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SOCIALLY AVAILABLE HEROIN POWDERS-THEIR CHEMICAL COMPOSITIONS AND REPRODUCTIVE AND BEHAVIOURAL EFFECTS ON FEMALE RATS

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ABSTRACT

Morphine is one of such substance which produce very intense euphoria and a prolonged period of relaxation and sedation. These opioids are known to use modifications in neuroendocrine system during different stages of life, ranging from fetal through postnatal to adult. Heroin, an opioid, is a derivative of morphine and its long-term use, both in males and females, is associated with alteration in sexual behaviour and changes in endocrine functions. In females, hormonal imbalance thus resulted, can lead to amenorrhea and in some cases infertility. In Pakistan, heroin is available mixed with different additives in a variety of forms. Two varieties (S_1 and S_2) procured from patients visiting Drug Rehabilitating Clinic in the Civil Hospital, Karachi, were analyzed for their qualitative and quantitative properties.

Introduction

Opioids are known to act on nervous tissues for relieving pain and stress (Stimmel, 1975). Morphine is one of such substances which produce very intense euphoria and a prolonged period of relaxation and sedation (Mirin et *al*, 1980). These opioids are known to cause modifications in neuroendocrine system during different stages of life, ranging from fetal through postnatal to adult (Lichtensteiger and Schlumpf, 1984). Opioid peptides have been shown to play an important role as physiological modulators of the function of the hypothalamo-anterior pituitary axis and for sexual behaviour associated with structural alterations in discrete regions of the brain (Lichtensteiger *et al*, 1984; Lichtensteiger and Schlumpf, 1985). The endogenous opiates have been linked to the central catecholamine systems (Lau *et al*, 1977; O'Callaghan and Holtzman, 1976) responsible for gonadostat resetting and sexual behaviour. Heroin, an opioid, is a derivative of morphine and its long-term use both in males and females is associated with alterations in sexual behaviour and changes in endocrine functions. In females, hormonal imbalance thus resulted, can lead to amenorrhea and in some cases infertility (Litto *et al*, 1983). In Pakistan, heroin is available mixed with different additives in a variety of forms. Two varieties (S_1 and S_2) procured from patients visiting Drug Rehabilitating Clinic in the Civil Hospital, Karachi, were analysed for their qualitative and quantitative properties. Based on the quantitative findings, S_2 was used to assess effects on reproductive and behavioural pattern in female rats after its prolonged exposure.

Material and Methods

Chemical studies

Component separation by HPLC
Sample St (White colour)

5 mg of S_1 was dissolved in 1 ml of acetonitrile-propanol (4:1) mature and separated on HPLC (Shimadzu, Japan). 100 NI of the sample was applied to the column (Nova Pak, Cm, Waters) using isocratic system of the solvent acetonitrile-propanol (4:1). The flow rate was maintained at 60 ml/hr. The elution pattern was recorded at 280 nm.

Sample S_2 (Brown colour)

5 mg of S_2 was dissolved in 1 ml of methanol. 100 μl of the clear solution was applied to the column as stated above using 40% methanol as isocratic system. The flow rate was adjusted at 60 ml/hr and the elution pattern recorded at 280 nm.

GC-Mass spectrometry

The presence of a number of components was confirmed by gas chromatography using the Hewelett-Packard 58901 instrument. Mass spectra of all the components from both the samples were obtained on double focusing mass spectrometer (JMS HX 110 Jeol, Japan) connected to PDP 11173 DEC (USA) computer system. Exact mass measurements were carried out through peak matching experiment. The GC-MS of the components was performed by applying the following conditions: Injector temperature 250°C, column temperature 70°C, column used, OV 101 (Waters), column size (25cm x 0.4cm). He at a flow rate of 32 m1/min and temperature programming 2 min at 8°C/min upto 220°C.

Thin layer chromatography

The contents of S_1 and S_2 and the purity of separated peaks were checked on TLC (Silica gel SIF-254, precoated aluminium cards) in $CHCl_3$-McOH (9:1) system.

Molecular ion peak matching

Molecular mass of the components obtained by GC-MS were exactly measured on mass spectrometer (MAT 311A, Finnigen MAT, Germany).

Biological effects

The Wistar rats used in this study were female adults weighing 150-180g. They were maintained under controlled temperature (21±1°C) and light (12 hour light-dark cy*cle).* A balanced diet formulated at National Institute of Health, Islamabad, along with water were available ad lib.

Animals were assigned to the following treatment regimen

A. Experimental animals (n = 8) were administered, intraperitoneally 5 mg/kg, daily of S_2 in 50 ml of 0.9% saline for a period of approximately 60 days.

B. Control animals (n=10) were administered 50 ml of 0.9% saline, through the same route for a period of 60 days.

All treatments were given between 8.30 to 10.30 hrs. Records were kept of body weight and vaginal cyclicity. Vaginal smears were taken daily during the treatment period and examined for the cytological changes. Only those animals were included for the study which exhibited normal 4-5 days regular oestrous cycle.

For the last 10 days of the treatment both experimental (exposed to prolonged S2 treatment) and control group of animals were left with adult, sexually active male rats. Mounting behaviour of the males towards both experimental and control animals as well as lordotic and defensive behaviour of the females in both groups were observed twice a day for a two h period (8:30-10: 30 his and 16:00 to 18:00 hrs).

After monitoring behavioural changes during the final 10-12 days of treatment control animals were killed by decapitation on the diestrous day (day 58-61) of the ovarian cycle whereas treated animals were killed on the last day of treatment with vehicle (60 days) when most of the females were in diestrous phase of the cycle. Trunk blood was collected in heparin zed tubes at room temperature. The blood was then centrifuged at 400xg at 5°C for 10 minutes and the plasma stored at -20°C until assayed. Progesterone concentrations present in the aliquots (0.5 ml) were measured by radioimmunoassay using a kit obtained from Diagnostic Products Corporation, Los Angeles. The infra-and inter-assay coefficient of variation for aliquots of a rat serum pool were 5.8 and 11.7, respectively. This kit is in extensive use for the measurement of steroid hormones in clinical laboratories.

Results and Discussion

Chemical Studies

Sample S_1 (White colour)

S^1 was completely soluble in acetonitr0e-propanol (4:1) system. It resolved into three peaks, one minor peak (PK-1) and 2 major peaks (PK-2 and PK-3) *by* reversed phase high performance liquid chromatography (Fig.1).

GC-MS of PK-1 revealed major isomers at m/z (%), 563 (59), 327 (125); PK-2 237, 284 (126), 285 (122); PK-3 327 (125), 563 (58) and 503 (96). The molecular formula, $C_{19}H_{21}NO_4$, was obtained through exact mass measurement of the molecular ion at m/z 327.146 which corresponds to morphine 3- or 6- monoacetate, being present in all the three peaks at PIC-1(125), PK-2 (126) and PK-3 (125).

The second major compound of the sample $_{St,}$ PK-2, has a molecular mass 284 (126) or 285 (122) whose exact mass measurement of the molecular ion at m/z 285.359 confirmed it to be morphine ($C_{17}H_{I9}NO_3$).

The fragment having a molecular mass 503 (96) which appeared from peak-3 could not be *identified.* A molecular ion peak of molecular mass 563 is found in PK-1 and PK-3 and is not a fragmentation ion peak of morphine or its related compounds, it may be an impurity or an additive. Thus the sample S1 (white colour) contains two major compounds, morphine monoacetate and morphine, in a ratio 1:12 and an additive which could not be identified.

Sample S_2 (Brown colour)

The methanol soluble fraction of sample S2 was found to be a crude mixture of four compounds. These were separated *by* HPI.0 (A, B, C and DX and were in the ratio 13:14:18:29 respectively (Fig. 2). GC-MS of peak-A displayed two major molecular ion peaks 327 (125) and 338 (145). The exact mass was found to *be* 327.146, corresponding to the molecular formula, $_{C19II21NO4,}$ and showed to be morphine 3- or 6- monoacetate, while the mass 338 (145) could not be identified but it is not a molecular ion peak of morphine fragment Peak-B was resolved into two fragments, again 327.146 (125) giving morphine 3- or 6-monoacetate and a mass 369 (131) indicating the presence of morphine diacetate ($C_{21}H_{23}NO_5$).

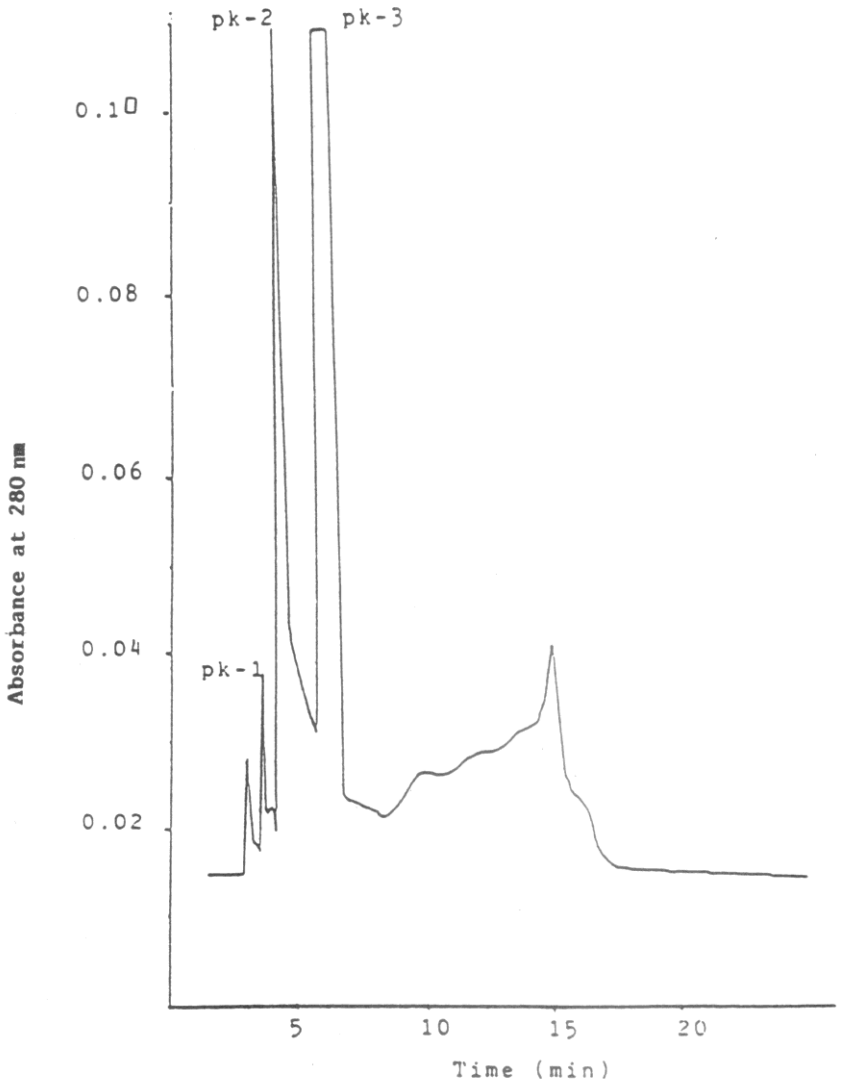

Figure 1. Separation of S_1 components by HPLC.

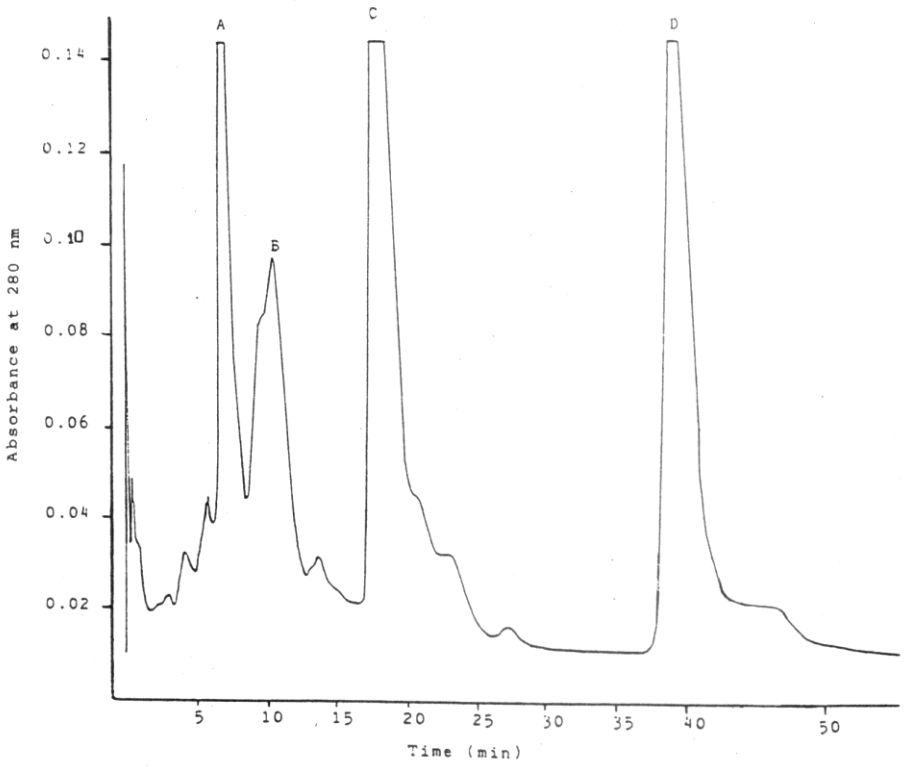

Figure 2. Separation of S_2 by HPLC

The major peaks of peak-C obtained from GC-MS were 341 (124) and 412 (161). Accurate measurement of mass 341.164 indicated the presence of o-acetyl codeine ($C_{20}H_{21}NO_4$). While the exact mass 412.1433 (161) enabled to propose possible molecular formula C25H28N2O4. Peak-D resolved into two major fragments having molecular mass 296 (118) and 563 (53). The compound determined after exact molecular mass measurement 296.106, having molecular formula, $C_{25}H_{19}NO_3$, is codeinone or morphothebaine. The peak showing molecular mass 563 (53) appeared to be a false one. Thus the major compounds detected in sample S_2 are morphine 3- or 6- monoacetate, morphine diacetate (heroin), o-acetyl codeine and codeinone or morphothebaine in a ratio of 13:14:18:29.

Biological effects

These were observed on three key parameters, i.e. vaginal cyclicity, sexual behaviour and plasma progesterone concentrations.

Control females (n=10) observed throughout the period of treatment, did not exhibit any variation in their vaginal cyclicity. In all the 8 animals, exposed to S2, a significant effect on the oestrous cycle was observed. All but one of these animals went into persistent diestrous by approximately the 10-11th day (1132.2, X Y S.D.) of treatment. This persistent diestrous suggests an involvement of the hypothalamic and / or pituitary ovarian axis. This is consistent with some of the recent reports that opioid peptides act on the hypothalamus to inhibit LHRH release and not on the pituitary since the response to exogenous LHRH is not blocked *in vivo* and LH release by pituitary cells incubated *in vitro* is unaffected (Adler *et al*, 1984; Akabori and Barraclough, 1986; Dyer *et al*, 1988). A direct action of opiates on the pituitary can, however, not be ruled out as evidence of the presence of opioid receptors in the pituitary have also been reported (Ching, 1983).

Opioid peptides may also act ind[i]rectly by inhibiting dopaminergic turn over rate in the tuberoinfundibular dopaminergic systems and augmenting serotoninergic turn over rate which may possibly increase noradrenergic turn over rate as well (Pattersen and Barraclough, 1986). This is probably the factor which maintains just the basal 1.H concentrations thus causing the animals to be in persistent diestrous. These animals had plasma progesterone concentrations of significant diminution ($P < 0.01$) (Fig. 3).

Alteration in the sexual behaviour has been another parameter which was observed in the group of animals exposed to heroin (S_2) for a prolonged duration. These observations were made in the last 10-12 days of the treatment period and are presented in Table 1. The control females left with adult males showed 53.7% lordotic and 14.1% defensive behaviour during the 37 tests examined.

Table 1

Sexual behaviour in control and heroin treated rats

Treatment	Rats	Test	% Positive tests with L*	% Positive tests with D*
Controls (0.9% saline)	10	37	53.7	14.1
Controls (in diestrous)	10	26	17.1	82.9
Treated (S_2, 5 mg/kg)	8	28	7.4	86.0

*L: Lordotic behaviour towards vigorous males
*D: Defensive behaviour trowards vigorous males

However, when the observations were grouped for the diestrous phase of control, vast majority (82.9%) of female rats exhibited defensive and aggressive response whereas still a nominal (17.1%) demonstrated lordotic inclinations but did not permit the mating. The treated animals, in contrast, were found to be non-receptive during the most number of tests. A very large percentage (86%) displayed defensive behaviour when exposed to the normal adult males. However, a very meagre percentage (7.4%) exhibited lordotic behaviour. This is peculiar with the diacetyl morphine (active component of heroin) to cross the blood-brain barrier and either exert a generalized sedative effect on the CNS or inhibit the hypothalamo - pituitary-gonadal axis (Hinz et al., 1978). The possibility of a direct effect on pituitary can also not be ruled out (Blank et al, 1986).

It has also been suggested that progesterone may exert its effect on sex behaviour via the serotoninergic system (Kow et al, 1974). In our study, chronic S2 treatment has resulted in a decline in plasma progesterone concentrations (Fig. 3). Sietniks and Meyerson (1982) have suggested that progesterone stimulates activity by increasing 5-HT turn over which then acts presynaptically inhibiting further serotoninergic transmission. This suggestion appears reasonable in view of the fact that 5-HT receptor antagonists stimulate sexual behaviour (Davis and Kohl, 1978), whereas some agonists (LSD) inhibit it (Miskiewicz *et al*, 1979). Diminished lordotic activity in the treated female rats in this study may thus be attributed to the enhanced serotininergic activity in the CNS occurred as a result of depressed progesterone milieu.

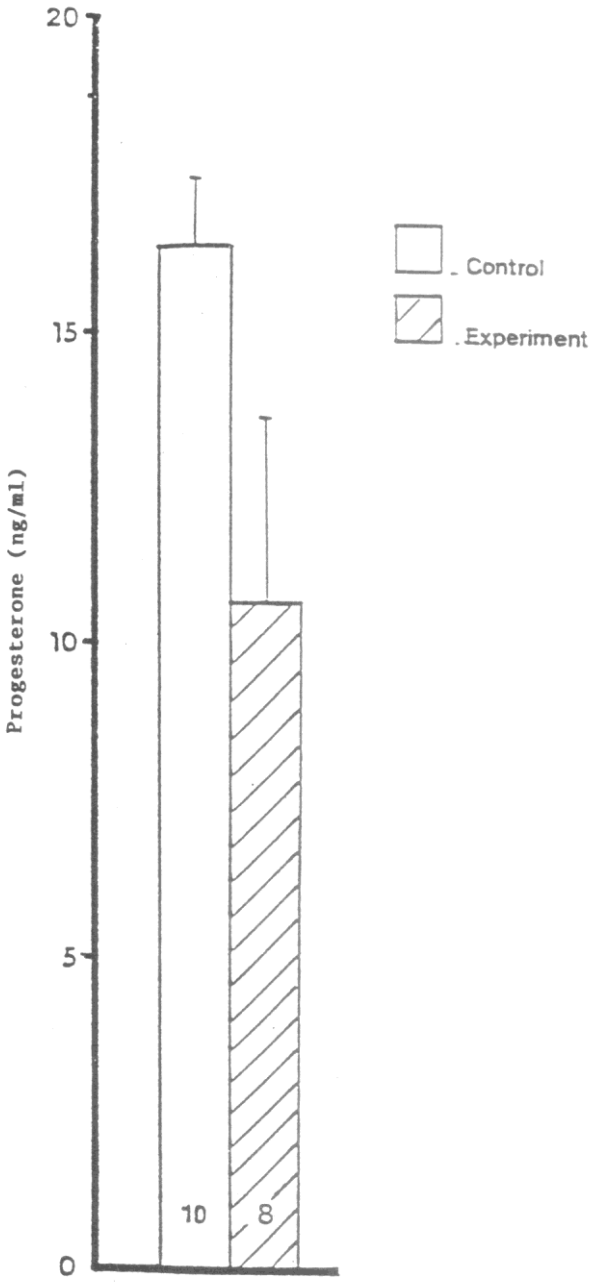

Figure 3. Plasma progesterone concentrations in heroin treated and control female rats (X± S.E.)

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