

**MATERIALS  
AND  
METHODS**

## **MATERIALS AND METHODS**

This study was carried out at the Drug Rehabilitation Centre, RHC Murad Memon Goth, Malir, Karachi and in the Department of Pharmacology, University of Karachi, under the supervision of Prof. Shahida P. Ahmed, Head of the Department of Pharmacology, University of Karachi.

A total of 50 male opiate addicts who were seeking treatment for opioid dependence were consecutively admitted between September 2001 to September 2003. All were admitted for 12 days to treat acute opiate withdrawal syndrome and then treated for opioid dependence as outpatients for 12 weeks.

### **SELECTION OF PATIENTS**

All patients in the study were selected according to the following criteria:

#### **Inclusion Criteria**

1. Males between 21 and 45 years of age seeking treatment for opioid dependence for a duration of three months.
2. Following routine clinical criteria indicating opioid addiction were observed:
  - a. Self reported duration of opioid dependence of at least four months.
  - b. An average of two or more episodes of opioid use per day.

- c. Physical evidence of recent intravenous drug use (tracks).
  - d. Urine toxicology positive for opiates at entry to the study.
  - e. A rating of two or greater on a self reported level of withdrawal scale 12 hours after the last opioid use.
3. Ability and willingness to give informed consent.

### **Exclusion Criteria**

1. Psychiatric illness e.g. anxiety, neurosis, phobia, obsessive compulsive neurosis, and hysteria.
2. Self reported current dependence on alcohol or other major drug of abuse like sedatives, hypnotics (including benzodiazepines), cocaine, or amphetamines.
3. Acute liver or cardiovascular diseases.
4. Current enrolment in an opiate treatment programme.
5. Any debilitating disease (Mendelson et al., 1996).

### **MATERIALS**

1. Dried black seeds of *Nigella Sativa* (Kalonji) were purchased from Majeed Brothers, Lajpat Road, Hyderabad, and were cleaned off from adulterant materials and were ground with an electric grinder into coarse powder.
2. Empty capsules manufactured locally were purchased from open market.

3. The frontline opiates test strips obtained from Boehringer Mannheim Pakistan (Pvt) Ltd., Ch-B/Lot No. 28739531 and expiry in February 2004.

### **Treatment Schedule**

The selected patients were divided into two groups:

#### **Group-I**

Twenty five patients with opioid dependence were kept on 500 mg Nigella Sativa orally TID.

#### **Group-II**

Twenty five patients with opioid dependence were kept on one gram Nigella Sativa TID.

The patients received single blind placebo capsule (orally) containing ferrous sulphate powder of same colour, size and shape for the drug, during day-1 and day-2 of admission. They were observed and rated for the presence and absence of opioid withdrawal signs and symptoms experienced during the previous 24 hours by an observer.

Thereafter each treatment, group received single blind capsule, containing either 500 mg or one gram Nigella Sativa on day-3 of admission (treatment day-1). After the initial administration, patients with a positive response to the drug in terms of opiate withdrawal signs and symptoms and without producing side effects were given the drug upto day-12 of admission (treatment day-10). Diazepam 5 mg was prescribed

for some patients having body aches. The ratings covered the withdrawal signs and symptoms during the previous 24 hours. All the patients received their respective treatment upto day-12 of admission during their stay in hospital. After that patients were discharged on the same treatment and advised to attend OPD weekly upto twelve weeks.

### **GENERAL PLAN OF THE STUDY (PROTOCOL)**

The study was carried out according to the following protocol:

1. Permission was obtained from the Incharge Medical Officer of the Rehabilitation Centre, R.H.C., Memon Goth, Karachi.
2. All the patients met inclusion and exclusion criteria for admission to the study.
3. Consents were obtained from all patients before they were enrolled in the study.
4. On entry in the study, the patients received complete physical examinations including electrocardiograms and laboratory screening tests (complete blood cell count, serum chemistry for hepatic functions and urine analysis) to exclude any pathology.
5. All the patients were admitted to the hospital for 10 days for the treatment of acute opiate withdrawal syndrome.
6. The inpatient records of each group were recorded on a proforma (Appendix-I).

7. The severity of opioid abstinence syndrome of each patient during admission and during follow up was recorded on a proforma especially designed for this study (Appendix-II).
8. All the patients were physically dependent on heroin, with a model dose range "between ¼ and ½ grams" (street doses) per day.
9. To ensure that the patients during the protocol did not covertly ingest other drugs. They were confined to a locked inpatient unit, and visitors were restricted. However, patients could discontinue their participation in the protocol and leave the unit at any time on request without prejudice to their future treatment.
10. Patients were divided into two groups. One group comprising 25 opiate addicts was treated with 500 mg Nigella Sativa and the other group comprising of 25 opioid addicts was treated with one gram Nigella Sativa.
11. The patients received single blind placebo capsule for each drug during day-1 and 2 of admission and they were observed and rated for the presence or absence of opioid withdrawal signs and symptoms experienced during the previous 24 hours.
12. On day-3 of admission single blind treatment with Nigella Sativa was assigned in a random manner.

13. After the initial *Nigella Sativa* administration, patients with a positive response to drug in terms of opiate withdrawal signs and symptoms and without producing side effects were given drug upto day-12 of admission.
14. Urine samples were collected, on day-1 and 12 of admission and tested immediately for opioids by test strips.
15. Patients were discharged after 15 days of admission and then assessed weekly upto twelve weeks.
16. Each patient received the treatment for eight weeks and then the doses of drug were gradually tapered off during next two weeks that is weeks-9 and 10.
17. During last two weeks, that is, weeks-11 and 12, patients were assessed without given any drug.
18. Urine samples were tested for opioids on weeks-4, 8 and 12 during the follow up period.
19. This study was carried out on 50 patients. Total period of study was two years.

Data was statistically evaluated.

## **METHOD**

### **Measures**

The selected patients were enrolled, data and progress of the patients were recorded as per Appendix-II, which includes the parameters for abstinence as well as protracted withdrawal for opiate dependence.

### **Subject-Reported Measures**

It was in the form of modified subjective opiate withdrawal scale (MSOWS), which contained 38 opiate withdrawal symptoms (Appendix-II). Subjects indicated the degree to which they had experienced each symptom during the past 24 hours on a five point scale in which 0=not at all, 1=a little, 2=moderately, 3=quite a bit, and 4=extremely (maximum possible total score was 152). The ratings for individual item were summed for a total score each scale (Hiltunen et al., 1995).

### **Observer Rated Measures**

It was in the form of objective opiate withdrawal scale (OOWS, Appendix-II) containing 18 observable physical signs. An independent observer observed and rated the presence and intensity of signs on a five point grade scale in which 0=not at all, 1=a little, 2=moderately, 3=quite a bit, and 4=extremely (Hiltunen et al., 1995).

## **PHYSIOLOGICAL PARAMETERS**

It includes the pulse rate, systolic blood pressure, diastolic blood pressure, temperature, respiratory rate, body weight, and caloric intake (Appendix-II) (Martin and Jasinski, 1969).

## **URINE ANALYSIS MEASURES**

Urine samples were collected on days-1 and 12 of admission and then on weeks-4, 8 and 12. All samples were collected under staff observation to deter bogus urine samples and tested immediately for opioids by using one-step dip and read chromatographic test strips (Frontline opiates test strips).

### **Principle**

The test is used for the immunological semi-quantitative detection of opiates in urine. The test principle is based on the GLORIA (Gold labeled Optical-read Rapid Immunoassay) technology. During immersion the strip absorbs the urine volume necessary for chromatography. The urine passes through a zone containing soluble antibody – gold conjugate that binds specifically to opiates. Excess conjugate is retained by an intercepting zone of immobilized morphine so that only the gold conjugate loaded with opiate metabolites reaches the detection zone, which develops a colour between cream.

## **Ingredients**

One test contains:

- i. 3.5  $\mu\text{gm}$  monoclonal antibodies to opiates labeled with colloidal gold.
- ii. 8.2  $\mu\text{gm}$  morphine polyhapten

## **TEST PROCEDURE**

Test strip is dipped into the urine sample such that the fluid level is between the two blue marks. After 3 to 5 seconds test strip is withdrawn and placed horizontally for chromatography. The result can be read after two minutes. The resulting colour is estimated by using a comparison scale (downward reading). The test strip offers three ranges for an estimation of the concentration on present in the sample:

- < 200 ng/ml negative
- > 200 ng/ml cut off, positive
- >1000 ng/ml highly positive

## **Stability**

Frontline opiates are stable upto the expiry date as specified when stored at +15°C to +25°C.

## **Interference**

Urine samples with acidic values (pH <3) may lead to false negative reading. Urine samples with alkaline values (pH >10) may lead to false positive readings.

## **STATISTICAL ANALYSIS**

The mean was calculated by adding up the observed values and dividing by the total number of observations. This is expressed by the following symbol:

$$\bar{x} = (\sum x) / n$$

Where

$\bar{x}$  signifies the mean,  $x$  is each of observations,  $n$  is the number of observations,  $\sum$  the Greek capital sigma denotes "sum of".

Standard deviation (SD) can be calculated by formula:

$$SD = \sqrt{\frac{\sum x^2 - (\sum x)^2}{n-1}}$$

Where

$x$	=	Individual observation
$n$	=	Number of individual values in series
$\sum$	=	Sum of

Standard error of mean (SE) or (SEM) was calculated by the formula:

$$SE = SD / \sqrt{n}$$

Where

SD	=	Standard deviation
$n$	=	Number of observations

### Student's 't' test

The statistical significance of difference between the mean values of the two groups was evaluated by student's 't' test.

The value of 't' was calculated by the formula:

$$t = \frac{x_1 - x_2}{\sqrt{(SE_1)^2 + (SE_2)^2}}$$

Where

$x_1 > x_2$	
$x_1$	= Mean of one group of observations
$x_2$	= Mean of second group of observations
$SE_1$	= Standard error of $x_1$
$SE_2$	= Standard error of $x_2$

Degree of freedom (d.f) was calculated as:

$$d.f. = n_1 + n_2 - 2$$

Where

$n_1$	= Number of observation in one group
$n_2$	= Number of observation in second group

### P Value

The degree of probability was computed by comparing the calculated value of 't' with tabulated values in the table of 't' distribution against the degree of freedom (d.f). The difference in the mean values of the two groups was regarded statistically significant if the P value was equal to or less than 0.05 and non-significant (NS) if the P value was greater than 0.05. It was highly significant if the P value was less than 0.001.