Journal of Clinical Medicine and Research Vol. 4(4), pp. 53-58, April 2012
Available online at http://www.academicjournals.org/JCMR
DOI: 10.5897/JCMR11.076
ISSN 2141-2235 ©2012 Academic Journals

Full Length Research Paper

In vitro and *in vivo* evaluation of acetylsalicylic acid in Khat (Qat) chewing healthy volunteers

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Accepted 20 January, 2012

Aspirin is being extensively used in Yemen as analgesic, antipyretic, anti-inflammatory and anti-platelets aggregation for prophylaxis of thrombotic heart diseases. The objective of this study was to evaluate 2 common brands of aspirin present in the market, weight variation, disintegration and dissolution, hardness and drug content and the effect of Khat chewing on their bioavailability. 28 healthy male volunteers (14 Khat chewing and 14 Khat non-chewing) were enrolled in the study; each received a single dose (600 mg) of aspirin. Urine samples were collected for 24 h. The urine concentrations of salicylic acid were then determined using UV-Visible Spectrophotometer. Results obtained revealed that as weight variation, hardness, friability, dissolution, disintegration and drug content for both brands (a, b) were closely related to each other and within the acceptable pharmacopeial limits. For *in vivo* study, the results obtained showed that higher cumulative percentage excreted after 24 h, higher peak height (mg/h), higher percentage of bioavailability and the higher extent of absorption in Khat non-chewing volunteers than Khat chewing volunteers. While longer time of elimination was observed in Khat chewing volunteers than Khat non-chewing volunteers. In conclusion, we suggest that Khat chewing had a worse effect on the bioavailability and pharmacokinetic properties of the studied drug.

Keywords: *In vitro*, *in vivo*, aspirin, Khat chewing.

INTRODUCTION

Aspirin is one of the most commonly used drugs due to its usefulness as an analgesic, anti-inflammatory, anti-thrombotic and antipyretic agent, and its ready commercial availability (Gordon et al., 1994). It was introduced in the late 1890's and has been to treat a variety of inflammatory conditions (Thun et al., 2002). However, the anti-platelet activity of this agent was not recognized until almost 70 years later (Awtry and Loscalzo, 2000). The ingestion of 325 mg of aspirin every other day reduced the incidence of myocardial infarction by over 40% in male physicians (Jimenz et al., 1992).

It has poor water solubility; hence its dissolution rate is the rate limiting step, thereby affecting its bioavailability (Bamigbola et al., 2009).

The pharmacological activity of acetylsalicylic acid is mainly due to salicylic acid, a metabolite formed after hydrolysis. The increase in the urinary pH produced an increase in the renal clearance of salicylate and thus produces reduced plasma level of the salicylate level (Awtry and Loscalzo, 2000; Clissold, 1986; Patrono et al., 1998).

After oral administration, 80 to 100% absorbed in the stomach and in the small intestine. However, bioavailability is lower because partial hydrolysis occurs during absorption and there is a "first-pass" effect in the liver **(**Borga et al., 1976).

Elimination half life: 4.7 to 9 h (average 6 h) and the half-life dose-related. Acetylsalicylic acid when administered to normal volunteers is reported to have a half-life of only 13 to 20 min after which it is immediately hydrolyzed to Salicylic acid (Done, 1960).

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Aspirin has various side effects on the gastro-intestinal tract, and primarily causes gastric lesions, ulcerations and erosions (Sung et al., 2000).

No report on effect of khat chewing on the availability of aspirin tablets since most of the Yemeni peoples are khat chewer, where supporters of Khat chewing claim that it is useful in diabetic patients because it lowers blood glucose, it acts as a remedy for asthma, it eases symptoms of intestinal tract disorders (Al-Meshal et al., 1985) and maintains social contact as a socializing herb (Kalix, 1984).

The objective of this study to evaluate 2 common brands of aspirin present in the market such as weight variation, disintegration and dissolution, hardness and drug content and the effect of Khat chewing on their bioavailability.

MATERIALS AND EQUIPMENTS

Aspirin®, 300 mg acetylsalicylic acid, uncoated tablet, brand A (Shaphaco -Yemen, Batch No 1140), brand B (Bayer-Germany, Batch No 00110928), aspirin standard (Porte-Pharma, Germany), hydrochloric acid, ethanol and sodium hydroxide (BDH ,England). Ferric chloride (Himedia Lab, India), sodium acetate trihydrate and phenol red (Brixworth-N.UK).

UV-Visible Spectrophotometer (UV-1601(PC) S220V, CAT NO. 20-67501-93, Shimadzu Corporation, assembled in Australia), dissolution tester, disintegration tester, hardness tester, friability tester (Pharma test: Germany) and electronic balance(Sartorius: USA).

Methodology

Weight variation, friability, hardness and disintegration were carried according to USPXXX methodology. Dissolution test was carried for the two brands using USPXXX (2007) methodology in which 500ml of 0.05M acetate buffer maintain at 50 rpm at 37± 0.5°C at 265 ± 2 nm. Drug contents was also carried out according to USP, in which a number of aspirin tablets was grinded and accurately weighed amount equivalent to 0.5 g of aspirin placed in flask with 50 ml of 0.5 N NaOH, boiled gently for 10 min. Phenol red was used as indicator and the excess of NaOH was titrated by 0.5 N hydrochloric acid till the solution became colourless. Blank determination was performed, using 0.5 N NaOH and 0.5 N hydrochloric acid. The amount of 0.5 N NaOH which actually reacted with aspirin was determined.

In vivo study in biological fluids

28 healthy male volunteers with no history of GI, liver or kidney diseases were shared in this study. Each volunteer was instructed to abstain from all medications or foods that might interfere with the drug such as (ethanol, caffeine, chocolate, and tea) for at least 24 h before getting aspirin dose. They also asked to abstain from taking any prescription drugs for 2 weeks before the study or any other drug including vitamins, for at least 1 week before until the end of study. Following an overnight fast, each volunteer was instructed to void his bladder and ingest 250 ml of water. In 1 h before and the 0 h. Urine samples were taken as control and 600 mg of aspirin was ingested with 250 ml of water. No foods or liquids other than water permitted for 4 h following ingestion of dose. Cumulative urine samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h. The volume of collected urine samples measured at each collection time and samples were refrigerated immediately. Each volunteer was instructed to drink 250 ml of water after each urine collection for the first 3 h and simple uniform meal was served after the 4 h sample (Emmanuel et al., 2009). 2 ml of urine sample was taken in a test tube and added 10 ml of analytical reagent (ferric chloride 1 g/100 ml distilled water). Spectrophotometer was maintained at 540 nm. And the pharmacokinetic parameters determined in this study were overall elimination rate constant, the biological half-life of elimination, the absorption rate constant and the biological half-life of absorption. The overall elimination rate constant was determined by the amount of drug remaining to be excreted method. The last few (terminal) points of cumulative amount excreted were subtracted from the total amount excreted and plotted versus time. The slope of the curve gave the overall elimination rate constant (K_e/2.303).

RESULT AND DISCUSSIONS

The data obtained for *in vitro* studies of the two brands (A) and (B), (weight variation, hardness friability and disintegration) within the acceptable limits. Dissolution test passed USP value which is 80% at 30 min and drug contents confirm the USP limit which is 90 to 110% as illustrated in Table1.

As shown in Table 2, the cumulative mg of salicylate excreted after 24 h, the urinary peak height (mg/h), the time to reach that peak (h) and the percent bioavailability were used as the bioavailability parameters to evaluate and compare Khat chewing and none chewing volunteers. It is well known that the cumulative urinary excretion data describe the extent of bioavailability of the drug (Ritshel, 1976).

As shown in Table 3 and Figure 1; the cumulative salicylate excreted after 24 h for Khat none chewing volunteers ranged from 433.94 to 521.39 mg with an average value of 477.67 mg, the results are in good accordance with that mentioned (Gadalla et al., 1989), but for all the Khat chewing volunteers ranged from 372.40 to 416.18 mg with an average value of

Table 1. Physicochemical parameters of the two brands of aspirin (A) and (B).

Parameters	Brand A	Brand B
Weight variation(mg)	360.70 ± 0.15	358.1 ± 0.26
Hardness (kg)	4.21 ± 0.07	7.12 ± 0.05
Friability %	0.94 ± 0.01	0.31 ± 0.01
Disintegration (min.)	1.10 ± 0.12	2.42 ± 0.19
Dissolution % (30 min.)	93.91 ± 0.31	88.92 ± 0.24
Drug Content %	102.43 ± 0.21	97.53 ± 0.34

Table 2. Average bioavailability and pharmacokinetic parameters obtained from the Khat none chewing and Khat chewing volunteers.

Parameters	Khat non chewing	Khat chewing	P value
Cumulative% excreted after 24 h	91.122	75.212	0.000
Peak height (mg/h)	67.834	55.381	0.013
Time of peaking (h)	2.501	2.433	0.874
Percent Bioavailability	91.082	75.211	0.000
Absorption rate constant(1/h)	0.764 ± 0.03	0.736 ± 0.05	0.934
Half-life of absorption (h)	0.915 ± 0.04	0.960 ± 0.08	0.753
Elimination rate constant (1/h)	0.209 ± 0.03	0.159 ± 0.02	0.023
Half-life of elimination (h)	3.302 ± 0.22	4.361 ± 0.30	0.016

Table 3. The cumulative and average Cumulative mg salicylate excreted after 24hr following oral administration of aspirin and the average urinary excretion rates of aspirin in Khat non-chewing and chewing volunteers.

Time	Average cumulative of salicylate (mg) excreted after 24 h Mean ± SE		The average urinary excretion rates of aspirin Mean ± SE	
H	Khat none chewing volunteers	Khat chewing volunteers	Khat none chewing volunteers	Khat chewing volunteers
1.0	8.96 ± 0.82	8.46 ± 1.08	17.926 ± 0.65	16.92 ± 3.21
1.5	26.33 ± 0.69	23.26 ± 0.93	34.74 ± 0.23	29.53 ± 1.05
2.0	51.36 ± 0.64	42.39 ± 0.18	54.05 ± 0.72	38.27 ± 0.91
3.0	94.36 ± 1.39	64.61 ± 0.23	65.07 ± 0.88	50.42 ± 0.98
4.0	137.56 ± 0.68	110.04 ± 0.77	53.56 ± 0.58	42.48 ± 0.61
6.0	190.62 ± 0.23	151.78 ± 0.46	53.06 ± 0.90	41.75 ± 2.82
8.0	275.09 ± 0.52	212.09 ± 1.36	42.24 ± 1.00	30.16 ± 0.56
10.0	347.35 ± 1.37	262.22 ± 0.90	36.13 ± 1.76	25.06 ± 0.98
12.0	402.72 ± 0.73	309.74 ± 0.33	27.92 ± 0.29	23.79 ± 0.91
24.0	447.70 ± 0.94	344.69 ± 1.08	22.48 ± 0.88	17.45 ± 2.02
1.0	477.67 ± 1.26	394.29 ± 1.10	2.425 ± 0.42	4.13 ± 1.26

394.29 mg. The Khat none chewing and Khat chewing's volunteers gave a different cumulative amounts excreted, it is of higher value for the Khat none chewing volunteers of 83.38 mg, Khat none chewing volunteers showed the greater extent of bioavailability, while Khat chewing volunteers showed the lower extent of bioavailability. The peak height of the urinary excretion rate curve as well as the time to reach the peak could be used as suitable parameters to describe the rate and extent of aspirin absorption.

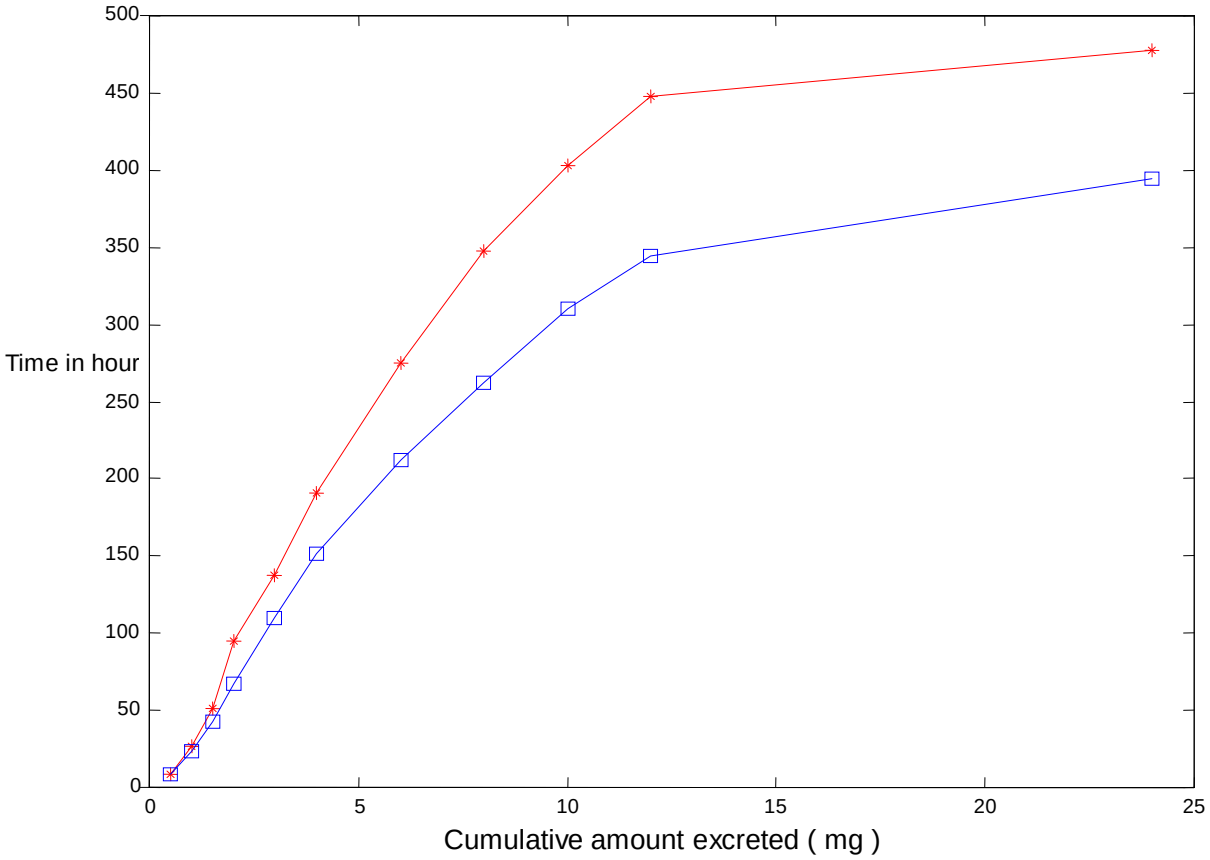

Figure 1. The average cumulative amount of aspirin excreted by Khat none chewing and Khat chewing volunteers.

The results indicated that the urinary peak height for the Khat non chewing volunteers ranged from 61.18 to74.44 mg/h with an average of 67.81 mg/h. For the Khat chewing volunteers ranged from 40.29 to 61.05 mg/h with an average of 50.67 mg/h as shown in Figure 2. So Khat non-chewing volunteers showed the higher peak height while Khat chewing volunteers showed the lower peak height.

As shown in Figure 2, the results indicated that time taken to reach peak urinary concentration for the Khat none chewing volunteers ranged from 1.5 to 4.0 h with an average of 2.5 h while for the Khat chewing volunteers ranged from 2.0 to 4.0 h with an average of 2.43 h. From the average values of the time to reach the peak, Khat none chewing volunteers showed the longer while Khat chewing volunteers showed the shorter time of peaking.

As illustrated in Table 1; the bioavailability of the oral dose aspirin shown that for the Khat non-chewing volunteers ranged from 82.74 to 100% with an average of 91.08%. But for Khat chewing volunteers ranged from 68.29 to 78.59% with an average of 75.21%.

The results indicated that physiological availability decreased significantly, at which Khat none chewing volunteers again showed higher bioavailability while Khat chewing volunteers again showed lower bioavailability.

Generally, on the basis of the calculated bioavailability parameters for commercial aspirin product brand (A) in Khat none chewing and Khat chewing volunteers. The Khat none chewing volunteers showed the best results while Khat chewing volunteers showed the worst results. That means, Khat none chewing had the higher values of the cumulative mg salicylate excreted after 24 h, peak height, percent of bioavailability as compare to the results obtained from Khat chewing volunteers.

The extent of absorption of aspirin in Khat chewing volunteers is lower than that of Khat none chewing volunteers and that may be attributed to the gastrointestinal tract disturbances which mostly described in chronic Khat chewers.

The stringent characteristic of tannins appears to be account for reports of periodontal diseases, stomatitis, esophagitis and gastritis.

Tannin is also believed to delay intestinal absorption (Report of WHO, 1980). Moreover, the ingredients of Khat leaves are numerous, but the major and most abundant ingredients include 6 major alkaloids, tannins (7

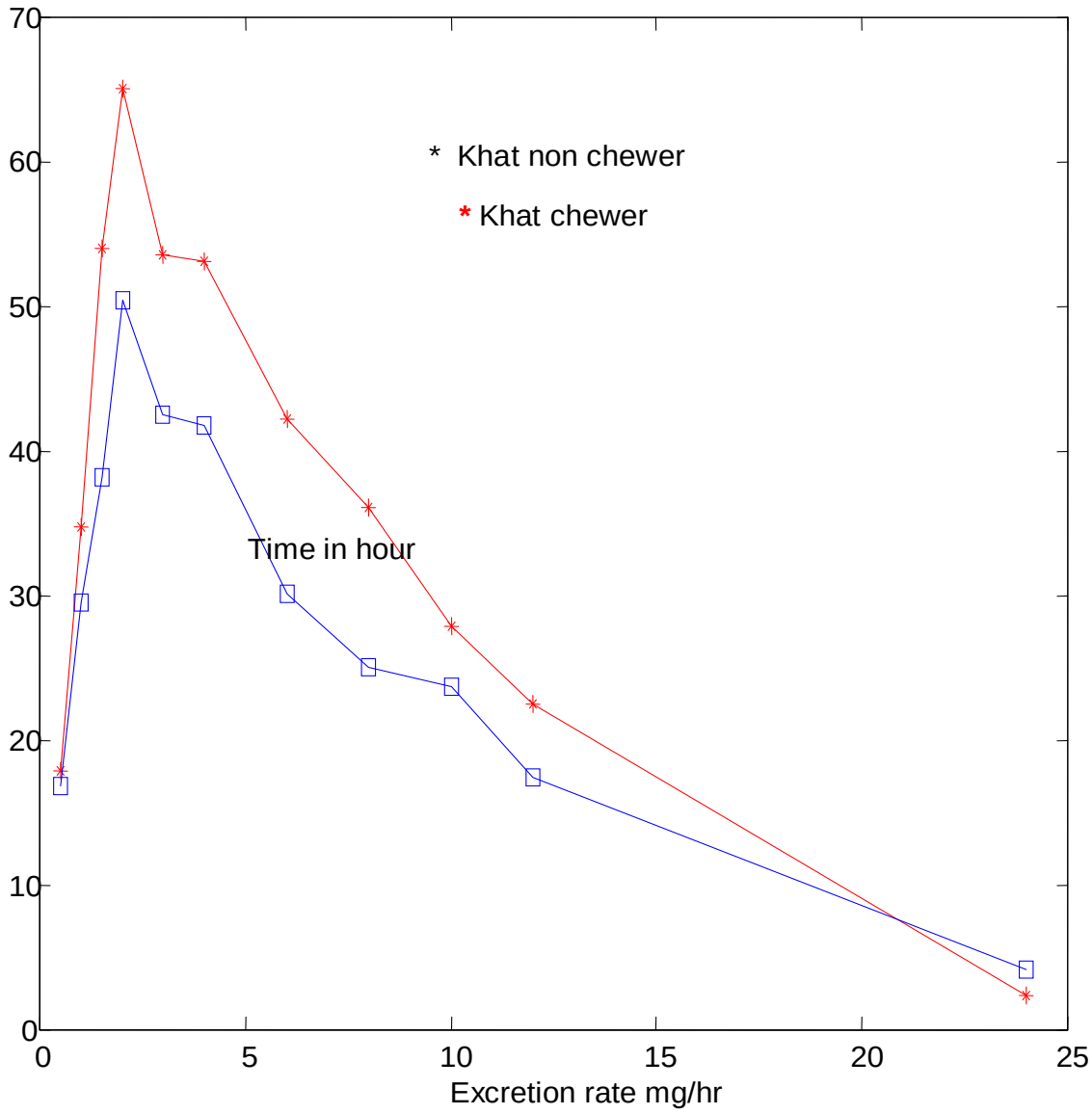

Figure 2. The average excretion rate mg/h of aspirin in Khat none chewing and Khat chewing volunteers.

to 14%) and flavonoids (Luqman and Danowski, 1976). In order to make this in vivo evaluation of aspirin product of more biological significance, some pharmacokinetic parameters were computed and these parameters were computed for a product and both Khat none chewing and Khat chewing volunteers, assuming first order elimination from single compartment (Fitzgerald, 1991; Lopez-Farre et al., 1995).

As shown in Table 1; the values of elimination rate constant Ke, elimination half life $t_{1/2}$ with standard errors in Khat none chewing and Khat chewing volunteers were shown to be (0.2096 ± 0.03), (3.30 ± 0.22) and (0.1590 ± 0.02), (4.36 ± 0.30) respectively.

Also the half life of elimination for a product in Khat none chewing volunteers was found to be ranged from 2.55 to 4.45 h with an average value of 3.30 h, which corresponded to an elimination rate constant of 0.2096 h^{-1}. While in Khat chewing volunteers it was found to be ranged from 5.53 to 3.71 h with an average value of 4.36 h, which corresponded to an elimination rate constant of 0.1590 h^{-1}. The results were in good accordance with that reported (Katzung, 1998).

The results show longer time of elimination in Khat chewing volunteer and this may attributed to that, Khat affects the urinary system by relaxation of bladder wall and closure of internal sphincter and urine retention may

also occur and maximum urine flow rate is reduced (Nasher et al., 1995).

The values of absorption rate constant K_a, absorption half-life $t_{1/2}$ with standard errors in Khat non-chewing and Khat chewing volunteers were shown to be (0.7635 ± 0.03), (0.0.919 ± 0.04) and (0.7356 ± 0.05), (0.96 ± 0.08) respectively.

The half life of absorption for brand (a) in Khat none chewing volunteers was found to be ranged from 0.79 t1.04 h with an average value of 0.915 h, which corresponded to an absorption rate constant of 0.7635(1/h). While in Khat chewing volunteers was found to be ranged from 0.71 to 1.21 h with an average value of 0.96 h, which corresponded to absorption rate constant of 0.7356 (h^{-1}).

In general, results in Table 2; indicated that closely related absorption rate constant in both Khat none chewing and Khat chewing volunteers. Khat none chewing volunteers had the higher extent of absorption, percent bioavailability than that of Khat chewing volunteers.

Comparison of the data obtained for the pharmacokinetic parameters (percent cumulative, percent bioavailability, high peak concentration, elimination rate constant (h^{-1}) and half-life of elimination (h) between Khat chewing and Khat none chewing volunteers revealed that the differences were statistically significant ($P < 0.05$). But the difference in the absorption rate constant (h^{-1}) and half life of absorption (h) was not statistically significant ($P > 0.05$) were shown in Table 2.

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