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Original Article

Estimation of *Catha edulis* (Vahl) Forssk. ex Endl. - antidiabetic drug interactions by using closed-loop Doluisio's method

[Estimación de la interacción *Catha edulis* (Vahl) Forssk. ex Endl. - fármacos antidiabéticos mediante el método de circuito cerrado de Doluisio]

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Abstract

Context: *Catha edulis* (khat) is a prevalent plant in Yemen and other African regions. In Yemen and other countries, most people chew this plant, which may change the pharmacokinetics of many drugs, such as anti-diabetics (glibenclamide and metformin).

Aims: To evaluate the effect of *C. edulis* presence in the gastrointestinal tract (GIT) on the intestinal perfusion and bioavailability of metformin and glibenclamide and its effectiveness.

Methods: The present study was conducted *in situ* using the closed-loop Doluisio's method. Rat intestinal perfusion of a reference standard of each test drug alone was compared to the drug intestinal perfusion in the presence of *C. edulis* (three types of *C. edulis* agriculture in different regions of Yemen). The type of *C. edulis* that produced the most influence on the absorption of the drug reference was then re-investigated using the same *in situ* method against a pharmaceutical dosage form (tablets) of the drug.

Results: The intestinal absorptions (perfusion) of reference standards of metformin and glibenclamide were decreased in the presence of *C. edulis* by 30.53-35.9% and 14.71-27.93%, respectively, while such decrease with pharmaceutical dosage form (tablets) of the two drugs was 24.24 and 19.77%, respectively.

Conclusions: Based on the results reached from this study, the anti-diabetic bioavailability was significantly reduced in the presence of *C. edulis*, and this effect may affect the drug efficacy.

Keywords: antidiabetics; *Catha edulis*; glibenclamide; intestinal absorption; khat; metformin.

Resumen

Contexto: *Catha edulis* (khat) es una planta predominante en Yemen y otras regiones africanas. En Yemen y otros países, la mayoría de las personas mastican esta planta, lo que puede cambiar la farmacocinética de muchos medicamentos, como los antidiabéticos (glibenclamida y metformina).

Objetivos: Evaluar el efecto de la presencia de *C. edulis* en el tracto gastrointestinal (TGI) sobre la perfusión intestinal y la biodisponibilidad de metformina y glibenclamida y su efectividad.

Métodos: El presente estudio se realizó *in situ* utilizando el método de Doluisio en circuito cerrado. La perfusión intestinal de rata de un estándar de referencia de cada fármaco de prueba solo se comparó con la perfusión intestinal del fármaco en presencia de *C. edulis* (tres tipos cultivares de *C. edulis* en diferentes regiones de Yemen). Luego, se volvió a investigar el tipo de *C. edulis* que produjo la mayor influencia en la absorción del fármaco de referencia utilizando el mismo método *in situ* frente a una forma de dosificación farmacéutica (tabletas) del fármaco.

Resultados: Las absorciones intestinales (perfusión) de los estándares de referencia de metformina y glibenclamida disminuyeron en presencia de *C. edulis* en 30,53-35,9% y 14,71-27,93%, respectivamente, mientras que dicha disminución con la forma de dosificación farmacéutica (tabletas) de los dos fármacos fue de 24,24 y 19,77%, respectivamente.

Conclusiones: Con base en los resultados de este estudio, la biodisponibilidad del antidiabético se redujo significativamente en presencia de *C. edulis*, y este efecto puede afectar la eficacia del fármaco.

Palabras Clave: antidiabéticos; *Catha edulis*; glibenclamida; absorción intestinal; khat; metformina.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by prolonged elevated blood sugar with disorders in the metabolism of fate, protein, and carbohydrates due to deficiency in insulin, resistance, or both. It may increase the rate of morbidity and mortality due to the associated severe problems (World Health Organization, 1999). There are three main types of diabetes: Type 1, type 2, and gestational diabetes (diabetes while pregnant) (CDC, 2023). Type 2 diabetes is the most common type, usually in adults with insulin resistance or does not produce enough insulin. In the previous three decades, type 2 diabetes has increased dramatically in nations of all income levels, and type 1 diabetes cannot presently be prevented. Effective methods are available to prevent type 2 diabetes and the complications and early death that can result from all types of diabetes. These comprise policies and practices across entire populations and within specific locations (school, home, workplace) that donate to good health for everybody, irrespective of whether they have diabetes, such as eating healthily, exercising regularly, controlling blood pressure and avoiding smoking, and lipids (World Health Organization, 2023). The biguanide metformin is an oral anti-hyperglycaemic agent that can be used in the treatment of patients with non-insulin-dependent diabetes mellitus (NIDDM). Metformin is the best option addition to the drug therapy options available for diabetes patients because it decreases blood sugar levels predominantly by reducing hepatic sugar production and release and also by increasing peripheral sensitivity of tissue to insulin. It does not stimulate the beta cells in the pancreas to secrete insulin. (Campbell et al., 1996). Glibenclamide is a second-generation sulfonylurea derivative that can be used to treat patients with type 2 diabetes mellitus. It is typically given to patients who cannot be managed with metformin, the standard first-line therapy (FDA, 2010). Glibenclamide stimulates insulin secretion by closing ATP-sensitive potassium channels on beta cells, raising intracellular potassium and calcium ion concentrations (Gribble and Reimann, 2006).

Catha edulis (Vahl) Forssk. ex Endl. is an evergreen tree from the family of *Celastraceae.* This plant has other names such as chat, qat, khat, miraa Abyssinian tea, African salad, African tea, and quaatka (Tolcha, 2020). This plant is commonly cultivated in the Arabian Peninsula and East Africa (e.g., Ethiopia and Kenya) (Issa et al., 2016).

C. edulis is chewed for its stimulatory effect due to the presence of more than 40 psychoactive substances contained in the fresh leaves of the herb (Geisshüsler and Brenneisen, 1987).

The most important parts are the young leaves and buds near the tip of the branch, and the most active substances are alkaloids with amphetamine-like properties (cathine and cathinone), which have euphoric and excitatory effects (Khan and Kalix, 1984). The dominant stimulator ingredients include cathinone, cathine, and norephedrine (Pantelis et al., 1989).

In Yemen, chewing *C. edulis* is a widespread routine; approximately 80–85% of adult males and 50-60% of adult females in North of Yemen chew *C. edulis* at least once a week. The simultaneous use of *C. edulis* with standard drugs is anecdotally purported to be commonly used in Yemen (Issa et al., 2016). There are different types of *C. edulis* according to the region in which it is grown, such as Arhabi khat, Shami khat, Hamdani khat, Khawlani khat, Ansi khat, Pallot khat, Herari khat (Ethiopian khat) and so on. The quality of *C. edulis* is based on the concentration of its components (alkaloids, flavonoids, and tannins) (Albaser et al., 2021).

In a systemic review (Albaser et al., 2021), several studies reported *C. edulis* interactions with many drugs. For instance, a study among healthy Yemeni adults who chew *C. edulis* revealed significant reduction in the bioavailability of antibiotics (e.g., ampicillin, amoxicillin, cephradine and ciprofloxacin) (Albaser et al., 2021; Attef et al., 1997; Kassem, 2004), phosphodiesterase type 5 (PDE5) inhibitors (sildenafil and tadalafil) (Al-Ghani et al., 2022a; 2022b) and the antimalarial drug chloroquine (Issa et al., 2016). Another study revealed a significant reduction in the pharmacokinetics properties of tetracycline-HCl among healthy Yemeni adults who chew *C. edulis* (Farah et al., 2015). Other studies established that *C. edulis* significantly increases the bioavailability of some drugs (clopidogrel, sertraline, clomipramine, vilazodone, aripiprazole), which might be attributed to the inhibition of their metabolic enzymes (Alhazmi et al., 2020; Bedada et al., 2018; Elkady et al., 2020). Another study in healthy adults suggested that chewing *C. edulis* had an inferior effect on the bioavailability and other properties of aspirin as their antiplatelet activity (Noman and Kadi, 2012). To the best of our knowledge, no previous studies have been concerned with the effect of *C. edulis* on the absorption of oral anti-diabetic drugs such as metformin and glibenclamide.

Due to the potential decrease of the drug absorption by oral intake of *C. edulis* and the reduction of drug efficacy, this study tried to investigate *C. edulis* interaction with two commonly prescribed oral anti-

Table 1. Scientific name, locality (coordinates and altitude), date of collection, vernacular name, and herbarium number of plant specimens (*C. edulis*).

No.	Scientific name	Location	Coordinates		Altitude	Date of collection	Vernacular name	Herbarium No.
			Longitude	Latitude				
1	*Catha edulis* (Vahl) Forssk. ex Endl.	Dhamar governorate (Anis District)	44°22'26.39"E	14°34'27.5"N	2400 masl	12, 17, 20, 24th Oct/2022	Khat Ansi	BHSS: 722/ cv.1
2	*Catha edulis* (Vahl) Forssk. ex Endl.	Sana'a governorate (Sana'a City)	44°11'27.6216"E	15°22'10.0020"N	2300 masl	15th Oct/2022	Khat Khawlani	BHSS: 722/ cv.2
3	*Catha edulis* (Vahl) Forssk. ex Endl.	Al-Jawf governorate	45°30'59.99"E	16°46'59.99"N	1134.96 masl	22th Oct/2022	Khat Pallot	BHSS: 722/ cv.3

masl: meters above sea level; cv: Cultivar

diabetic drugs. The significance of this study is greater for people who use oral anti-diabetic drugs and, at the same time, chew *C. edulis* like Yemeni people.

MATERIAL AND METHODS

Material

Metformin standard (98.4%) and glibenclamide standard (99.2%) were purchased from Shiphaco Pharma Company, Yemen. Metformin tablets (Denk, Germany) and Daonil tablets (Sanofi-Aventis, France) were purchased from the drug market. All other materials were at least of analytical grade. *C. edulis* samples were recognized and classified by the associate professor, Hassan Ibrahim (h.ibrahim@su.edu.ye) in Table 1.

The samples of three types of *C. edulis* were Ansi (khat I), Khawlani (khat II), and Pallot (khat III) collected from Dhamar, Sana'a, and Al-Jawf regions, as demonstrated in Table 1.

Instrumentations

HPLC (High-Performance Liquid Chromatography; Waters, model: Pump, 1525, detector, 2998, Germany) and an Inertsil® ODS-3V C_{18} column (250 mm × 4.6 mm, 5 µm), Japan was used. Electric balance (Radwag, Poland), mixer (JJ-1mixer, China). Water bath (HH-4, China), centrifuge (China).

Collection and preparation of *C. edulis* samples

The young leaves and buds near the tip of the branch were picked in the early morning from the three mentioned regions and transferred to Sana'a City on the same day of picking. 500 mg from the fresh leaves and buds were cut into smaller pieces (1 mm) using an appropriate tool. 200 mg of the small pieces were macerated with 200 mL of distilled water for 24 hours. The mixture amount was then filtered to obtain a clear solution. The resultant filtrate was mixed 1:1 with a physiological solution used in the experiments. The used physiological solution (pH 6.8) consisted of potassium dihydrogen phosphate 0.029 mol/L + sodium hydroxide to pH 6.8 + sodium taurocholate (bile salt) 5×10^{-3} mol/L + lecithin 1.5×10^{-3} mol/L + potassium chloride 0.22×10^{-3} mol/L + distilled water up to 1000 mL (Thabet and Al-Ghani, 2019).

Preparation of drug test samples

Metformin aqueous standard solution

Metformin HCl reference standard (641.3 mg, equivalent to 500 mg of metformin) was dissolved in 50 mL water to produce aqueous drug solution with a 10 mg/mL concentration. This solution was diluted as 1:1 with the physiological solution (pH 6.8) to produce a final concentration of the drug of 5 mg/mL.

Glibenclamide aqueous standard solution

Five mg of glibenclamide reference was dissolved in 50 mL water to produce an aqueous drug solution with a 0.1 mg/mL concentration. This solution was diluted as 1:1 with the physiological solution (pH 6.8) to produce a final drug concentration of 0.05 mg/mL.

Metformin-tablet aqueous solution

Ten tablets were ground to powder, and an amount of the powder equivalent to 1000 mg of the drugs was dissolved in 80 mL of distilled water and then filtered. The filtrate was diluted to 100 mL with the same solvent to produce an aqueous solution of the drug with a concentration of 10 mg/mL. This solution was diluted as 1:1 with the physiological solution (pH 6.8) to produce a final drug concentration of 5 mg/mL.

Glibenclamide-tablet aqueous solution

Ten tablets were ground to powder, and an amount of the powder equivalent to 10 mg of the drug was dissolved in 80 mL of distilled water and then filtered. The filtrate was diluted to 100 mL with the same solvent to produce an aqueous solution of the drug with a concentration of 0.1 mg/mL. This solution was diluted as 1:1 with the physiological solution (pH 6.8) to produce a final drug concentration of 0.05 mg/mL.

Animal models and *in situ* investigation

Ethical approval was done by Institutional Animal Care and Use Committee (Al-Razi U-IACUC)-Al-Razi University, Yemen.

Fifty healthy male rats weighing between 120-150 g were selected for the experiment from an inbred colony maintained under the controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%), and light (10 and 14 hours of light and darkness, respectively). The animals were fasted overnight but had free access to water prior to the experiment.

The animals were anesthetized by chloroform. The intestine of the rat was exposed by a midline abdominal incision. In the small intestine, two incisions were made, the first at the beginning of the duodenal segment and the second about 15 cm distant. A cannula was placed at the duodenal incision (input cannula) and another at the other end (output cannula). In order to remove the intestinal contents from the selected intestinal section, a physiological solution followed by water was introduced through the input cannula to flush the section thoroughly, and the fluid was then withdrawn through the output cannula.

The test of estimation of intestinal perfusion was carried out using *in situ* perfusion method (the closed loop Doluisio's method) as reported in the literature (Cabrera-Pérez et al., 2018; Caldeira et al., 2018; 2021; Chaturvedi et al., 2020; Chen et al., 2020; Christfort et al., 2020; Hens et al., 2022; Liu et al., 2019; Lozoya-Agullo et al., 2015; 2016; 2017; Luo et al., 2013; Marinho Dezani et al., 2017; Miranda et al., 2022; Ram et al., 2007; Ruiz-Picazo et al., 2020) as follows:

Ten mL of each *C. edulis* sample and other test solutions were introduced and sampled either individually or as *C. edulis*-drug combination into the selected section of the intestine with the aid of the syringes through the input cannula. The system was left for 30 minutes. Then, the fluid was withdrawn through the output cannula.

Analysis of samples

Standard calibrations

Metformin: Metformin HCl reference standard (128.26 mg, equivalent to 100 mg of metformin) was dissolved in 100 mL volumetric flasks with 50 mL distilled water and completed the volume to 100 mL with the distilled water to prepare standard stock solution of 1 mg/mL.

Ten milliliters of stock solution were passed to another 100 mL volumetric flask, and the volume was completed to 100 mL with distilled water to prepare 100 µg/mL solution. Then, six 10 mL volumetric flasks 1, 2, 3, 4, 5, and 6 mL of (100 µg/mL) concentration were taken and completed all to 10 mL with mobile phase solution (buffer and acetonitrile (95:5) (USP 43/NF 38, 2020) to prepare 6 solutions of the following concentration: 10, 20, 30, 40, 50, and 60 µg/mL. Chromatographic separation was performed under the stated chromatographic conditions. Calibration curves related to the obtained peak areas of the drug to the corresponding concentrations were made, and the regression equations were calculated. Measure the (high performance liquid chromatography) HPLC chromatogram at 235 nm for six concentrations and repeat each one three times and write the required chromatographic data.

Glibenclamide: 100 mg of glibenclamide standard were dissolved in 100 mL volumetric flasks with 50 mL methanol and completed the volume to 100 mL with the distilled water to prepare standard stock solution of concentration (1 mg/mL).

Five milliliters of stock solution were added to another 50 mL volumetric flask and completed the volume to 50 mL with distilled water to prepare solution of 100 µg/mL concentration. Six 10 mL volumetric flask 0.5, 1, 1.5, 2, 2.5, and 3 mL of 100 µg/mL concentration were taken, and complete all to 10 mL with selected mobile phase solution (buffer (ammonium phosphate) and acetonitrile (45:55) (USP 43/NF 38, 2020) to prepare 6 solutions of the following amounts 5, 10, 15, 20, 25, and 30 µg/mL. Chromatographic separation was then performed under the stated chromatographic conditions. Calibration curves relating the obtained peak areas of the drug to the corresponding concentrations were made, and the regression equations were calculated. HPLC chromatogram was measured at a wavelength of 228 nm (Bachri and Sitohang, 2019) for six concentrations, repeated thrice, and the chromatographic data were recorded.

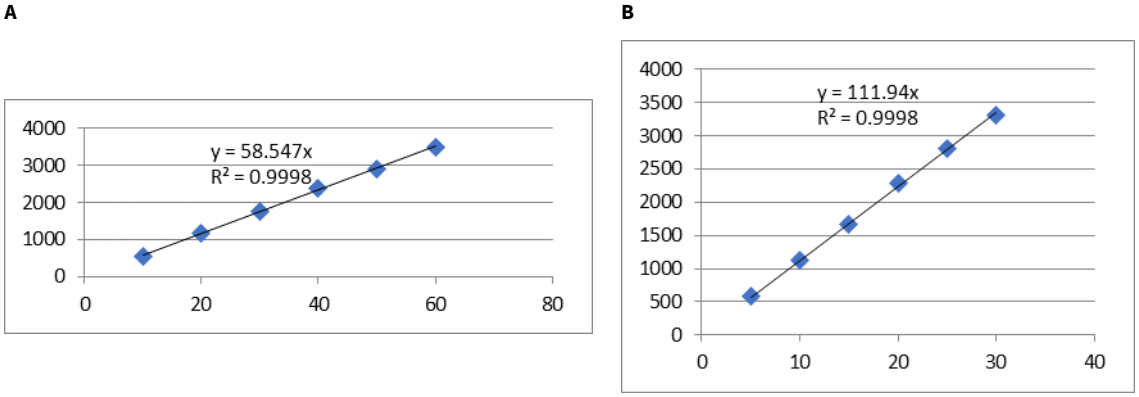

Figure 1. Calibration curves for the HPLC determination of **(A)** metformin (10-60 µg/mL) using buffer and acetonitrile (95:5), adjusted with phosphoric acid to a pH of 3.0 as the mobile phase at a flow rate of 1.5 mL/min and UV detection at 235 nm; and **(B)** glibenclamide (5-30 µg/mL) using buffer (ammonium phosphate) and acetonitrile (45:55), with pH of 5.3, as the mobile phase at a flow rate of 2 mL/min and UV detection at 228 nm.

Test samples

The withdrawn output solutions obtained from *in situ* perfusion test were first treated preanalytically with centrifugation at 4000 rpm for 15 min, followed by filtration of the supernatant clear solution. The filtrate was diluted properly with HPLC mobile phase. Then, 20 µL was injected into the HPLC system. The drug calculation in the test samples was carried out the same way as applied to standard solutions. These concentrations of test samples related to the unabsorbed portion of the drug were subtracted from the original concentrations introduced into the experiment to calculate the absorbed concentration of the drug (intestinal perfusion). The percentage of intestinal perfusion (IPP) of the drug was then calculated as per formula [1].

$$\text{Intestinal perfusion percentage (IPP)} = 100 \times Ca/Co \quad [1]$$

Where Ca and Co were the absorbed and original drug concentration, respectively.

The influence of *C. edulis* on the IPP of the drug was calculated according to formula [2].

$$R = IPP_a - IPP_{a+K} \quad [2]$$

Where R was the reduction percentage in the absorbed concentration percentage represented by IPP, IPP_a and IPP_{a+k} were the intestinal perfusion percentages of the drug alone and in the presence of *C. edulis*.

Statistical analysis

The Statistical Package for Social Sciences (IBM SPSS) version 27.0 was used to perform statistical analysis. Single-way analysis of variance (ANOVA) was used for comparison of the intestinal perfusion percentage profiles for each anti-diabetic drugs. The results were considered statistically significant, when the p-values less than 0.05.

RESULTS AND DISCUSSION

As shown in Fig. 1A-B, the HPLC standard calibration curves of metformin and glibenclamide demonstrated two straight curves with linearities of 0.9998 and 0.9998, respectively. The regression equations of the two curves were (y = 58.547x) and (y = 111.940x) and were used to calculate practical concentrations of the drugs in the test samples. In these equations, Y was the area under the peak in the HPLC chromatogram, and X was the concentration of the drug.

Table 2 shows the mean of intestinal perfusion percentage (IPP) of metformin alone, metformin in the presence of khat I and khat II, which were 88.47%, 52.52% and 57.94%, respectively. The reduction percentages of khat I and khat II on IPP of the drug were 35.95 and 30.53%. These findings indicated that both types of *C. edulis* reduced the intestinal perfusion of that drug, but the influence of khat I was greater. Therefore, khat I was selected for investigation of the effect of *C. edulis* on the IPP of tablets dosage forms of the drug. In this respect, the IPP of the drug (as tablets, metformin-Denk®) were 87.65% and 63.41% for the drug-tablets alone and in the presence of khat I. The influence of *C. edulis* on metformin-tablet perfusion was 24.24%, which was less than that obtained with the drug standard. This could be attributed to the presence of excipients in tablets that enhance the absorption, e.g., disintegrating agents (Allen and Ansel, 2017).

Regarding glibenclamide, Table 3 shows the mean of intestinal perfusion percentage of glibenclamide alone in the presence of khat I and khat III, which were 89.69, 61.75 and 74.98%, respectively. The influence of khat I and khat III on the IPP of the standard drug were 27.93 and 14.71%. These findings indicated

Table 2. The results of intestinal perfusion percentage of metformin alone and with *C. edulis* (khat) obtained *in situ* by using the closed loop Doluisio's method.

Sample	Standard Met. (IPP)	Met. with khat I (IPP)	R	Standard Met. (IPP)	Met. with khat II (IPP)	R	Met. tab (Denk)	Met. (Denk) with khat I (IPP)	R
1	88.506	52.519	35.987	88.506	60.376	28.13	87.464	62.767	24.697
2	88.181	51.324	36.857	88.181	57.302	30.879	87.617	64.646	22.971
3	88.369	53.544	34.825	88.369	55.764	32.605	87.686	61.742	25.944
4	88.813	52.690	36.123	88.813	58.326	30.487	87.822	64.475	23.347
Mean	88.467	52.519	**35.948**	88.467	57.942	**30.525**	87.647	63.408	**24.240**
SD	0.2662	0.9143	0.8405	0.2662	1.9344	1.8429	0.1489	1.3973	1.3565
SEM	0.1331	0.4572	0.4203	0.1331	0.9672	0.9215	0.0745	0.6986	0.6782
IP-reduction %			**35.948**			**30.525**			**24.240**

Met.: Metformin. SD: Standard deviation. SEM: Standard error of mean. IPP: Intestinal Perfusion Percentage. R: Reduction percentage in intestinal perfusion percentage.

Table 3. The results of intestinal perfusion percentage of glibenclamide alone and with *C. edulis* (khat) obtained *in situ* by using the closed loop Doluisio's method.

Sample	Standard Glib. (IPP)	Glib. with khat I (IPP)	R	Standard Glib. (IPP)	Glib. with khat III (IPP)	R	Glib. tab (Daonil) (IPP)	Glib. (Daonil) with khat I (IPP)	R
1	90.319	60.232	30.086	90.319	74.441	15.877	84.480	65.773	18.707
2	89.097	61.186	27.912	89.097	74.531	14.567	84.599	63.867	20.733
3	89.991	64.015	25.976	89.991	75.693	14.299	84.599	64.611	19.988
4	89.336	61.573	27.763	89.336	75.246	14.090	84.692	65.028	19.664
Mean	89.686	61.752	27.93	89.686	74.978	14.708	84.593	64.820	**19.773**
SD	0.5664	1.6109	1.6830	0.5664	0.5973	0.8035	0.0867	0.7967	0.8397
SEM	0.2832	0.8055	0.8415	0.2832	0.2986	0.4017	0.0433	0.3984	0.4199
IP-reduction %			**27.93**			**14.708**			**19.773**

Glib.: Glibenclamide. SD: Standard deviation. SEM: Standard error of mean. IPP: Intestinal Perfusion Percentage. R: Reduction percentage in intestinal perfusion percentage.

Table 4. Statistical analysis between the results of intestinal perfusion percentage of metformin (Met.) alone and with *C. edulis* (khat) obtained *in situ* by using the closed loop Doluisio's method.

Statisticals	Standard Met.	Met. with khat I	Standard Met.	Met. with khat II	Met. (Denk) tab	Met. (Denk) tab with khat I
Mean	88.467	52.519	88.467	57.942	87.647	63.408
SD	0.2662	0.9143	0.2662	1.9343	0.1489	1.3973
SEM	0.1331	0.4572	0.1331	0.9671	0.0745	0.6986
N	4	4	4	4	4	4
P	**0.366×10^{-8} ▲**		**0.00000071■**		**0.0000004□**	

SD: Standard deviation.; SEM: Standard error mean; ▲,■: ANOVA-single way -test (between intestinal perfusion of metformin alone and with the presence of khat I, khat II respectively) indicated sig variation ($p<0.05$); □: ANOVA-single way -test [between intestinal perfusion of metformin tablets (tab) and with the presence of khat I] indicated significance variation ($p<0.05$).

Table 5. Statistical analysis between the results of intestinal perfusion percentage of glibenclamide (Glib.) alone and with *C. edulis* (khat) obtained *in situ* by using the closed loop Doluisio's method.

Statisticals	Standard Glib.	Glib. with khat I	Standard Glib.	Glib. with khat III	Glib. tab (Daonil)	Glib. tab. with khat I
Mean	89.686	61.752	89.686	74.978	84.593	64.82
SD	0.5664	1.611	0.5664	0.5973	0.0867	0.7967
SEM	0.2832	0.8054	0.2832	0.2986	0.0433	0.3984
N	4	4	4	4	4	4
P	**5.42×10^{-8} ▲**		**3.25×10^{-8} ■**		**4.65×10^{-9} □**	

SD: Standard deviation; SEM: Standard error of mean; ▲, ■: ANOVA-single way -test (between intestinal perfusion of glibenclamide alone and with the presence of khat I, khat III, respectively] indicated sig variation ($p<0.05$); □: ANOVA-single way-test [between intestinal perfusion of glibenclamide tablets (tab) and with the presence of khat I] indicated significance variation ($p<0.05$).

that both types of *C. edulis* reduced the intestinal perfusion of that drug, but the influence of khat I was greater. Therefore, khat I was selected for investigation of the effect of *C. edulis* on the IPP of tablets dosage forms of the drug. In this respect, the intestinal perfusion percentages of the drug (as tablets; Daonil®) were 84.59 and 64.82% for the drug-tablets alone and in the presence of khat I. The influence of *C. edulis* on glibenclamide-tablet perfusion was, therefore, 19.77%, which was less than that obtained with the standard drug. This finding was similar to that obtained with metformin tablets and could be attributed to the same causes.

From the present-day study, the co-administration of the two oral anti-diabetic drugs (metformin and glibenclamide) with *C. edulis* was found to significantly affect the pharmacokinetics of these drugs. This was comparable to reported reductions in the bioavailability of chloroquine, ampicillin, amoxicillin, tadalafil, sildenafil and the pharmacokinetic activity of tetracycline hydrochloride when co-administered with *C. edulis* (Al-Ghani et al., 2022a; 2022b; Attef et al., 1997; Farah et al., 2015; Issa et al., 2016). The mechanisms underlying this interaction are unknown. However, there is a possible mechanism related to the interaction of these drugs with some components of *C. edulis,* such as tannic acid, cathinone, and cathine, which are known to cause the formation of insoluble complexes and non-absorbable compounds (Attef et al., 1997; Farah et al., 2015).

Statistical analysis of the results obtained by the suggested methods for the comparison of intestinal perfusion percentage of the standard drug alone, with *C. edulis* and drugs tablet (metformin-Denk and Daonil) alone and with *C. edulis* as shown in Tables 4 and 5 revealed significant differences between them.

CONCLUSION

Based on the results obtained from the present study, the intestinal perfusion (absorption) of oral anti-diabetic drugs (metformin and glibenclamide) is significantly reduced in the presence of *C. edulis* (khat-chewing). Moreover, khat I, grown in Dhamar City, has more reducing influence than other types of *C. edulis*.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Al-Ghani AM	Al-Khawlani MA	Thabet AAM
Concepts or ideas	x		
Design	x	x	
Definition of intellectual content		x	x
Literature search	x		
Experimental studies	x	x	x
Data acquisition	x		
Data analysis	x	x	x
Statistical analysis	x		x
Manuscript preparation	x		x
Manuscript editing	x	x	x
Manuscript review	x	x	x

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