation of the oxidized DNA is followed by the excretion of excised deoxyribonucleotides in the serum and urine. Multiple studies have reported the presence of 8-OHdG as an important biomarker of oxidative stress in bodily fluids.[26] 8-OHdG has been reported as the predominant biomarker of free radical-induced

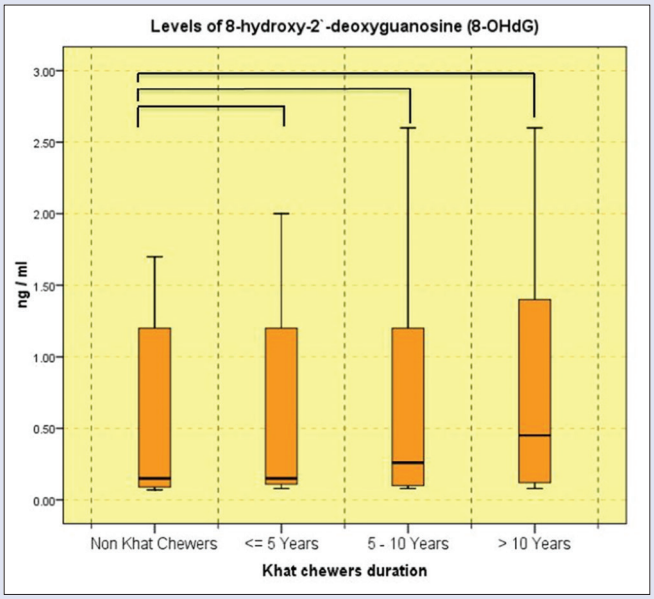

Figure 3: Box plot graph for the levels of 8-hydroxydeoxyguanosine, representing the highest, lowest, and median values in the non-khat chewers group and khat groups according to the short- and long-term chewing. U-test has showed no significance results between the non-khat chewers and others mentioned categories

oxidative damage of both mitochondrial and nuclear DNA. In addition, it is extensively utilized as a biomarker for assessing the chances of various neoplasms and degenerative diseases in clinics where it functions as an initiation and promotion factor of carcinogenesis. Recently, 8-OHdG started being used as a biomarker for the quantification of oxidative DNA impairment and to assess the risk of developing many diseases and cancers, especially after exposure to environmental carcinogens endogenously.[27]

Although our study presented a marginally significant rise in the levels of 8-OHdG in CKCs, it may still explain, at least in part, the possible correlation between CKC and the oxidative DNA damage through the negative impact on the levels of the antioxidant enzymes. To the best of our knowledge, no previous study has reported the relationship between CKC and the level of 8-OHdG as a surrogate biomarker of DNA damage in human blood.

Studies have showed that the consumption of khat is not always alone; sometimes, it is chewed together with tobacco and or alcohol. It has been, however, reported that khat consumption, especially if accompanied by alcohol and/or tobacco, might be a potential cause of oral neoplastic events and genotoxic in nature. This notion was consolidated by the 8-fold increase in the micronucleated buccal mucosal cells among khat chewers.[28] However, this observation was concluded by highlighting the influence of smoking effects, which is a definite cause of increasing micronuclei.[22]

In line with our findings, a very recent study has demonstrated that treatment with 400 μg/ml of khat extract was able to induce cell death via apoptosis mechanism in breast cancer, with an increase in the expression of the proapoptotic mitochondrial protein, Bax, and a decrease in the expression of B-cell lymphoma.[2] In addition, a decrease in ROS levels in a time-dependent manner was also reported. The authors have suggested that khat induces cell death in MDA-MB-231 cells via MAPK activation and triggering the mitochondrial (intrinsic) pathway of apoptosis. MAPK is an enzyme which regulates cell proliferation, survival, and cell differentiation.[29]

One limitation we faced during this study was the availability of nonchewer individuals. It was difficult to obtain a khat nonchewing subject (never chew khat) from our study region since khat chewing is a very popular habit. Accordingly, the age matching was not perfect between the CKCs and non-chewers groups.

CONCLUSION

Our study showed that that chronic daily chewing of khat certainly induces ROS production, potentially caused oxidative toxicity. Both enzymatic and nonenzymatic antioxidants were responsible for protection against this oxidative toxicity. ROS endogenous formation increased in CKCs in correlation with a decreased SOD level. Meanwhile, GR activity elevation reflects the presence of highly toxic compounds. Habitually, long-term khat chewers will be susceptible to oxidative toxicity and possible to DNA damage; therefore, they are strongly recommended giving up khat chewing**.** This study showed an increase in the levels of 8-OHdG in CKCs that may have been statistically insignificant, yet it may still explain an important role played by CKC in the DNA damage process.

CKCs found to be at high risk of oxidative stress with DNA damage. The endogenous oxidative damage to DNA initiates and promotes the carcinogenic process. This result adds to the role of CKC as a potential risk for health. Khat chewers are highly recommended to quit this habit for better health and quality of life. More studies are required to investigate the role of khat chewing short and long duration on the genotoxicity and antioxidant defense mechanism.

Acknowledgements

The authors would like to express their thanks and appreciation to the Deanship of Scientific Research, Jazan University (Project #1436-54-21.).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Beckerleg S. Khat in East Africa: Taking women into or out of sex work? Subst Use Misuse 2008;43:1170-85.
2. Al-Habori M. The potential adverse effects of habitual use of *Catha edulis* (khat). Expert Opin Drug Saf 2005;4:1145-54.
3. Alsanosy RM, Mahfouz MS, Gaffar AM. Khat chewing among students of higher education in Jazan region, Saudi Arabia: Prevalence, pattern, and related factors. Biomed Res Int 2013;2013:487232.
4. Ageely HM. Prevalence of Khat chewing in college and secondary (high) school students of Jazan region, Saudi Arabia. Harm Reduct J 2009;6:11.
5. Alfaifi H, Abdelwahab SI, Mohan S, Elhassan Taha MM, Syame SM, Shaala LA, *et al.* *Catha edulis* Forsk. (Khat): Evaluation of its antidepressant-like activity. Pharmacogn Mag 2017;13:S354-8.
6. Hoffman R, Al'Absi M. Khat use and neurobehavioral functions: Suggestions for future studies. J Ethnopharmacol 2010;132:554-63.
7. Sheikh KA, El-Setouhy M, Yagoub U, Alsanosy R, Ahmed Z. Khat chewing and health related quality of life: Cross-sectional study in Jazan region, Kingdom of Saudi Arabia. Health Qual Life Outcomes 2014;12:44.
8. Douglas H, Boyle M, Lintzeris N. The health impacts of khat: A qualitative study among Somali-Australians. Med J Aust 2011;195:666-9.
9. El-Menyar A, Mekkodathil A, Al-Thani H, Al-Motarreb A. Khat use: History and heart failure. Oman Med J 2015;30:77-82.
10. Al-Akwa AA, Shaher M, Al-Akwa S, Aleryani SL. Free radicals are present in human serum of *Catha edulis* Forsk (Khat) abusers. J Ethnopharmacol 2009;125:471-3.
11. Al-Qirim TM, Shahwan M, Zaidi KR, Uddin Q, Banu N. Effect of khat, its constituents and

restraint stress on free radical metabolism of rats. J Ethnopharmacol 2002;83:245-50.

12. Abid MD, Chen J, Xiang M, Zhou J, Chen X, Gong F. Khat (*Catha edulis*) generates reactive oxygen species and promotes hepatic cell apoptosis via MAPK activation. Int J Mol Med 2013;32:389-95.
13. Marinello PC, da Silva TN, Panis C, Neves AF, Machado KL, Borges FH, *et al.* Mechanism of metformin action in MCF-7 and MDA-MB-231 human breast cancer cells involves oxidative stress generation, DNA damage and transforming growth factor β1 induction. Tumour Biol 2016;37:5337-46.
14. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy 2016;12:1-222.
15. Mian OY, Khattab MH, Hedayati M, Coulter J, Abubaker-Sharif B, Schwaninger JM, *et al.* GSTP1 Loss results in accumulation of oxidative DNA base damage and promotes prostate cancer cell survival following exposure to protracted oxidative stress. Prostate 2016;76:199-206.
16. Franken C, Koppen G, Lambrechts N, Govarts E, Bruckers L, Den Hond E, *et al.* Environmental exposure to human carcinogens in teenagers and the association with DNA damage. Environ Res 2017;152:165-74.
17. World Medical Association. World medical association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ 2001;79:373.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
19. Naji KM, Al-Maqtari MA, Al-Asbahi AA, Abdullah QY, Babu RN, Devaraj VR. Effect of daily chewing soft buds and leaves of *Catha edulis* (Khat) on the antioxidant defense system and oxidative stress markers in blood. Arab J Sci Eng 2015;40:1-6.
20. van Zwieten R, Verhoeven AJ, Roos D. Inborn defects in the antioxidant systems of human red blood cells. Free Radic Biol Med 2014;67:377-86.
21. Hassan AA, Abdullah SM, Khardali IA, Oraiby ME, Shaikain GA, Fageeh M, *et al.* Health impact of khat chewing and pesticides: Detection of 8 pesticides multi-residues in khat leaves (*Catha edulis*) from Jazan region, KSA. Adv Environ Biol 2016;10:30-6.
22. El-Setouhy M, Loffredo CA, Radwan G, Abdel Rahman R, Mahfouz E, Israel E, *et al.* Genotoxic effects of waterpipe smoking on the buccal mucosa cells. Mutat Res 2008;655:36-40.
23. Costea DE, Evelyn N. Khat (*Catha edulis*) induces reactive oxygen species and apoptosis in normal human oral keratinocytes and fibroblasts. Toxicol Sci 2008;103:311-24.
24. Carvalho F. The toxicological potential of khat. J Ethnopharmacol 2003;1:1-2.
25. Takane M, Sugano N, Ezawa T, Uchiyama T, Ito K. A marker of oxidative stress in saliva: Association with periodontally-involved teeth of a hopeless prognosis. J Oral Sci 2005;47:53-7.
26. Rai B, Anand S. Gingivitis and periodontitis: 8-hydroxydeoxyguanosine. Adv Med Den Sci 2008:4-7.
27. Purdy M, Kokki M, Anttila M, Aspinen S, Juvonen P, Selander T, *et al.* Does post-surgery placement of rectus sheath block analgesia alter the oxidative stress biomarker 8-OHdG concentrations: A randomised trial of patients with cancer and benign disease. Cancer Genomics Proteomics 2016;13:239-44.
28. Kassie F, Darroudi F, Kundi M, Schulte-Hermann R, Knasmüller S. Khat (*Catha edulis*) consumption causes genotoxic effects in humans. Int J Cancer 2001;92:329-32.
29. Lu Y, Li Y, Xiang M, Zhou J, Chen J. Khat promotes human breast cancer MDA-MB-231 cell apoptosis via mitochondria and MAPK-associated pathways. Oncol Lett 2017;14:3947-52.