Current Science
CS 5 (4), 4219-4230 (2025)
Received 25 May 2025 | **Revised 20 June** 2025 | **Accepted 22 July**. 2025 | **Online Available 30 August** 2025
https://doi.org/10.52845/CS/2025-5-4-69

Current Science

Current Science

OPEN ACCESS

ISSN (O) 2795-8639

Original Article

Estimation of *Catha Edulis*-Anti-Platelet Drugs Interaction by HPLC and Using Closed-Loop Doluisio's Method

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Abstract:

Background: Khat (*Catha edulis*) is a widespread tree in different areas of the world like Yemen and Africa. In these countries, Khat is chewed by most people, which may lead to alteration in pharmacokinetics of a large numerous drugs like anti-platelet aggregation (aspirin, clopidogrel and dipyridamole).

Objective: To assess the Khat-Antiplatelet drugs interaction in the gastrointestinal tract (GIT) on the bioavailability and absorption of aspirin, clopidogrel and dipyridamole in intestinal tract and also its effect on their activity.

Methods: Currently the study was conducted *In Situ* by using Deloisio closed-loop method. High-performance liquid chromatography (HPLC) was used to estimate the intestinal perfusion (concentration of absorbed drugs). The absorption of the standard for each experimented drug was compared to the absorption of the drug in the presence of Khat (three types of Khat culture in the different Yemen's areas). The type of Khat that had the greatest effect on the reference drug absorption was then reinvestigated using the similar used method versus a dosage form of the drug (tablets).

Results: The references of aspirin, clopidogrel and dipyridamole absorptions (perfusion) were induced in the presence of Khat by 20.2185 ± 0.3323%, 7.9250 ± 1.3499% and 9.1193 ± 4.3749% respectively while such increasing with tablets dosage form of the three drugs were 6.4272 ± 0.3580%, 8.9455 ± 2.2412% and 6.8605 ± 2.7936%, respectively.

Conclusions: Depending on the findings reached from the proposed study, the absorption of the antiplatelet aggregation was significantly induced in Khat presence, which may affect the drug efficiency and toxicity.

Keywords: Antiplatelet; Aspirin, Catha Edulis, Clopidogrel, Dipyridamole, Intestinal absorption, Khat.

Introduction

Cardiovascular diseases (CVD) remain the major clinical and public health problem in developed countries, and this problem is progressively increasing worldwide. Heart disease and stroke are the main manifestations associated with cardiovascular diseases. The World Health Organization estimates that cardiovascular diseases will be the leading cause of death and disability worldwide by 2020[1].

Antiplatelet drugs are medical agents that work to stop blood clots from forming. They work in preventing platelets from sticking together. Antiplatelet drugs are used to prevent blood clots, which can lead to strokes and heart attacks *https://my.clevelandclinic.org/health/drugs/22955-antiplatelet-drugs*

Aspirin, (*CAS Number*. *50-78-2*) also known as acetylsalicylic acid, is one of the most widely used medications in the world. It is derived from white willow bark extract, and its therapeutic potential has been known in Egypt since 1534 BC. Historically, Aspirin pharmacological effects have been secondary to its anti-inflammatory and platelet-inhibiting properties[2].

Clopidogrel, (*CAS Number*: 120202-66-6) is a thienopyridine antiplatelet drug used in thromboembolic disorders. Similar to ticlopidine, it is a platelet P2Y12 -receptor antagonist that acts by inhibiting adenosine diphosphate-mediated platelet aggregation (Brayfield, 2014).

Dipyridamole (*CAS Number*: 58-32-2) is an adenosine reuptake and phosphodiesterase inhibitors with antiplatelet and vasodilating activity used in thromboembolic disorders. Oral Dipyridamole is used for the prophylaxis of thromboembolism after cardiac valve replacement and in the management of stroke (below). It has also been used in the management of myocardial infarction[3].

Catha edulis is an evergreen tree in the Celastraceae family. This plant has other terms such as Qat, Khat, Chat, African tea, Miraa Abyssinian tea, African salad, and Quaatka[4]. This plant is commonly cultured in Arabian Peninsula and East Africa (e.g. Ethiopia and Kenya) [5-7].

Khat is chewed for its stimulatory effect as a result of the presence of more than 40 psychoactive substances found in the fresh leaves of the herb[8-10].

The most important parts are the tender leaves and buds near the tip of the branch, and the most active substance is the alkaloids with amphetamine-like properties (cathine and cathinone) that have euphoric and exciting effects [11]. The dominant stimulator ingredients include Cathinone, Cathine, and Norephedrine[12].

In Yemen, chewing Khat is a common habit; about 80-85% of adult men and 50-60% of adult women in northern Yemen chew Khat at least once a week. Concomitant use of Khat with standard medications is thought to be common in Yemen[5]. There are different types of Khat depending on the area in which it is cultured, such as Hamdani, Arhabi, Shami, Khawlani, Ansi, Pallot, Harari (Ethiopian) Khat, etc. The Khat quality depends on the concentration of its components (alkaloids, flavonoids and tannins) [13].

The previous studied indicated that Khat interacts with many medications. For example, a study in healthy adult people chewing Khat revealed a significant reduction in the antibiotics bioavailability (e.g., ampicillin, amoxicillin, cephradine, ciprofloxacin)[13-15], drugs inhibit phosphodiesterase type 5 (tadalafil and sildenafil) [16, 17] and drug of the malaria, chloroquine[5]. Another study revealed a significant reduction in the pharmacokinetic properties of tetracycline hydrochloride among healthy Yemeni adults chewing Khat[18]. Other studies have shown that Khat significantly reduces the bioavailability of some antidiabetic drugs such as metformin and glibenclamide[19] and induces some drugs bioavailability (sertraline, clopidogrel, vilazodone, aripiprazole, clomipramine), which might be accredited to the inhibition of their metabolic enzymes[20-22]. Another study in healthy adults indicated that chewing Khat had minimal influence on the bioavailability and other properties of Aspirin, such as its antiplatelet activity[23]. To our knowledge, there have been no previous studies related to the influence of Khat on the oral antiplatelet drugs absorption such as aspirin, clopidogrel, and dipyridamole.

Because drug absorption is increased with oral administration of Khat and the drug efficacy and possibly toxicity are enhanced, this study attempted to investigate the interaction of Khat with three commonly prescribed oral antiplatelet drugs and is consistent with previous studies. The significance of this study is greater for patients who administered antiplatelet drugs and simultaneously chew Khat like Yemenis.

2. Materials and Instrumentation:

2.1. Material:

Aspirin standard (96%), clopidogrel standard (99.5%) and dipyridamole standard (98.6 %) were purchased from Shiba Pharma-Yemen. **Aspirin-75 mg tablet (**(Aspirin Wockhardt-UK)**),** clopidogrel 75 mg tablet (Plavix 75mg -Sanofi-Aventis-France) and dipyridamole-75 **tablets (**Dipyridamole-Zydus-India) were obtained from the market of drug. All other chemicals were at least of analytical grade. Khat samples were identified and classified by the associate

professor, Hassan Ibrahim (h.ibrahim@su.edu.ye) (Table 1).

Table 1: Scientific name, Locality (coordinates and altitude), Date of Collection, Vernacular, and Herbarium Number of Plant specimens (Khat)

No.	Scientific name	Location	Coordinates		Altitude	Date of collection	Vernacular Name	Herbarium No.
			Longitude	Latitude				
1	*Catha edulis* Forssk.	Dhamar governorate (Anis District)	44°22'26.39 "E	14°34'27.5 "N	2400 m asl	10th Nov/2023	Khat Ansi	BHSS: 722/ cv.1
2	*Catha edulis* Forssk.	Hajja governorate (Al-Mahabisha District)	43° 32′ 0″ E	15° 55′ 0″ N	1800 m asl.	11th Nov/ 2023	Khat Shami	BHSS: 722/ cv.4
3	*Catha edulis* Forssk.	Sana'a governorate (Sana'a City)	44O11'27.62 16"E	15°22'10. 0020"N	2300 m asl.	(9, 14, 21, 25, 29)th Nov/ 2023	Khat Hamdani	BHSS: 722/ cv.5

asl: above sea level; cv: Cultivars

Three samples of each type of three Khat types: Ansi (I), Shami (II) and Hamdani (III) were collected from Dhamar, Hajjah and Sana'a areas, respectively, as shown in Table 1.

2.2. 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. Mixture (JJ-1mixer, China), Electric balance (Radwag, Poland), Centrifuge (China), Water bath (HH-4, China) was also used.

3. Methods

3.1. Khat Preparation and Collection:

Early in the morning from the aforementioned areas, the leaves and young shoots close the branch tip were collected and transported to Sanaa City on the same collection day. From the fresh shoots and leaves (500 mg) were censored into smaller pieces (1 mm) by using the suitable device, and the pieces (200 mg) was soaked. The mixture was combined with 200 ml of distilled water for 24 hours. Then, the mixture was filtered to obtain a clear solution. The resulting solution was mixed in a 1:1 ratio with the physiological solution, and this mixture was used in tests. The physiological solution used (pH 6.8) and is composed of sodium hydroxide at pH 6.8 + potassium dihydrogen phosphate 0.029 mol/L + potassium chloride 0.22×10−3 mol/L+ sodium taurocholate (bile salt) 5×10−3 mol/L + lecithin 1.5×10−3 mol/L and complete to 1000 ml with distilled water[24].

3.2. Preparation of Drug Test Samples

Standard Solution of Aspirin:

Aspirin reference (100 mg) was dissolved in methanol (50 ml) to produce solution of a drug with (2 mg/ml) concentration and then distilled water was used to dilution of drug solution to solution with (0.2 mg/ml) concentration. Also, another dilution in ratio 1:1 with the physiological solution (pH 6.8) to result a final drug with (0.1 mg/ml) concentration.

Standard Solution of Clopidogrel:

Clopidogrel bisulfate reference (130.44 mg) (equivalent to 100 mg clopidogrel) was dissolved in water (50 ml) to produce drug solution with (2 mg/ml) a concentration, this solution was diluted as 1:1 with the physiological solution (pH 6.8) to produce a final drug concentration of 1 mg/ml.

Standard Solution of Dipyridamole:

Dipyridamole reference (100 mg) was dissolved in 50 ml mobile phase (Methanol: buffer (0.77 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.0) in ratio (70:30)) to produce drug solution with (2 mg/ml) a concentration, this solution was diluted as 1:1 with the physiological solution (pH 6.8) to produce a final drug concentration of 1 mg/ml.

Aspirin-Tablet Aqueous Solution:

Ten tablets were crushed into powder. Then, a quantity of powder equivalent to 100 mg of the

drug was dissolved in 80 ml of distilled water. Next, the solution was filtered and diluted to 100 ml with the same solvent to yield an aqueous solution of the drug with a concentration of 1 mg/ml. This solution was diluted in a ratio of 1:1 with physiological saline (pH 6.8) to yield a final drug **Clopidogrel-Tablet Solution:**

Ten tablets were crushed into powder and a quantity of the powder equivalent to 130.44 mg of clopidogrel bisulfate (equivalent to 100 mg of the clopidogrel drug) was dissolved in 80 ml distilled water then filtered. The filtrate was diluted to 100 ml with the same solvent to yield aqueous solution of drug with concentration of 1 mg/ml. This solution was diluted as 1:1 with the physiological solution (pH 6.8) to produce a final drug concentration of 0.5 mg/ml.

Dipyridamole-Tablet Solution:

Ten tablets were ground to powder and a quantity of the powder equivalent to 100 mg of the drugs was dissolved in 50 ml methanol. The solution was filtered and diluted to 100 ml with the distilled water to yield aqueous solution of drug with concentration of 1 mg/ml. This solution was diluted as 1:1 with the physiological solution (pH 6.8) to yield a final concentration of the drug 0.5 mg/ml.

3.3. Animal Models Study:

For the experiment, fifty-five healthy male rats weighing 120 to 150 g were selected from a homogeneous group kept under controlled states of humidity (50 ± 5%), temp. (23 ± 2 °C) and light (14 and 10 h of dark and light, respectively). The animals were fasted overnight but had free access to water before the experimentation.

The animals were anesthetized with chloroform and the rat intestine was exposed through a midline abdominal incision. Two incisions were made in the small intestine; the first at the beginning of the duodenal segment and the second at a distance of approximately 15 cm. A cannula was placed in the duodenal incision (inlet cannula) and another at the other end (outlet cannula). To remove intestinal contents from the selected intestinal section, a physiological solution followed by water was introduced through the inlet cannula to completely wash the section, and then the liquid was removed through the outlet cannula.

The intestinal perfusion estimation test was performed using the *In Situ* perfusion method (Deloisio closed-loop method) as stated in the literature[25-37] as follows:

Ten ml of each Khat sample and other test solutions was introduced and sampled either individually or as a mixture of Khat and drug into the selected part of the intestine with the help of syringes through the inlet cannula. The system was left on for 30 minutes. The liquid was then withdrawn through the outlet cannula.

3.4. Analysis of Samples (HPLC)

Standard Calibrations

Aspirin:

100 mg of aspirin reference solution was dissolved in 100 ml volumetric flasks with 50 ml distilled water, and the volume was made up to 100 ml with distilled water to prepare a standard solution (1 mg/ml).

Ten ml of the stock solution was taken in another 100 ml volumetric flask and, the volume was made up to 100 ml with distilled water to prepare 100 µg/ml solutions. take to six 10 ml volumetric flasks 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml of concentration (100 µg/ml) were taken. All were made up to 10 ml with mobile phase solution (2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Then, it was adjusted with glacial acetic acid to pH 3.4.)[38], to prepare 6 solutions of the following concentration: 5 µg, 10 µg, 15 µg, 20 µg, 25 µg, 30 µg. Chromatographic separation was performed under the above chromatographic conditions. Calibration curves relating the obtained peak areas of the drug to the corresponding concentrations were made and regression equations were calculated. High performance liquid chromatography (HPLC) chromatogram was measured at 280 nm for six concentrations, repeated every three times and the required chromatographic data were recorded.

Clopidogrel:

130.44 mg of clopidogrel bisulfate reference (equivalent to 100 mg clopidogrel) was dissolved in 100 ml volumetric flasks with 50 ml methanol. The volume was completed to 100 ml with the distilled water to prepare standard stock solutions of concentration (1 mg/ml).

Five ml of stock solution was taken to another 50 ml volumetric flask, and the volume was completed to 50 ml with distilled water to prepare solutions of 100 µg/ml concentration taking to six 10 ml volumetric flask 0.5 ml, 1 ml, 1.5 ml, 1.75 ml, 2 ml, and 2.5 ml of (100 µg/ml) concentration, and completing all to 10 ml with selected mobile phase solution (Acetonitrile and Buffer (1.36 g/L of monobasic potassium phosphate in water) (25:75)) [38], to prepare 6 solutions of the following amounts 5 µg, 10 µg, 15 µg, 17.5 µg, 20 µg, 25 µg. Chromatographic separation was then achieved under the specified chromatographic conditions. Calibration curves relating the obtained peak areas of the drug to the corresponding concentrations were made and the regression equations were calculated. The HPLC chromatogram was measured at wavelength (220 nm) (USP 43/NF 38, 2020) for six concentrations and repeated every three times, and the chromatographic data were recorded.

Dipyridamole:

100 mg of dipyridamole reference standard was dissolved in 100 ml volumetric flasks with 50 ml methanol. The volume was completed to 100 ml with the distilled water to prepare standard stock solutions of concentration (1 mg/ml).

Take 5 ml of stock solution to another 50 ml volumetric flask, the volume was completed to 50 ml with distilled water to prepare solutions of 100 µg/ml concentration taking to six 10 ml volumetric flask 0.2 ml, 0.4 ml, 0.6 ml, 1 ml, 1.2 ml, and 1.6 ml of (100 µg/ml) concentration, and completing all to 10 ml with selected mobile phase solution (mobile phase (Methanol: buffer (0.77 g/L of ammonium acetate. Then, it was adjusted with glacial acetic acid to a pH of 4.0) in ratio (70:30)**)** (USP 43/NF 38, 2020) to prepare 6 solutions of the following amounts 2 µg, 4 µg, 6 µg, 10 µg, 12 µg, 16 µg. Chromatographic separation was then achieved under the specified chromatographic conditions. Calibration curves relating the obtained peak areas of the drug to the corresponding concentrations were made, and regression equations were calculated. The HPLC chromatography was measured at wavelength (288 nm)[38] for six concentrations, repeated each concentration three times, the chromatographic data were recorded.

Chromatographic separation was then achieved under the specified chromatographic conditions. Calibration curves relating the obtained peak areas of the drug to the corresponding concentrations were made and the regression equations were calculated. The HPLC chromatogram was measured at wavelength (288 nm) [38] for six concentrations and repeated each three times. The chromatographic data were written down.

3.5. Test Samples

The withdrawn outlet solutions obtained from the *In Situ* perfusion test were first processed prior to analysis by centrifugation at 4000 rpm for 15 minutes. This was followed by filtration of the upper clear solution. The filtrate was appropriately diluted using mobile phase HPLC. 20 µl was injected into the HPLC system. The calculation of the drug in the test samples was performed in the same manner as applied to the standard solutions. The concentrations of these test samples related to the unabsorbed fraction of the drug and subtracted from the original concentrations were entered into the experiment to calculate the absorbed concentration of the drug (intestinal perfusion). The percentage intestinal perfusion (PPI) of the drug was then calculated as follows:

Intestinal Perfusion Percentage (IPP) = 100* Ca/Co; where Ca and Co were the concentration of drug absorbed and original, respectively.

The influence of Khat on IPP of the drug was calculated as follows:

R = IPP_a - IPP_{a+K}; where R was the reduction percentage in the absorbed concentration percentage that represented by (IPP).

IPP_a and IPP_{a+k} were the intestinal perfusion percentages of the drug alone and in the presence of Khat.

Ethical Approval

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

4. Results and Discussion

The HPLC standard calibration curves of aspirin, clopidogrel and dipyridamole, as shown in Figure (1), (2) and (3), respectively, demonstrated three straight curves with linearities of 0.9998, 1.0 and

0.9991, respectively. The regression equations of the three curves, respectively were (Y = 497.68 * X), (Y = 69.386 * X) and (Y = 99.64 * X) and were used to calculate practical concentrations of the drugs in the test samples. In these equations, (Y) was an area under the peak in HPLC chromatogram, and (X) was the concentration of drug.

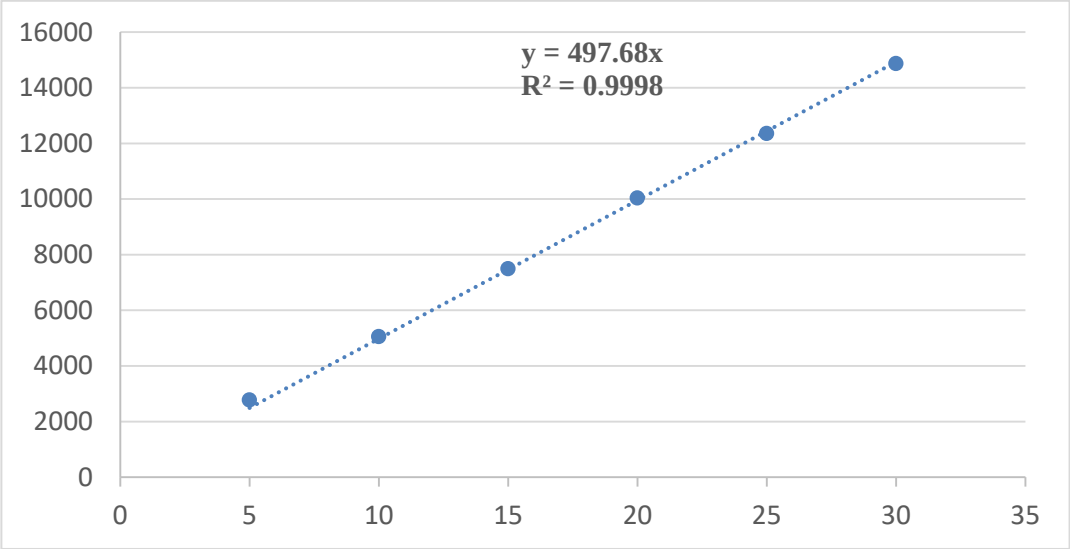

Figure 1. Calibration curve for the HPLC determination of aspirin (5-30 µg/ml) using Buffer and acetonitrile (85:15). Adjust with glacial acetic acid to a pH of 3.4. as the mobile phase at a flow rate of 2 ml/min and UV detection at 280 nm.

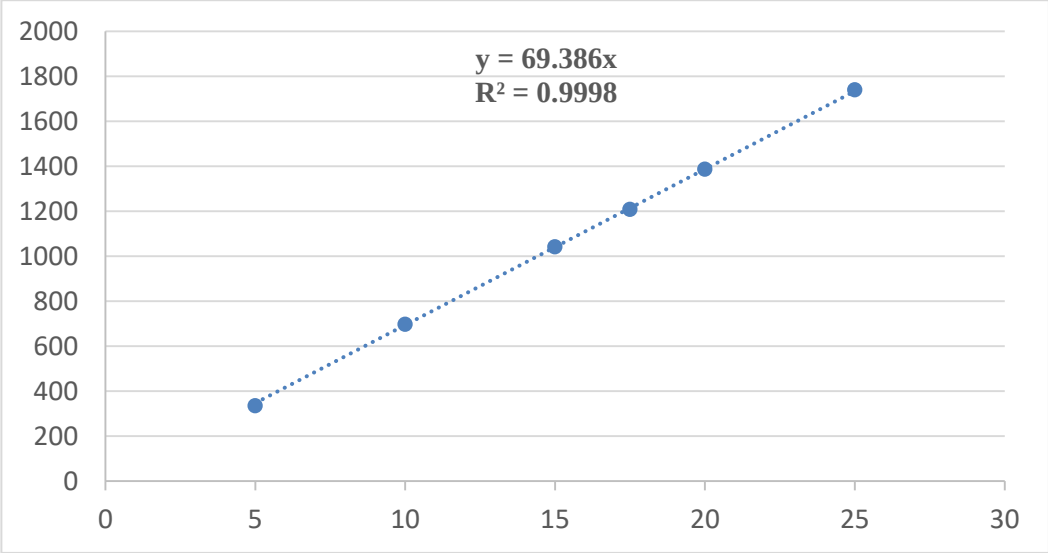

Figure 2. Calibration curve for the HPLC determination of clopidogrel (5-25 µg/ml) using Buffer (monobasic potassium phosphate) and acetonitrile (75:25). as the mobile phase at a flow rate of 1 ml/min and UV detection at 220 nm.

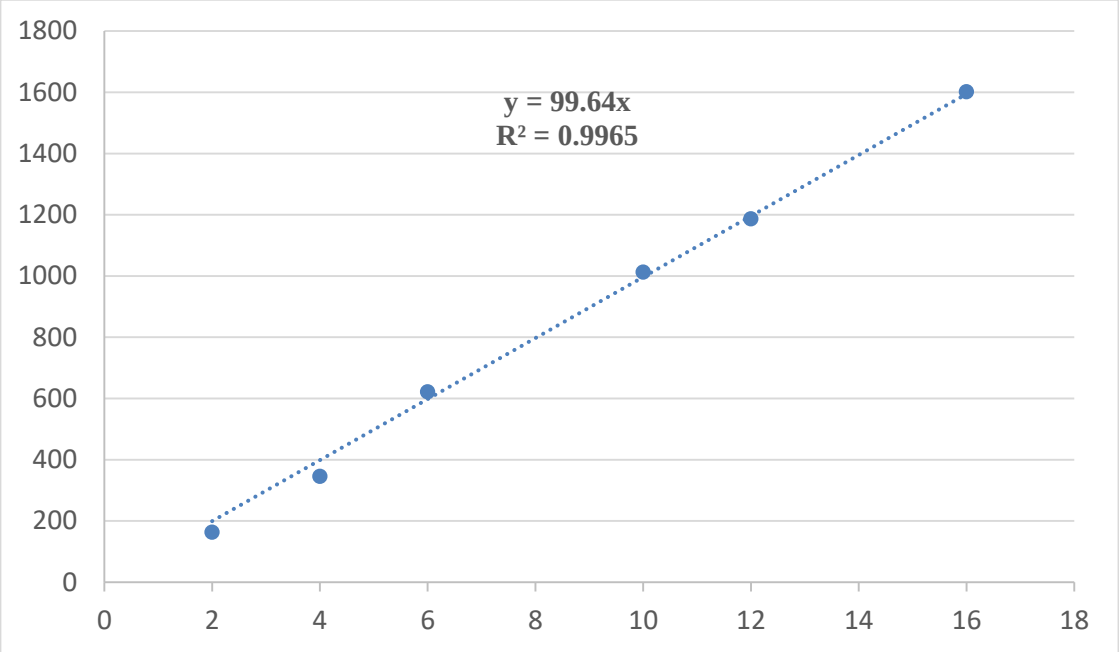

Figure 3. Calibration curve for the HPLC determination of dipyridamole (2-16 µg/ml) using Buffer (ammonium acetate) and methanol (30:70). with pH of 4.0. as the mobile phase at a flow rate of 1.2 ml/min and UV detection at 288 nm.

Table (2) shows the average intestinal perfusion percentages for aspirin alone and aspirin in the presence of Khat I, Khat II and Khat III were 41.8893%, 54.9598%, 60.6682% and 62.1078%, respectively. The induction percentages of Khat I, Khat II and Khat III on the intestinal perfusion percent of the drug were 31.2025%, 44.8301% and 48.2666%, respectively. These results indicated that all types of Khat induce intestinal perfusion of that drug, but the effect of Khat III was greater. Therefore, Khat III was chosen to investigate the effect of Khat on the intestinal perfusion percentage of tablet dosage forms. In this regard, the intestinal perfusion percentage for the drug (Aspirin® tablets) was 60.8522% and 67.2793% for the tablets of the drug alone and in the presence of Khat III. The effect of Khat on the perfusion of aspirin tablets was 10.5620%, which is higher than that obtained with the standard drug. This can be attributed to the presence of excipients in the tablets that improve absorption such as disintegrating agents.[39].

Table 2: The results of intestinal perfusion percentage of aspirin alone and with khat obtained in *In-Situ* by using the closed loop Doluisio's method

Sample	Stand Aspirin (IPP)	Aspirin with khat I (IPP)	Aspirin with khat II (IPP)	Aspirin with khat III (IPP)	RI	RII	RIII	Aspirin tab	Aspirin Tab. with khat III (IPP)	Ritab
1	42.425	55.212	60.702	63.045	12.787	18.277	20.620	60.635	67.597	6.962
2	41.421	53.47	60.902	61.706	12.049	19.481	20.285	61.036	67.263	6.227
3	42.023	54.74	60.501	62.175	12.717	18.478	20.152	60.501	66.727	6.226
4	41.688	56.417	60.568	61.505	14.729	18.88	19.817	61.237	67.530	6.293
Mean	41.8893	54.9598	60.6682	62.1078	13.0705	18.7790	20.2185	60.8522	67.2793	6.4272
SD	0.4339	1.2186	0.1768	0.6849	1.1546	0.5309	0.3323	0.3427	0.3954	0.3580
SE	0.2169	0.6092	0.0887	0.3425	0.5773	0.2655	0.1661	0.1715	0.1977	0.1790
IP-induction %					31.2025	44.8301	48.2666			10.5620

Stand: Standard. SD: Standard deviation. SE: Standard Error. IPP: Intestinal Perfusion Percentage. RI, II, III: Reduction percentage in intestinal perfusion percentage from Khat I, Khat II and Khat III respectively.

Regarding the clopidogrel drug, Table (3) shows the average percentages of intestinal perfusion for the clopidogrel drug alone and in the presence of greater effect Khat I, which were 77.7978% and

85.7228%, respectively. The effect of Khat III on the intestinal perfusion percentage of the standard drug was 10.1867%. These results indicated that Khat was a cause of induction of intestinal perfusion with that drug, and Khat III was chosen to investigate the effect of Khat on the intestinal perfusion percentage of tablet dosage forms. In this regard, the intestinal perfusion percentage of the drug (such as Plavix® tablets) was 75.4925% and 84.4380% for the tablets of the drug alone and in the presence of Khat III. Thus, the effect of Khat on the perfusion of clopidogrel tablets was 11.4895%, higher than that obtained with the standard drug. This result was similar to that obtained with aspirin tablets and can be attributed to the same reasons.

Regarding dipyridamole, Table (4) shows the average intestinal perfusion percentages for dipyridamole alone and in the presence of more effect Khat III, which were 12.0653% and 21.1845%, respectively. The effect of Khat III on the intestinal perfusion percentage of the standard drug was 75.5828%. These results indicated that Khat was a cause of induction of

Table 3: The results of intestinal perfusion percentage of Clopidogrel alone and with the more effect khat obtained in *In-Situ* by using the closed loop Doluisio's method

Sample	Stand Glop (IPP)	Glop with khat III (IPP)	R	Glop tab (Plavix) (IPP)	Glop (Plavix) with khat III (IPP)	R
1	76.849	86.167	9.318	73.679	84.918	11.239
2	79.395	85.543	6.148	79.203	85.255	6.052
3	77.329	85.062	7.733	75.937	84.390	8.453
4	77.618	86.119	8.501	73.151	83.189	10.038
Mean	**77.7978**	**85.7228**	**7.9250**	**75.4925**	**84.4380**	**8.9455**
SD	1.1106	0.5239	1.3499	2.7529	0.9056	2.2412
SE	0.5554	0.2619	0.6749	1.3764	0.4527	1.1206
IP-Induction %			**10.1867**			**11.4895**

Stand: Standard. Glop: Clopidogrel. SD: Standard deviation. SE: Standard Error. IPP: Intestinal Perfusion Percentage. R: Reduction percentage in intestinal perfusion percentage.

Table 4: The results of intestinal perfusion percentage of dipyridamole alone and with the more effect khat obtained in *In-Situ* by using the closed loop Doluisio's method

Sample	Stand Dip (IPP)	Dip with khat III (IPP)	R	Dip tab. IPP)	Dip tab. with khat III (IPP)	R
1	9.639	23.025	13.386	17.671	21.017	3.346
2	11.647	16.667	5.020	10.643	20.348	9.705
3	13.320	19.009	5.689	14.324	22.691	8.367
4	13.655	26.037	12.382	18.340	24.364	6.024
Mean	12.0653	21.1845	**9.1193**	15.2445	22.1050	**6.8605**
SD	1.8406	4.1663	4.3749	3.5351	1.7997	2.7936
SE	0.9203	2.0832	2.1875	1.7676	0.8999	1.3968
IP-Induction %			**75.5828**			**45.0031**

Stand: Standard. Dip: Dipyridamole. SD: Standard deviation. SE: Standard Error. IPP: Intestinal Perfusion Percentage. R: Reduction percentage in intestinal perfusion percentage.

Intestinal perfusion with this drug, and the Khat III was chosen to investigate the effect of Khat on intestinal perfusion percentage for tablet dosage forms. In this regard, the intestinal perfusion percentage of the drug (in the form of dipyridamole® tablets) was 15.2445% and 22.1050% for the tablet drug alone and in the presence of Khat III, respectively Thus, the effect of Khat on the perfusion of dipyridamole tablets

was 45.0031%, higher than that obtained with the standard drug. This result was similar to that obtained with Aspirin tablets and can be attributed to the same reasons.

From the current study, it was found that co-administration of three oral antiplatelet drugs (aspirin, clopidogrel and dipyridamole) with Khat significantly affects the pharmacokinetics of these drugs. This was similar to what was reported in previous studies that revealed the induction of aspirin and clopidogrel bioavailability by inhibiting the cytochrome P-450 enzyme[20-22] [23] and a comparison with other studies reveals a decrease in the bioavailability of chloroquine, ampicillin, amoxicillin, tadalafil, sildenafil and the pharmacological activity of tetracycline hydrochloride when taken with Khat[5, 14, 16-18] the mechanisms underlying this interaction are unknown. However, there is a possible mechanism related to the interaction of these drugs with some components of Khat, such as tannic acid, cathinone and cathine, which are known to cause the formation of insoluble complexes and non-absorbable compounds[14, 18] and to other unknown possible mechanism. Statistical analysis of the results obtained by the proposed methods for comparing the intestinal perfusion percentage of the standard drug alone, with Khat and medicinal tablets (Aspirin, Plavix, Dipyridamole) alone and with Khat showed a significant difference between them (Tables 5,6).

Table 5: Statistical analysis between the results of intestinal perfusion percentage of Aspirin alone and with khat obtained *In-Situ* by using the closed loop Doluisio's method

Statistical term	Stand. Aspirin	Aspirin with khat I	Stand. Aspirin	Aspirin with khat II	Stand. Aspirin	Aspirin with khat III	Aspirin Tab	Aspirin tab with khat I
Mean	41.8893	54.9598	41.8893	60.6682	41.8893	62.1078	60.8522	67.2793
SD	0.4339	1.2190	0.4339	0.1771	0.4339	0.6849	0.3430	0.3956
SE	0.2169	0.6090	0.2169	0.0887	0.2169	0.3425	0.1715	0.1978
N	4	4	4	4	4	4	4	4
P	**0.954×10^{-6} ▲**		**0.254×10^{-9} ■**		**0.436×10^{-8} ✱**		**0.299×10^{-6} □**	

SD: Standard deviation.; SE Standard Error; ▲, ■, ✱ : ANOVA-single way -test (between intestinal perfusion of aspirin alone and with the presence of khat I, Khat II and khat III respectively) indicated sig variation ($p < 0.05$): □ANOVA-single way -test (between intestinal perfusion of aspirin tablets and with the presence of khat I) indicated sig variation ($p < 0.05$).

Table 6: Statistical analysis between the results of intestinal perfusion percentage of (clopidogrel and dipyridamole) alone and with khat obtained *In-Situ* by using the closed loop Doluisio's method

Statistical term	Clopidogrel				Dipyridamole			
	Stand. Clop	Stand. Clop with khat I	Clop tab (Plavix)	Clop tab (Plavix) with khat I	Stand. Dip	Stand. Dip with khat I	Dip tab (Dipyrid-amole)	Dip tab with khat I
Mean	77.7978	85.7228	75.4925	84.4380	12.0653	21.1845	15.2445	22.1050
SD	1.1109	0.5238	2.7528	0.9054	1.8407	4.1666	3.5350	1.7997
SE	0.5554	0.2619	1.3764	0.4527	0.9203	2.0833	1.7676	0.8998
N	4	4	4	4	4	4	4	4
P	**0.000013 ▲**		**0.00083■**		**0.0071□**		**0.0135✱**	

SD: Standard deviation.; SE Standard Error; ▲, ■, : ANOVA-single way -test (between intestinal perfusion of clopidogrel alone and with the presence of khat I and clopidogrel tablet alone and with the presence of K\khat I, respectively) indicated sig variation ($p < 0.05$); □, ✱: ANOVA-single way -test (between intestinal perfusion of dipyridamole alone and with the presence of khat I and dipyridamole tablet alone and with the presence of K\khat I, repectively)) indicated sig variation ($p < 0.05$).

5. Conclusions

According to the results obtained in the proposed study, intestinal absorption (perfusion) of the oral antiplatelet drugs (aspirin, clopidogrel and dipyridamole) is significantly induced in Khat presence (chewing of Khat). Moreover, due to the difference in the percentage of chemical components of Khat that cultured in different regions, Khat III, cultured in Sana'a region, possessed more effect than other types of Khat. This induction in bioavailability of drugs may be led to increasing in efficacy and toxicity of drugs.

Authors' Contributions

The author who prepared the report studied the idea, developed the theory and performed calculations for the presented work. All authors participated in carrying out the experiments, discussed the results and contributed to the final manuscript.

Declarations

Conflicts of interest: The authors declare that they have no conflicts of interest in the publication of this article.

Authors Funding: No funding.

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