Effect of fasting on serum lithium levels: An experimental Study in animal models

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Abstract: Muslims throughout the world observe dawn to dusk fast in the month of Holy Ramadan. This study aims to investigate the effect of fasting on serum lithium levels in an animal model under typical conditions of Ramadan. Animals were categorized into oral and intraperitoneal groups. Each group was divided into fasting and non fasting groups along with their controls having six animals each. Mean serum lithium levels of non-fasting and fasting rats were assessed. Mean serum lithium levels of oral non-fasting rats was 0.23±0.004 mequiv/L, (n=6) compared to oral fasting rats 0.20±0.002 mequiv/L, (n=6) mean difference=0.003. The mean difference between mean serum lithium level of intraperitoneal non fasting (0.246±0.015 mequiv/L, n = 6) and intraperitoneal fasting rats (0.206±0.020 mequiv/L, n = 6) was 0.02. These differences were statistically non significant (P>0.05). The mean serum lithium is not grossly affected by fasting in rats under 25ºC and fasting for almost 12 hours which is consistent with a previous clinical study. Lithium can be used by fasting bipolar patients but, will require careful supervision.

Keywords: Ramadan, fasting, lithium, rats, atomic absorption.

INTRODUCTION

It has been over 60 years that lithium (Li^+) is an agent of preference in the treatment and prophylaxis of mood disorders. Indeed, it remained a standard, against which new mood stabilizers have been measured; in spite of its narrow therapeutic window and the lack of precision about its mechanism of action (Chiu *et al.*, 2007; Ellenhorn and Barceloux, 1988; Han *et al.*, 2004; Price and Heninger, 1994; Yatham *et al.*, 2005). In Bipolar Affective Disorder, plasma lithium concentration range is 0.6–1.2 mmol/L. Close monitoring of therapy is essential because there are possible risks of relapses with concentrations lower than 0.6 mmol/L, while toxic effects begin with concentrations over 1.5 mmol/L (Miao, 2002).

The Ramadan is the ninth month of the Islamic lunar calendar, in which all adult Muslims throughout the world practice fasting. They refrain from taking any food item, beverages and oral drugs between dawn and sunset. The limited choice of hours to eat freely brings changes in the therapeutic regimen and dosing time table of the fasting patients which may interrupt the course of illness of bipolar patients (Aadil *et al.*, 2004; Kadri *et al.*, 2000; Khaled *et al.*, 2006). Fasting in a condition of hot climate for long hours can cause decrease glomerular filtration and dehydration, which might increase serum lithium level (Farooq *et al.*, 2010). It is therefore imperative to study the serum lithium level in diverse conditions such as fasting especially during Ramadan.

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However, there is limited literature on the relationship between fasting and serum lithium levels. This is surprising in view of the fact that lithium is potentially toxic drug and the effect of fasting during Ramadan have been studied comprehensively in other conditions, like in relation to electrolyte imbalance, diabetes mellitus, daytime alertness, sleep wake cycle and other physiological parameters (Mustafa *et al.*, 1978; Pinar, 2002; Young and Macritchie, 2004). We previously investigated the effect of fasting on serum lithium levels and toxicity associated with fasting and relapses in patients suffering from Bipolar Affective Disorder, who were stable on prophylactic lithium throughout the month of Ramadan (Farooq *et al.*, 2010). In this study, we aimed to examine the effect of fasting on the serum lithium levels in rats which were bred and fed in conditions mimicking Ramadan.

METHOD

In house Sprague dawley rats of both sexes, weighing between 150 to 200gm were housed under controlled conditions of light, temperature and humidity in well furnished animal house of Department of Pharmacy, University of Peshawar, Peshawar, Pakistan. There was 12 hr Light cycle; 12 hours light/12 hours dark with light on at 8 am and off at 8 pm. The temperature was maintained at 77ºF ± 2º (25ºC) and 40% - 60% humidity was assured in the animal house. Rats were fed ad libitum with well balanced freshly prepared food. Experiments were carried out in accordance with the accepted guidelines of Animal (Scientific Procedures) Act UK 1986. Rats chosen for the

experiments were categorized into three groups; oral, intraperitoneal and normal. Each group was further classified into the following groups.

(A) Oral: This group, which received the dose orally (lithium/saline) and was further categorized, in the following groups.
IA) Fasting on lithium (n=6): These rats were administered lithium and kept at fast.
IIA) Fasting Saline (n=6): These rats were administered saline and kept at fast. This group served as control for group IA.
IIIA) Non Fasting lithium (n=6): These rats were on routine diet and administered lithium.
IVA) Non Fasting Saline (n=6): These rats were on routine diet and administered saline. This group served as control for group IIIA.
(B) Intraperitoneal: In this group, route of administration was Intraperitoneal ((lithium/saline) and was further categorized as:
IB) Fasting lithium (n=6): These rats were administered lithium and kept at fast.
IIB) Fasting Saline (n=6): These rats were administered saline and kept at fast. This group served as control for group IB.
IIIB) Non Fasting lithium (n=6): These rats were on routine diet and administered lithium.
IVB) Non Fasting Saline (n=6): These rats were on routine diet and administered saline. This group served as control for group IIIB.
C) Normal Groups: This group contained normal rats of the same breed and were kept under observation without any intervention. The group was included in order to observe the serum lithium levels and effect of fasting in normal rats which received neither lithium nor saline. These rats were grouped as:
IC) Fasting (n=6): These rats were on routine diet and kept at fast without any therapy.
IIC) Non Fasting (n=**6):** These rats were on routine diet without any therapy.

Fasting groups were kept at fast for at least 12 hours during the same period which correspond to the month of Ramadan (1427 Hegirian calendar, corresponding to September, 2006). Supply of food and water was suspended at dawn (seher) till sunset (Iftar) just like the rules of religious fasting. All rats were marked on tail for proper identification and properly handled well before the start of experiment.

Because of the low therapeutic index, lithium has different dose ranges when used for prophylaxis and treatment of bipolar disorder. Concentrations between 0.6 to 1.25 mEq per liter is considered effective and acceptably safe. The range of 0.9 to 1.1 mEq per liter is favored for treatment of acutely manic or hypomanic patients and 0.6 to 0.75 mEq per liter are considered adequate for prophylaxis of recurrent manic-depressive illness (William & Leo, 2006). As some patients may not relapse at concentrations as low as 0.5 to 0.6 mEq per liter and lower levels usually are better tolerated so 0.5 mEq Li/Kg body weight dose of lithium was used in the form of Li2Co3 (William & Leo, 2006). Controls received the volume of saline according to the body weight.

Animals were sacrificed by concussion/cervical dislocation and blood samples were collected 12 hours after the administration of last dose through cardiac puncture. All the procedures were carried out in accordance with the formal ethical rules of ethical committee of Department of Pharmacy, University of Peshawar, Peshawar, Pakistan.

ANALYSIS

Blood samples were collected in plastic tubes and allowed to clot at room temperature without anticoagulant. After clotting, samples were centrifuged for 10 minutes at 4000 RPM to separate serum. Test sera were diluted to 10 times its volume with deionized water. All the solutions were prepared in de-ionized water with specific resistance of at least 106 ohms at 25°C. Analytical grade lithium carbonate (Li_2CO_3) was used as reference standard. Sodium and potassium were added to all blanks and standard solutions in the form of analytical grade sodium chloride and potassium chloride to match the physiological matrix of the serum. All the sera were analyzed on atomic absorption spectrophotometer according to the prescribed method (Pybus and Bowers, 1970; Rocks *et al.*, 1982). Following solutions were made to analyze the sera.

Stock blank 140 mEq NaCl/L and 5 mEq KC1/L was prepared.
Working blank 14 mEq NaCl/L and 0.5 mEq KC1/L was made from stock blank.
Stock standard 1.00 mEq Li/L with Li_2CO_3 was prepared
Working standard 0.10 or 0.20 mEq Li/L in 14 mEq NaCl/L and 0.5 mEq /L were made.

RESULTS

Mean serum lithium level of non-fasting groups on lithium therapy were compared with fasting groups on lithium therapy along with respective control groups (table 1).

The mean value for oral non-fasting group on lithium was (0.23 ± 0.004, n=6) and that of oral fasting group on lithium was (0.20 ± 0.002, n=6). The difference between their mean values was 0.003, ($P<0.5213$) fig. 1.

Similarly, the mean value for intraperitoneal non fasting group on lithium was (0.246 ± 0.015, n=6) and that of intraperitoneal fasting group on lithium was ($0.206\pm$

0.020, n=6). The difference between their mean values was 0.02, (P<0.1430) fig. 2.

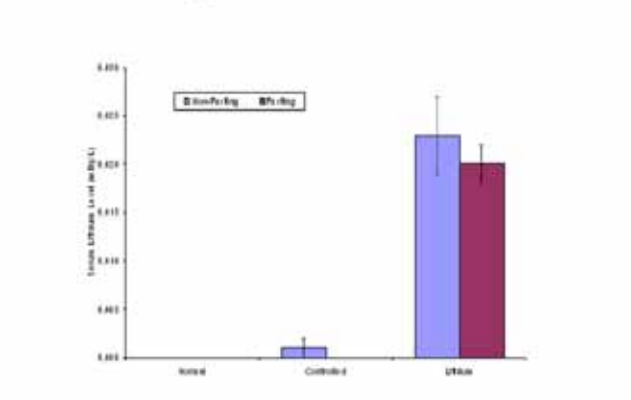

Fig. 1: Effect of Fasting on Serum lithium levels in rats during fasting and non fasting conditions after oral administration. Mean value for oral non-fasting group on lithium was (0.23+0.004, n=6) and that of oral fasting group on lithium was (0.20+0.002, n=6). The difference between their mean values was 0.003, (P <0.5213).

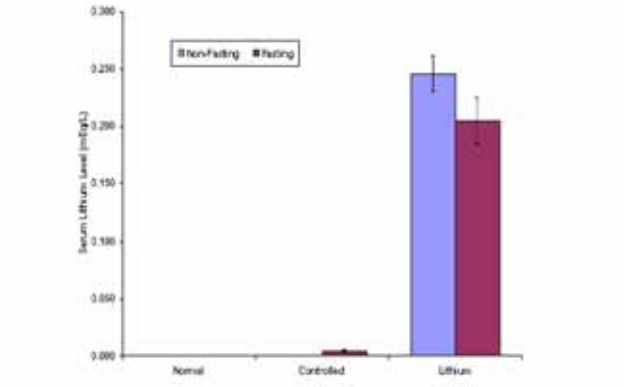

Fig. 2: Effect of Fasting on Serum lithium levels in rats during fasting and non Fasting conditions after intraperitoneal administration. Mean value for intraperitoneal non fasting group on lithium was (0.246± 0.015, n=6) and that of intraperitoneal fasting group on lithium was (0.206±0.020, n=6). The difference between their mean values was 0.02, (P<0.1430).

DISCUSSION

To the best of our knowledge, this is the first study to evaluate the effects of fasting on the lithium levels in experimental animals. As we could not find similar studies on this subject in animal models, we are not able to compare our results with other studies and will focus on our previous clinical studies on this subject. These results are consistent with earlier clinical research (Farooq *et al.*, 2010). In a clinical study we recruited 62 patients suffering from Bipolar affective Disorder and were stable on prophylactic lithium for at least three months Farooq *et al.*, 2010). Blood samples of the patients were assessed for lithium one week before the month of Ramadan (Pre Ramadan assessment), during the month at fasting (Mid Ramadan assessment) and a week after the month (Post Ramadan assessment). Mean serum lithium level of Mid Ramadan assessment was compared with Pre and Post Ramadan assessments. We also measured serum electrolytes and serum creatinine levels, as impairment of renal functions and electrolyte disturbances associated with fasting can affect lithium levels**.** The average serum lithium level increased in Mid Ramadan assessment (0.510) compared with pre Ramadan (0.490) and post Ramadan (0.441) assessments, but the mean difference between these assessments was not statistically significant. The results of these measurements are given in table 2. The side effects of lithium at three points were also not significantly different (Nazar *et al.*, 2009). However, these results could be attributed to relatively low dose of lithium at three points as the mean lithium dose was; Pre Ramadan, 784mg (SD=146), Mid Ramadan, 768mg (SD=145), and Post Ramadan, 792mg (SD=142) (P =0.662).

In another clinical study Kadri *et al.* (2000) reported relapses in 45% patients suffering from Bipolar Affective Disorder. However, these high relapse rates were not related to serum lithium concentrations. Daiasley *et al.* (1990) reported a case report of lethal lithium toxicity during fasting in which a patient presented with lithium toxicity when he continued with 5th day of a religious fast, only taking a glass of milk and a tea cup of daily porridge daily while taking lithium Carbonate 600 mg in morning and 900 mg in the evening.

Fasting during the month of Ramadan represents one of the five pillars of Islam and considerable reduction occurs in frequency and the number of meals during this month (Afrasiabi *et al.*, 2003) which may affect the serum lithium levels of patients using lithium. Majority of the Muslims, living in hot tropical climate observe the fasting, despite the fact that patients are exempted from fasting according to religious injunctions. The patients suffering from bipolar affective disorders need education and support for following this religious obligation, if they wish to fast.

The use of the rats in our study was to evaluate the validity of our clinical results. Since, patients take different medicines as well as various sorts of foods and Ramadan is especially associated with significant changes in dietary habits as food consumed during Suhur and Iftar is quite different from that used in non Ramadan days which may affect the serum lithium levels, therefore, the inbred animals were used in addition to the fasting subjects who take foods of known composition and their therapeutic schemes were well known. Besides, the accuracy of results in clinical samples may be affected by other factors such as co existing physical conditions like hypertension or kidney diseases etc.

Table 1: Mean serum lithium levels of both oral and Intraperitoneal non-fasting groups on lithium therapy compared with fasting groups on lithium therapy along with respective controls

Group	Oral		Intraperitoneal		Normal n=6
	Control n=6	Lithium n=6	Control n=6	Lithium n=6	
Fasting	0.00+0.00	0.20+0.002	0.004+0.00	0.20+0.020	0.00+0.00
Non-Fasting	0.001+001	0.23+0.004	0.005+0.001	0.24+0.015	0.00+0.00

Table 2: Mean serum Lithium, electrolyte and Creatinine levels of Bipoar patients at three assessments during Ramadan (Clinical study)

	Pre Ramadan (mEq/L)	Mid Ramadan (mEq/L)	Post Ramadan (mEq/L)
Serum Lithium (Li^+)	0.4862±0.031 (n=53)	0.5208±0.028 (n=53)	0.4474±0.032 (n=53)
Serum Sodium (Na^+)	142.153±7.0045 (n=53)	143.397±1.6647 (n=53)	141.229±1.5640 (n=53)
Serum Potassium (K^+)	4.037±0.2610 (n=53)	4.308±0.4246 (n=53)	4.283±0.6390 (n=53)
Serum Chloride (Cl^-)	105.256±1.9804 (n=53)	104.652±12.6844 (n=53)	105.875±3.0365 (n=53)
Serum Creatinine	0.86±0.0227 (n=53)		

Clinical guidelines recommend lithium as a first-line treatment (Canadian Network for Mood and Anxiety Treatments 2006; National Institute for Health and Clinical Excellence, 2006) and it is still the cheapest available mood stabilizer However, the use of lithium for the treatment of bipolar disorder is declining (Young and Hammond, 2007). Practical problems such as use of lithium during fasting may be responsible for this as there is little evidence based guidance on this subject. Our study provides preliminary evidence that the fasting for up to 12 hours in average temperature of 25ºC may be safe when lithium is used in prophylactic doses.

As Muslims follow the lunar calendar for religious occasions, the month of Ramadan varies from year to year. In large Muslim countries such as Egypt and Saudi Arabia, the month of Ramdan in peak summer months such as June and July, the fasting day can last up to 17 hours in temperatures which may peak up to 45 ºC. In these conditions the serum lithium levels could become toxic. It is therefore essential that further studies are carried out in clinical and animal models to examine the effects of lithium in such conditions. Patients suffering from Bipolar Affective Disorder may be using a number of drugs along with lithium, which could affect the serum lithium levels of bipolar patients. Therefore, the effect of other drugs on lithium levels during fasting also needs to be evaluated. The present study provides the empirical methods which can also be used for studying the effects of fasting on serum levels of other mood stabilizers and antipsychotics in animal models and clinical samples.

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