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EFFECT OF RAMADAN FASTING AND LIFE HABITS ON THE ANTIPYRINE TEST, URINE VOLUME AND pH

N. Aadil [*1], K. Benyassine [1], B. Benaji [2] and Y. Benchekroun [3]

Laboratory of Pharmacology [1], Biotechnology and Microbiology [2], Faculty of Sciences Ain Chock. Hassan II University of Casablanca. Route d'El jadida. BP 5366, Casablanca, Morocco.
ENSET Ecole Normale Supérieure de l'Enseignement Technique [3], Université Mohamed V Rabat, Morocco. Pharmacist Ph.D, Pharmaceutical Industry. Casablanca, Morocco

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Correspondence to Author:
Dr. N. Aadil

Laboratory of Pharmacology, Biotechnology and Microbiology, Faculty of Sciences Ain Chock. Hassan II University of Casablanca. Route d'El jadida. BP 5366, Casablanca, Morocco

E-mail: n.aadil@fsac.ac.ma

ABSTRACT: The purpose of this study was to investigate the effects of the Ramadan fasting and life rhythm eating rhythm on some elimination parameters, such as the antipyrine clearance, urinary volume and pH. A single oral dose of Antipyrine was administred to 14 healthy volunteers at 08:00 p.m. All the volunteers were submitted to four treatment phases; the first one was carried out 1 week prior to Ramadan (PR), the second on the first week of Ramadan (R1), the third on the third week of Ramadan (R3) and the last one 3 weeks after Ramadan (AR). The salivary kinetic of antipyrine was determined for each treatment throughout the 48 hours following drug intake. Eight urine collections were performed after each antipyrine intake, their volumes and pH were immediately determined. Saliva antipyrine concentrations were determined by high-pressure liquid chromatography (HPLC). No significant differences were found in salivary antipyrine elimination half-life t1/2 and clearance between the four periods studied. The 24 hours urine volume was not significantly modified during Ramadan. However, its circadian variations showed a significant increase during the night ($F = 4,046$, $p < 0,05$) and a significant decrease in the afternoon ($F = 3,245$, $p < 0,05$) during Ramadan. The urine pH is significantly influenced by the data collection time and also by Ramadan with ($F = 3, 1789$, $p < 0,05$) and ($F = 18,3133$, $p < 0,0001$), respectively.

INTRODUCTION: Despite the importance of the problem which concerns millions of people throughout the world, physiological and clinical studies on Ramadan fasting remain relatively rare. It is, thus, difficult to suggest a rational therapeutic behavior able to address both fasting and therapeutic goals.

In fact, Ramadan is a month of fasting in which life habits and subjects' synchronization change.

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These changes concern mainly the rest/activity cycle and the fasting habits. Several chronobiological studies have shown that such life habits induce temporal structure alterations of biological rhythms, like those of body temperature [1, 2], cortisol [2, 3], melatonine [2, 4, 5], among other things. Pharmacology wise, physiological alterations as well as drug intake schedule changes, which are exclusively nocturnal, could have an effect on the pharmacokinetics of drugs or even a clinical impact, particularly in the case of narrow-therapeutic range drugs [6].

Certain chronopharmacological studies conducted on Ramadan have shown pharmacokinetic variations of the absorption and elimination of some drugs, namely theophylline [7] and valproic acid [8]. Given that the biotransformation of drugs is

important for their elimination, the present study seeks to investigate the oxidative metabolic pathways used by most drugs during Ramadan.

To do so, an antipyrine clearance test was carried out in healthy volunteers before, during and after the month of Ramadan. It is a sensitive test, which is specific for the oxidative hepatic function investigation, and which is based on the evaluation of the antipyrine clearance.

Antipyrine, it should be noted, has been extensively used as a model compound to study the influence of disease, drugs and environmental factors on hepatic drug-metabolizing capacity. This molecule is rapidly and completely absorbed from the gastrointestinal tract and extensively (90%) metabolized by the cytochrome P-450 liver enzymes. Its elimination is independent of protein binding and its clearance is not limited by liver blood flow. Thus, antipyrine clearance constitutes a sensitive indicator of hepatic microsomal enzyme activity that can provide specific information of hepatic function during and outside Ramadan [9, 10].

This test involves administering a dose of antipyrine to a given individual, be it human or animal, and follow the kinetics of its plasma or salivary elimination in order to establish the main pharmacokinetic parameters, i.e., the apparent elimination half-life (t1/2) and total clearance (Clt). Several studies have shown a close correlation between plasma clearance of antipyrine and its salivary clearance [11, 12], a fact which further facilitates the use of this test in clinical research.

Owing to its low pKa and its small degree of plasma protein binding, antipyrine is distributed in total body water; highly reproducible significant correlations between its saliva and plasma concentrations have been reported in young subjects, since antipyrine plasma and saliva clearances appear not to differ significantly [12, 13, 14].

MATERIALS AND METHODS:

It is a healthy volunteer open assay, including four therapeutic sequences. Each sequence corresponds to a single oral antipyrine dose followed by 48 hours kinetic determination. Urinary samplings have equally been conducted every 4hours subsequent to each antipyrine intake.

Definition of Subjects:

Sixteen healthy volunteer fasters were included in this study; they were all completely informed about the goal and the description of the assay. A written informed consent was obtained after a detailed explanation of the protocol. The volunteers were informed that they could leave the study at any time. The study protocol was approved by the local ethics committee of the faculty of Medicine and Pharmacy in Casablanca. All the volunteers were healthy young males, whose age ranged from 20 to 28 years (average age of 24.8 years old) and whose weight varied between 60 and 87 Kg (mean weight 73 Kg). None of them has any record of cardiovascular diseases, or epilepsy, diabetes, renal and hepatic failures and gastro duodenal ulcer. The subjects were neither obese nor alcoholic; they were not smokers either. They were all included in the study after they passed a clinical test (blood pressure, pulse measure) and a biological test, including blood count, creatinine, transaminases, urea, glycemia and total proteins.

Each of the subjects was informed that they were not allowed to take any medication one week prior to or within the assay apart from the antipyrine destined for the study.

Two subjects (S2 and S16) left the study after the first phase for personal reasons. Our results are, therefore, based on the evaluations conducted in the 14 remaining subjects during the four periods.

In order to highlight any changes that may be caused by the life rhythm of the month of Ramadan, the choice of subjects and experimental conditions must be rigorous. Indeed, subjects who participated in our study were all males to avoid interruption of fasting observed in women during menstruation, and also avoid any drug interaction with oral contraceptives that cause decreased clearance antipyrine [15].

Antipyrine administration:

Each sequence corresponds to a 1000 mg antipyrine capsule intake. The antipyrine is administred at 08:00 pm, two hours after the break

of fasting during Ramadan. Outside Ramadan, the antipyrine is administrated at the same time.

Sequences are as follows:

- Sequence I: one week prior to the month of Ramadan (PR)
- Sequence II: at the end of the first week of Ramadan (R1)
- Sequence III: at the end of the third week of Ramadan (R3)
- Sequence IV: three weeks after the end of Ramadan (AR)

In order to avoid any food influence on antipyrine absorption, we made sure the composition of meals preceding antipyrine intake during and outside Ramadan was standardized and taken at the same time (i.e., 06:00 pm) during the four phases of the test. The adopted food composition was consistent with the meal usually taken for breaking the fast during Ramadan. This meal was taken two hours prior to the administration of antipyrine (06:00 pm).

Salivary and urine collections:

5 to 8 ml salivary collection were needed to carry out the antipyrine kinetic at 0.25, 0.5, 1, 1.5, 2,2.5, 3, 4, 6, 8, 12, 18, 24, 36 and 48 hours after drug intake. The reference collection was taken before the antipyrine intake (t0). The salivary test samples were centrifuged, and two items of each acqueous phase were kept at – 90°C until analysis.

Before performing urine collections in each phase of the study, healthy volunteers all emptied their bladders immediately before taking the test antipyrine dose and this urine collection was eliminated. Eight other urine collections were then made conducted at the following time intervals: 0 to 4 hours (0 corresponding to antipyrine intake time), 4-8h, 8-12h, 12-16h, 16-20h, 20-24h, 24-36h and 36-48 hours.

Each urine collection was carefully shaken, its volume was measured using a 2000 ml graduated cylinders (class A) and its pH was measured using a pH meter. 10ml aliquots were then frozen in duplicate at - 20°C for subsequent determination of antipyrine metabolites. To prevent oxidation of these metabolites, urine aliquots were frozen after addition of sodium pyrosulphite.

Dosage method:

The antipyrine salivary concentrations were determined through high performance liquid chromatography (16). Antipyrine was extracted from 500 µl salivary samples using dichloromethane – pentane (50/50) in an alkaline medium. Phenacetine was the internal standard. The mobile phase was made of phosphate buffer – acetonitrile (100/30, V/V); separation was carried out through a Lichrosorb RP18 column (Merck). A UV detection (Shimadzu SPD – 6A) was used at 240 nm.

The method was validated according to the International Conference on Harmonization guidelines for validation of analytical procedures [17]. **Table 1** shows the analytical validation criteria and results.

TABLE 1: ANALYTICAL VALIDATION RESULTS

Analytical validation criteria	Results
Linearity:	
Range	[5 – 50] mg/l
Linearity equation	Y = 0.069X – 0.057
Correlation coefficient (r)	0.0997
Precision:	
Repeatability (5, 20, 50 mg/l)	0,22 – 0,60 – 0,44 (%)
Reproductibility (5, 20, 50 mg/l)	4,95 – 3,45 – 2,79 (%)
Accuracy:	
Mean recouvrement	99,266 %
Recouvrement range	91,11 – 107,88 %
Limit of detection	1,91 mg/l
Limit of quantification	2,45 mg/l

Pharmacokinetic analysis:

Antipyrine individual salivary concentrations were processed according to a mono-compartment model and by means of a pharmacokinetics modeling and calculation software program (Kinetica®). The pharmacokinetic parameters studied were the apparent elimination half -life t1/2 (h: hours) , the distribution volume Vd (L: Liter) and total elimination clearance Clt (L/ h). Total clearance was calculated according to the following formula CL = ln2 x Vd / t1 / 2. The volume of distribution was estimated based on the dose and concentration extrapolated to time zero (C_0 mg/l).

The parameters of absorption (C_{max} and T_{max}) were not considered in the present study. Some research works have stressed that the determination of pharmacokinetic parameters of antipyrine must be limited to the elimination phase [18]. Indeed, the difference in antipyrine concentration between arterial blood and venous blood is the source of increased salivary concentrations immediately subsequent to its administration.

During the absorption phase, the concentration of antipyrine in the arterial blood irrigating salivary glands are greater than the concentrations in the venous blood collected for assays . This difference disappears once the distribution is complete [18, 19].

Statistical analysis:

Inter-period comparison of urine volume in 24 hours (diuresis), urine volume issued in the morning (08:00 am – 12:00 am) , afternoon (12:00 am – 16:00 pm) and night (16:00 pm – 08:00 am) as well as antipyrine pharmacokinetics parameters were conducted by means of variance analysis of a controlled factor (Ramadan factor).

A two-factor controlled ANOVA was used to study the effects of Ramadan and temporal variations in urinary pH. These statistical tests were carried out with a first type risk α equal to 5%.

RESULTS:

Time-based evolution of mean salivary antipyrine concentrations, corresponding to the four periods studied is shown in **Fig. 1**.

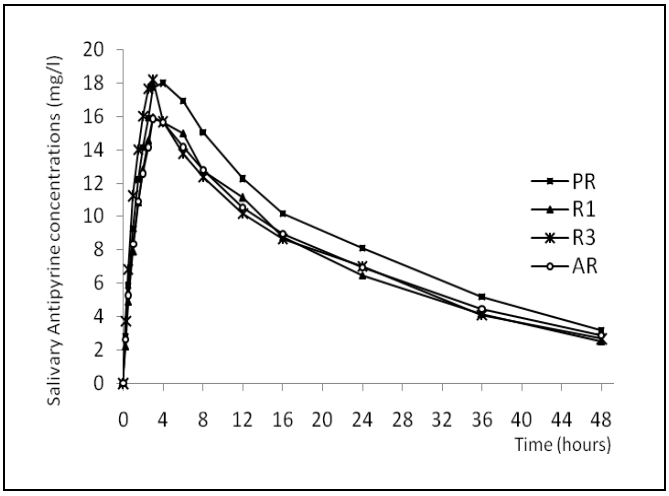


FIG. 1: MEAN SALIVARY CONCENTRATIONS OF ANTIPYRINE (mg/l) BEFORE, DURING AND AFTER RAMADAN.

Mean values (± SD) of pharmacokinetic parameters calculated from the salivary concentrations are reported in **Tables 2**.

TABLE 2: MEAN VALUES (± SD) FOR ANTIPYRINE KINETIC PARAMETERS BEFORE, DURING AND AFTER RAMADAN.

Periods	Vd (L) ± SD	Clt (ml/min) ± SD	t1/2 (h) ± SD
PR	56,31 ± 18,90	35,10 ± 10,67	18,72 ± 4,24
R1	61,46 ± 15,88	42,35 ± 12,27	17,39 ± 4,84
R3	62,07 ± 13,74	39,59 ± 7,78	18,24 ± 3,02
AR	63,55 ± 11,87	38,23 ± 6,95	19,46 ± 3,65

Controlled –factor variance analysis does not show a significant effect of Ramadan so much on the apparent elimination half-life t1/2 ((F = 0,656, p = 0,583) as on the total clearance Clt (F = 1,3656, p = 0,264) of antipyrine .

The mean values (± SD) of the 24 hours urine volume corresponding to the four phases of the study are shown in **Fig. 2**. Controlled-factor variance of the analysis does not show a significant Ramadan effect on this parameter (F = 1,934, p = 0,136).

To examine the temporal variations in urine volume over the 24 hours, we have divided it into three phases of the day: morning (08:00 am to 12:00 am), afternoon (from 12:00 am to 04:00 pm) night (04:00pm to 08:00 am). The mean values of those volumes (±SD) corresponding to the fours periods studied are shown in **Fig.2**. Controlled-factor variance analysis showed a significant decrease in the urine output in the afternoon (F = 3,245, p < 0,05) and a significant increase in urine output at night (F = 4,046 , p < 0,05) during Ramadan. The variation in urine volume in the morning was still not significant (F = 1,359, p = 0,136).

Fig. 3 shows the mean values of urinary pH (± SD) measured during the four periods studied. ANOVA II showed that Ramadan and temporal variations during the 24 hours have a significant effect on urine pH with (F = 3,179, p < 0,05) and (F = 18,313, p < 0,0001), respectively .

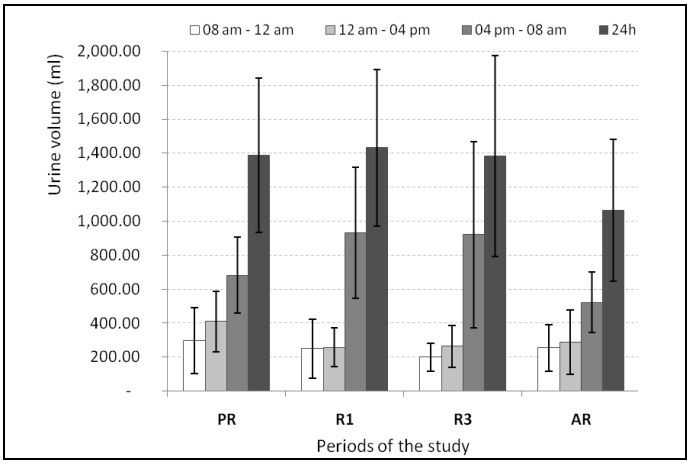
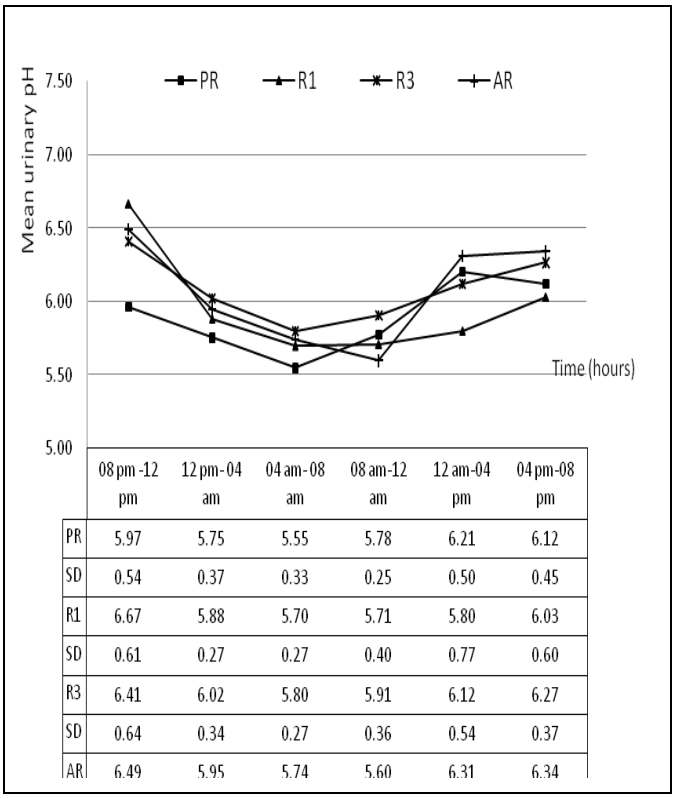


FIG.2: MEAN VALUES (± SD) OF URINE VOLUME EXCRETED BEFORE, DURING AND AFTER RAMADAN. MORNING (08:00 am -12:00 am) COLLECTION, AFTERNOON (12:00 am – 04:00 pm) COLLECTION, OVERNIGHT (04:00 pm – 08:00 am) COLLECTION AND TOTAL 24 HOURS VOLUME.



	08 pm -12 pm	12 pm-04 am	04 am-08 am	08 am-12 am	12 am-04 pm	04 pm-08 pm
PR	5.97	5.75	5.55	5.78	6.21	6.12
SD	0.54	0.37	0.33	0.25	0.50	0.45
R1	6.67	5.88	5.70	5.71	5.80	6.03
SD	0.61	0.27	0.27	0.40	0.77	0.60
R3	6.41	6.02	5.80	5.91	6.12	6.27
SD	0.64	0.34	0.27	0.36	0.54	0.37
AR	6.49	5.95	5.74	5.60	6.31	6.34

FIG.3: CIRCADIAN VARIATIONS OF URINARY pH MEAN VALUES (+SD) BEFORE, DURING AND OUTSIDE RAMADAN.

The interaction between experiment or test period and temporal variations of the pH was also significant ($F = 1,377$, $p < 0,05$). Indeed, the circadian variations in urine pH showed a nocturnal decrease (from 08:00 pm to 08:00 am) during and outside Ramadan with higher values during the fasting period.

Day time variations (08:00 am to 08:00 pm) showed a gradual increase in urine pH during the two periods studied (during and outside Ramadan). It is important to note the existence of a peak pH in the range 12:00 am - 04:00 pm during the normal period (before and after the month of Ramadan).

DISCUSSION: Measuring of antipyrine clearance (plasma or saliva) makes it possible to explain interindividual and intra-individual variations drug response variations, which may be genetic [20], secondary to a pathological situation [21], a drug interaction [22] or related to exogenous factors, such as diet [23, 24], tobacco [25], physical activity [26] and exposure to chemical substances [27].

Life rhythm during Ramadan may be among these exogenous factors that could impact oxidative drug metabolism. Indeed, pharmacokinetic studies conducted during this month of fasting, with regard to certain narrow- therapeutic drugs, showed variations in their elimination. A study on a sustained-release theophylline [7] showed a significant prolongation of its elimination half-life after administration to 04 am during Ramadan. Another study on valproic acid [8] reported a significant decrease in its half-life after administration to 05 am during Ramadan. It is, therefore, interesting to explore the metabolic pathways during Ramadan to test the hypothesis of their possible involvement in these pharmacokinetic variations.

The results of the present study showed no significant change in the total clearance and apparent elimination half-life (t1/2) of antipyrine during the Ramadan month. We can thus consider that the reversal of the feeding rhythm and alteration of rest / activity cycle characterizing this month of fasting, do not impact the hepatic drug-metabolizing capacity.

It should be emphasized, however, that antipyrine is primarily metabolized by cytochrome P450 and that 95 % of an orally administered dose is excreted as 4- hydroxyantipyrine, norantypirine, 3-hydroxy methylantipyrine and 3 - carboxy - antipyrine. According to Engel.G et al 1996, these metabolites are formed by the intervention of six isozymes (CYP1A2, CYP2B6, CYP2CB, CYP2C9,

CYP2C18 and CYP3A4) [28]. In fact, antipyrine total clearance is the result of the activity of these different oxidases. The determination of antipyrine metabolites' urinary profiles and their partial clearances in a second phase of this study will enable us to follow the circadian variations in the activity of these enzymes and possibly supplement the results we obtained.

It is, moreover, is important to stress that establishing a correlation between these results and the elimination of other drugs via oxidative metabolic pathways remains very delicate. Indeed, antipyrine has traditionally been considered as a model drug for evaluating drug metabolizing capacity, but the hepatic oxidative metabolism involves numerous P450 subfamilies, all of which do not necessarily contribute to the metabolism of antipyrine [29].

The results obtained based on urinary collections show that the alteration of the rest/activity cycle and the change in the feeding rhythm and habits during the month of Ramadan do not significantly impact the 24-hour urine volume (diuresis). However, when this volume is spread over the three previously mentioned phases (morning, afternoon and evening), the urine output during Ramadan shows a significant increase at night, a significant decrease in the afternoon while it remains unchanged in the morning. The lack of significant change in 24 hours urine output during Ramadan could be explained by compensation between the nocturnal increase and the diurnal decrease in urine volume. Our results were consistent with those reported by a study conducted in 16 Sudanese Muslims, where the daytime urine volume and total daytime urinary sodium excretion were decreased during Ramadan fasting [30].

In 20 Malaysian Muslims, where the urine was collected before, during and after Ramadan fasting each in the morning (08.00 am-12.00 am), afternoon (12.00 am-04.00 pm) and overnight (04.00 pm - 08.00 am).The authors found that Ramadan fasting did not affect the overnight urine volume (values not given in the paper) or osmolality (means: 649–781 mosm/kg). Over the morning and afternoon collection periods, however, urine volume, sodium, potassium and total solute excretion were lower, and urinary osmolality was higher during Ramadan than either before or after Ramadan. During Ramadan, the osmolality of the urine samples collected in the afternoon were very high, indicating effective water conservation both by maximum urinary concentration and a decreased obligatory urine output [31]. Another study conducted before and during Ramadan in fifty-seven men aged 30 to 55 years old, including 37 recurrent calcium calculus formers and 20 with no history of kidney calculi showed that the total excretion of calcium, phosphate, and magnesium in 24-hour urine and also urine volume during fasting were significantly lower than those in the non fasting period [32].

The significant decrease of urinary volume that we observed in the afternoon during Ramadan, can be explained by the adaptation of the body water balance to the intermittent dehydration during this fasting month. In most situations where water turnover rate is altered, the total body water content is usually conserved [33]. In a recent study using an isotopic tracer technique in Malaysian Muslims [34], it was demonstrated that total body water content was conserved during Ramadan although daily water turnover was reduced. The decrease in water turnover in this study appeared to be due to a reduction in recorded fluid intake, but hydration was maintained by a drop in non renal losses [35].

The circadian variations in urine pH observed in our study during and outside Ramadan are consistent with those reported in the literature. We generally notice a decrease during the rest phase and an increase in the activity phase [36]. The circadian rhythm of pH is thus preserved during Ramadan but with an increase in nocturnal values compared to the normal period. This could be explained by diet which is exclusively nocturnal and activity phase that lasts throughout the month of fasting. Our results also show a peak in the pH of 12.00 am - 04.00 pm interval during the two phases, before and after Ramadan. Such results could also be explained by the dietary factor, because the urinary collection corresponding to this time interval is performed four hours after lunch.

According to Cameron et al, 2012, changes in urine pH during certain hours of the day appear to be

temporally related to food intake. These authors have approximately noticed an increase in urine pH within 4 hours of each meal [37]. These postprandial peaks reported by Cameron et al were not detected by previous studies [38, 39]. The author explains the peaks by the fact that urine collections in his study were more frequent and pH measurement was performed using a pH meter and not a pH indicator paper. Cameron et al's results, thus, seem to support the hypothesis of the involvement of dietary factor in the regulation of circadian variations in urine pH which remains essentially controlled by the internal biological clock.

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