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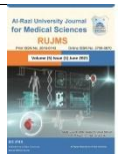

Effect of *Catha Edulis* (Khat) on the Bioavailability of Sildenafil: An *Ex-Vivo* Study

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Abstract:

Background: Catha Edulis (Khat) is extensive plant in certain countries include Yemen. This plant taken by utmost people in Yemen and other countries and can effect on the bioavailability of a large number of drugs such as sildenafil. **Objectives:** The present study aimed to evaluate the effect of the khat plant on the bioavailability of sildenafil in the GIT. **Methods:** The present study was carried out in *ex-vivo* by using everted gut sac method. In comparative studies for absorption of stand. sildenafil alone and stand. sildenafil in presence of more than one type of khat (Ansi and Hamdani) and confirm this study by comparative study for sildenafil (Viagra) alone and in presence of the more effective type of khat (Ansi). **Results:** In the present studies, by the calibration curve equation was used to calculation of Conc. %, the difference means in Conc.% of stand. sildenafil alone and stand. sildenafil in presence of khat Ansi and stand. sildenafil in presence of khat Hamdani, were 14.14 ± 2.922 and 8.316 ± 0.868 respectively, and the difference means in Conc.% of sildenafil (Viagra) alone and sildenafil (Viagra) in presence of khat Ansi was 19.986 ± 3.981. **Consolation:** Based on the results obtained from the study, the bioavailability of sildenafil was significantly reduced in the presence of khat. The rate of reduction was affected by the type of khat, with khat ansi being more effective than khat hamdani in reducing the bioavailability of sildenafil.

Keywords: Bioavailability; *Catha Edulis*; Everted sac intestinal system; *Ex-vivo*; Khat; Sildenafil.

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Introduction

Khat (Catha Edulis) is a plant from Celastraceae family and recognized as Chat, Qat, Miraa Abyssinian tea, African tea, Quaatka and African salad[1]. This plant is extensively cultured in the south of Arabian Peninsula and East Africa (e.g. Kenya and Ethiopia)[2]. Khat is a natural inspirational plant, the main parts of which are the young leaves and buds near the branch of the twig, and the most active substances are alkaloids with amphetamine properties (cathine and cathinone) which have exhilarating and exciting effects[3].

Chewing khat is a common routine in Yemen. About 80-85% of adult males and 50-60% of adult females in northern Yemen chew khat at least once a week. Simultaneous use of khat with standard drugs is reportedly expected to be commonly used in Yemen[2].

There are different type of Khat as stated by the region in which it is grown such as Khat Hamdani, Khat Ansi, Khat Shami, Khat Herari Khat (Ethiopian Khat) ….and so on. The quality of Khat base on their components concentrations (alkaloids, flavonoids, tannin…)[4].

Sildenafil is one of the important oral phosphodiesterase type 5 (PDE5) inhibitors that remain the standard pharmacologic therapy for erectile dysfunction (ED). ED, a prevalent disorder that is more common in men with old year more than 40 years , can have significant consequences for quality of life and self-esteem[5].

Sildenafil citrate is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1 Hpyrazolo [4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate[6] with chemical structure showed in figure 1.

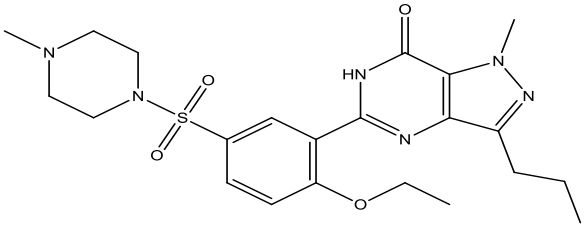

Figure 1: Chemical structure of sildenafil

In my previous study that carried out on rabbits by using everted gut sac intestinal permeability method that showed significantly reduction in bioavailability of drug from the same therapeutic family of sildenafil that is tadalafil.[7] In Khat-drug interaction: a systemic review[4]. Previous studies have revealed that among healthy Yemeni adults who chew khat the bioavailability of certain antibiotic drugs such as ampicillin, amoxicillin, cefradine and ciprofloxacin is significantly reduced[8,9,10], and antimalarial drug as chloroquine[2]. Another study revealed a significant decrease in the pharmacokinetics of tetracycline-HCl among Yemeni adults who chew khat[11]. Other studies found that Khat significantly increase bioavailability of some drugs (Clopidogrel, Sertraline, Clomipramine, Vilazodone, Aripiprazole) which might be attributed to inhibition of their metabolic enzymes[12-14]. Another study in healthy adults suggested that chewing of khat had an inferior effect on the bioavailability and other properties of aspirin as their antiplatelet activity[15]. This study aimed to investigate the effect of khat chewing on the bioavailability of sildenafil. In addition to identifying the type of khat that has the greatest effect on the bioavailability of sildenafil.

This study was carried out in ex-vivo by using everted gut sac intestinal permeability method according to previous studies[16-29].

Materials and Methods

Material:

Sildenafil standard (98.7%) were purchased from Shiba pharma. Company-Yemen. Distilled water, medium of stomach (TC 199 solution containing 145 mM NaCl, 4.56 mM KCl, 1.25 mM $CaCl_2$. $2H_2O$ and 5 mM $NaHPO_4$. Pure Chemical Co. India and UNI-CHEM.Co. Naslovna Strana), $CHCl_3$ (Pure Chemical Co,). All khat types were purchased from the local market. Sildenafil product (Viagra tablet-*Pfizer*-USA) were purchased from the drug market**.**

Instrumentations:

UV spectrophotometer (Lasany international, India). Electric balance (Radwag, Poland), Mixture(JJ-1mixer, China). Water bath (HH-4, China), Centrifuge(China). Rabbit intestinal permeability test system was constructed in our lab as described in the literature[16-28].

Methods

Standard calibration curve:

Procedure:-

100 mg of sildenafil standard is dissolve in 100 ml volumetric flask with 50 ml D.W and complete the volume with the D.W to 100 ml to prepare standard stock solutions of concentration (1 mg/ml).

Take 10 ml of stock solution to another 50 ml volumetric flask and complete the volume to 50 ml with D.W to prepare solutions of 50 µg/ml concentration, to six 10 ml volumetric flask take 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, and 3 ml of (100 µg/ml) concentration, and complete all to 10 ml with TC 199 solution to prepare 6 solutions of the following concentration 2.5 µg, 5 µg, 7.5 µg, 10 µg, 12.5 µg, 15 µg. Measure the UV spectrophotometric absorbance at 220 nm for six concentrations and repeat each conc. three times and write the absorbance data.

Everted gut sac test:

The test was carried out based on adopted methods described in literature[16-29].

Animal model

The animals used in the test were 20 male rabbits with weight (800-1200 g). The animals were incubated in an appropriate cage. Before the test, they were fastened overnight with an excess of free water. The animals were anesthetized with chloroform. The intestinal section included the duodenum and jejunum 15 cm long and a surface area (1 cm^2) was removed. The animal was sacrificed with an overdose of chloroform.

The intestine of the animal was exposed via an abdominal incision and 15 cm of the jejunum was excised (starting 20 cm below the pylorus) and immediately cooled, cleaned by DW and then placed in an oxygenated TC199 buffer solution at 37 °C. using a glass rod for eversion of the excised segment. The lower end was tied with a lace.

Test apparatus

The apparatus consisted of a volumetric beaker (1000 ml) placed on a water bath at 37.5 ^{0}C. The beaker contained 500 ml of buffer. The buffer was prepared in volumetric flask by mixing and the volume was made up to 1000 ml with distilled water, this solution known as TC 199 buffer solution[30]. The everted gut sac was filled with 10 ml of a buffer solution (for testing the intestinal permeability). The sac was incubated for 45 minutes in the buffer medium which was kept oxygenated and stirred all over the experiment at a rate of 85 rpm.

In Ex-Vivo Procedures:

The experiment was conducted as described in the literature[15-28] with appropriate modifications. Prior to the intestinal permeability test, a standard drug calibration curve had to be performed in the incubation medium used in the experiment. Therefore, a stock solution (50 µg / ml) of sildenafil acetate standard in TC 199 solution was prepared. Serial dilution of the stock solution was performed to prepare 6 diluted standard solutions of concentration (2.5–15 µg / ml). The UV absorbance of those solutions was measured at 220 nm and then a calibration curve was formed, from which the regression equation was determined.

The UV absorbance of the liquid was measured by a UV spectrophotometer at 220 nm. The test was achieved in triplicate. Similarly, tests were achieved only for the standard, for the standard with ansi khat, for the standard with hamdani khat, for the brand-only product (Viagra) and for the product (Viagra) with ansi khat. Its UV

absorbance was also measured at 220 nm. Their UV absorption averages were calculated and entered into the calibration regression equation to calculate the working concentration (Cp µg / mL) of the drug in the everted gut and to calculate the percentage of concentration from which the percentage of decrease in bioavailability was calculated.

Results

Figure 1, shows the calibration curve at 220 nm, of stand. sildenafil in TC 199 solution for the everted intestinal sac test. The standard curve is optimally linear 0.9993 and their regression equation used to calculate the practical drug concentration in the sample was (y = 0.0934x + 0.0175).

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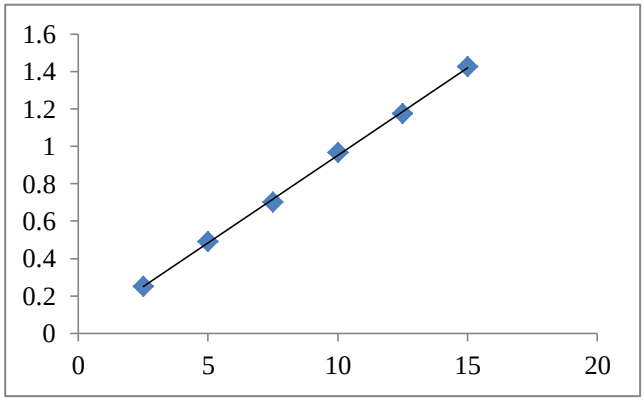

Figure 1. Calibration curve of sildenafil in TC 199 solution at 220 nm

From the proposed study, The co-administration or presence of sildenafil with khat was establish to significantly affect the bioavailability of sildenafil in *Ex-vivo* study. This was similar to reported reductions in the reduced bioavailability of chloroquinone, ampicillin, amoxicillin and the pharmacokinetic effects of tetracycline hydrochloride when co-administered with khat[2,8,11]. There is a possible interaction between sildenafil and khat, as indicated by the significantly reduced sildenafil AUC and rate of recovery of concentration values observed with and without khat used in this study. The mechanisms underlying this interaction are unknown; However, a possible mechanism may be related to the interaction of the drug with certain components of khat-tannic acid, cathinone and cathine, which are known to cause the formation of insoluble complexes and non-absorbable compounds[8,11].

In the present study, comparing concentration % result of standard sildenafil alone and that of sildenafil with khat (ansi) and khat (hamdani) as shown in Table 1, and the difference between them with khat (ansi) and with khat (hamdani) as shown in Table 1. This decrease in drug concentration implies a decrease in the bioavailability of sildenafil by khat (ansi) more than by khat (hamdani) as shown in Table 1. Also established this results, the results obtained by comparing the % conc. of sildenafil (Viagra) alone and its % conc. in the presence of khat (ansi) and the difference between them as shown in Table 1. Therefore, there was a significant decrease in the bioavailability of the drug. In this study, sildenafil concentration levels decreased significantly in the presence of khat compared to the absence of khat and this effect varies between types of khat, as khat (ansi) has a greater effect than khat (hamdani), and this is attributed to the difference in the percentage of khat concentration components.

STATISTICAL ANALYSIS:

Statistical analysis of the results obtained by the methods proposed for the comparative study of recovery concentration % standard sildenafil, sildenafil tablet (Viagra) with and without khat was performed using Student's t-test and F-test of proportional variance at P=0.05. As shown in Table (2), the calculated t and F values were higher than the tabulated ones, indicating a significant difference between them.

Table 1: The results of sildenafil conc.% alone and with khat obtained In Ex-vivo by using everted sac methods

Sample	Stand Sild (Conc. %)	Sild with khat ansi (Conc. %)	Difference (Conc. %)	Stand Sild (Conc. %)	Sild with khat hamdani (Conc. %)	Difference (Conc. %)	Sild tab (Viagra) (Conc. %)	Viagra with ansi khat (Conc. %)	Difference (Conc. %)
1	72.698	62.634	10.064	72.698	64.561	8.137	95.182	87.045	8.137
2	70.985	54.283	16.702	71.985	62.42	9.565	109.957	78.694	31.263
3	74.697	58.994	15.703	74.697	67.131	7.566	103.105	82.548	20.557
4	72.698	58.637	14.091	72.698	64.704	7.994	102.677	82.762	19.915
Mean	72.769	58.640	**14.140**	72.769	64.704	**8.316**	102.730	82.762	**19.986**
SD	1.518	3.419	2.922	1.518	1.629	0.868	6.037	3.413	3.981
SE	0.759	1.709	1.461	0.759	0.963	0.434	3.019	1.706	1.991
Bioavailability-reduction %			**19.43**			**11.43**			**19.45**

Stand: Standard Sild: Sildenafil. Conc: Concentration. SD: Standard deviation. SE: Standard Error.

Table 2: Statistical analysis between the results of sildenafil conc.% alone and with khat obtained In Ex-vivo by using everted sac methods

Statistical term	Stand. Sild	Sild with ansi khat	Stand. Sild	Sild with hamdani khat	Sild tab (Viagra)	Viagra with ansi khat
Mean	72.769	58.64	72.769	64.704	102.730	82.762
SD	1.518	3.419	1.518	1.629	6.037	3.413
SE	0.759	1.709	0.759	0.963	3.019	1.706
N	4	4	4	4	4	4
***F(0.108)**	0.197		0.386		0.319	
***t(2.447)**	7.557		7.384		5.759	
P	0.00027		0.00032		0.0012	

SD; Standard deviation. SE Standard Error. N: Number of samples

*Figures in parentheses are the theoretical t and F values at (p=0.05).

Conclusion

Based on the results obtained from this study, the bioavailability of sildenafil was significantly reduced in the presence of khat (khat-chewing). The absorption rate of the product (Viagra) was higher than that of standard sildenafil, but the presence of khat reduced the bioavailability of the standard drug by the same percentage. The rate of reduction was influenced by the type of khat, as ansi khat was more effective than hamdani khat in reducing the bioavailability of sildenafil.

Authors' Contributions

The correspondent author conceived the idea and developed the theory and performed the calculations of the presented work. All authors participated in conducting experiments, discussing the results, and contributing to the last manuscript.

Declarations

Conflicts of interest: The authors declare that there are no conflicts of interest of publishing this article.

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Ethical Approval: Ethical approval done by Institutional Animal Care and Use Committee (AL-Razi U-IACUC) -AL-Razi University-Yemen.

References

1. Tolcha, P.T., Khat marketing and its export performance in the Ethiopian economy. Science Research, 2020. 8(4): p. 90.
2. Issa, F.H., Al-Habori, M., and Chance, M.L., Effect of khat (Catha edulis) use on the bioavailability, plasma levels and antimalarial activity of chloroquine. Sultan Qaboos University Medical Journal, 2016. 16(2): p. 182–188.
3. Khan, I. and Kalix, P., Khat, a plant with amphetamine-like effects. Trends in Pharmacological Sciences, 1984. 5: p. 326-328.
4. Albaser, N.A., Mohamad, A.-W.H., and AL-Kamarany, M.A., Khat-drug interactions: A systematic review. Journal of Pharmacy & Pharmacognosy Research, 2021. 9(3): p. 333-343.
5. Medicines, A., Oral PDE5 inhibitors for erectile dysfunction. US Pharm, 2018. 43(6): p. 29-33.
6. Abdulla, M., Mallah, E., et al., Influence of Energy Drinks on Pharmacokinetic Parameters of Sildenafil in Rats. Biomedical & Pharmacology Journal, 2018. 11(3): p. 1317-1328.
7. AL-Ghani, A., M Thabet, Anes AM and Albaser, N.A., Study of the effect of khat on the bioavialability of tadalafil (ex-vivo) by using everted sac method. Al-Razi University Journal of Medical Sciences, 2022. 6(1): p. 17-22.
8. Attef, O., Ali, A.-A., and Ali, H.M., Effect of Khat chewing on the bioavailability of ampicillin and amoxycillin. The Journal of antimicrobial chemotherapy, 1997. 39(4): p. 523-525.
9. Kassem, A.K.Y., Cephradine Bioequivalence and its Interaction with Khat and Food (Al-Sayadeyah) in Yemen, 2004, University of Khartoum.
10. AG, A.-M., The effect of chewing khat on drug absorption: A case study of pharmacokinetic profiles of ciprofloxacin 500 mg tablets. Int J Pharm Pharm Res, 2020. 18(4): p. 415–428.
11. Farah, F.H., Attef, O.A., and Ali, A.-A.A., The influence of khat on the in-vitro and in-vivo availability of tetracycline-HCl. Research Journal of Pharmaceutical Dosage Forms and Technology, 2015. 7(1): p. 1-6.
12. Bedada, W., de Andrés, F., et al., Effects of Khat (Catha edulis) use on catalytic activities of major drug-metabolizing cytochrome P450 enzymes and implication of pharmacogenetic variations. Scientific reports, 2018. 8(1): p. 1-10.
13. Alhazmi, H.A., Kadi, A.A., et al., Exploring the effect of khat (Catha edulis) chewing on the pharmacokinetics of the antiplatelet drug clopidogrel in rats using the newly developed LC-MS/MS technique. Open Chemistry, 2020. 18(1): p. 681-690.
14. Elkady, E.F., Fouad, M.A., et al., Validated LC–MS/MS method for the determination of some prescribed CNS drugs: Application to an in vivo pharmacokinetic study of drug-herb metabolic

interaction potential of khat. Microchemical Journal, 2020. 158: p. 151-162.
15. Noman, M.A. and Kadi, H.O., In vitro and in vivo evaluation of acetylsalicylic acid in Khat (Qat) chewing healthy volunteers. Journal of Clinical Medicine and Research, 2012. 4(4): p. 53-58.
16. Tariq, M., Alam, M.A., et al., Biodegradable polymeric nanoparticles for oral delivery of epirubicin: in vitro, ex vivo, and in vivo investigations. Colloids and Surfaces B: Biointerfaces, 2015. 128: p. 448-456.
17. Bothiraja, C., Pawar, A., and Deshpande, G., Ex-vivo absorption study of a nanoparticle based novel drug delivery system of vitamin D 3 (Arachitol Nano™) using everted intestinal sac technique. Journal of Pharmaceutical Investigation, 2016. 46(5): p. 425-432.
18. Nunes, R., Silva, C., and Chaves, L., Tissue-based in vitro and ex vivo models for intestinal permeability studies, in Concepts and Models for Drug Permeability Studies2016, Elsevier. p. 203-236.
19. Shailender, J., Ravi, P.R., et al., Tenofovir disoproxil fumarate loaded PLGA nanoparticles for enhanced oral absorption: Effect of experimental variables and in vitro, ex vivo and in vivo evaluation. Colloids and Surfaces B: Biointerfaces, 2017. 158: p. 610-619.
20. Masiiwa, W.L. and Gadaga, L.L., Intestinal permeability of artesunate-loaded solid lipid nanoparticles using the everted gut method. Journal of drug delivery, 2018(3): p. 1-9.
21. Suvarna, V.M. and Sangave, P.C., HPLC estimation, ex vivo everted sac permeability and in vivo pharmacokinetic studies of darunavir. Journal of chromatographic science, 2018. 56(4): p. 307-316.
22. Dey, T.K., Koley, H., et al., Effects of nano-sizing on lipid bioaccessibility and ex vivo bioavailability from EPA-DHA rich oil in water nanoemulsion. Food chemistry, 2019. 275: p. 135-142.
23. Kontogiannidou, E., Karavasili, C., et al., In vitro and ex vivo assessment of microporous Faujasite zeolite (NaX-FAU) as a carrier for the oral delivery of danazol. Journal of Drug Delivery Science and Technology, 2019. 51: p. 177-184.
24. Martins, E., Silva, V., et al., Newly synthesized oxygenated xanthones as potential P-glycoprotein activators: in vitro, ex vivo, and in silico studies. Molecules, 2019. 24(4): p. 707.
25. Tambe, A., Mokashi, P., and Pandita, N., Ex-vivo intestinal absorption study of boswellic acid, cyclodextrin complexes and poloxamer solid dispersions using everted gut sac technique. Journal of pharmaceutical and biomedical analysis, 2019. 167: p. 66-73.
26. Thabit, A.A., Ali, O.K., et al., Predicting the Bioequivalence of Ciprofloxacin HCL Tablet-Brands Using In Vitro Dissolution and Rat Intestinal Permeability Tests. Journal of Chemical and Pharmaceutical Research, 2019. 11(7): p. 13-19.
27. Chaturvedi, S., Garg, A., and Verma, A., Nano lipid based carriers for lymphatic voyage of anti-cancer drugs: An insight into the in-vitro, ex-vivo, in-situ and in-vivo study models. Journal of Drug Delivery Science and Technology, 2020: p. 101899.
28. Wang, Y., Chen, Z., et al., Effect of Short-Term Exposure to Titanium Dioxide Nanoparticles on Intestinal Absorption of Glucose by Ex Vivo Everted Rat Gut Sac Model. Journal of Nanoscience and Nanotechnology, 2021. 21(9): p. 4586-4595.
29. Zancanella, P., Oliveira, D.M., et al., Mitotane liposomes for potential treatment of adrenal cortical carcinoma: ex vivo intestinal permeation and in vivo bioavailability. Pharmaceutical development and technology, 2020. 25(8): p. 949-961.
30. Al-Awar MAS, Salih EMA, Ismailovichc DC. Role of Catha Edulis (Khat) in Free Radicals Formation in Vivo and in Vitro Study. London Journal of Research in Science: Natural and Formal, 2017; 17 (1): 37-41.
31. Al-Awar MSA, Al-Eryan MAY. Effect of the ethanolic extract of Catha edulis leaves on the electrical activity of some brain centers of male rabbits. International Biological and Biomedical Journal, 2017; 3 (3): 133-137.