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Responses of parathyroid gland, C cells, and plasma calcium and inorganic phosphate levels in rat to sub-lethal heroin administration

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Abstract: *In order to record the effects of heroin on plasma calcium (Ca) and inorganic phosphate (Pi) levels as well as parathyroid gland and C cells, two sub-lethal doses (0.50 LD_{50} and 0.75 LD_{50}) of the drug were administered intramuscularly in Rattus norvegicus for 30 days. Plasma Ca level of control rats ranged between 9.53±0.32 - 9.88±0.22 mg 100 ml^{-1} while plasma Pi concentration fluctuated between 4.55±0.18 - 4.71±0.24 mg 100 ml^{-1}. Sub-lethal heroin administration induced progressive increase in plasma Ca level during the first seven days ($p < 0.001$), thereafter the level declined on day 15 and 30. However, plasma Pi level of the heroin-treated rats registered increase with the peak value ($p < 0.001$) on day 30. The treatment elicited degenerative changes in parathyroid gland as evident by cytoplamic vacuolization, presence of more pycnotic nuclei and occurrence of patchy areas among the chief cells. Degenerative changes were also noticed in cristae of mitochondria, Golgi complex and endoplasmic reticulum. There was decrease in chromatin material in the nucleus and loss of hormone granules in the cytoplasm. Oxyphil cells of the heroin-treated rat depicted dilation of endoplasmic reticulum and damaged cristae. Sub-lethal heroin administration in the rat for 30 days induced dilation in endoplasmic reticulum and loss of secretory granules in C cells.*

Key words: *Heroin, Plasma calcium, Plasma inorganic phosphate, Parathyroid gland, Oxyphil cells, C cells, Rattus norvegicus*
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Introduction

Heroin abuse is the burning problem of the society world over and associated with multiple poor health manifestations, reduced quality of life and encourage criminal behaviour (Sawynok, 1986; Neri-Serneri and Modesti, 1991; Sporer, 1999; Cami and Farre, 2003; Brown *et al*., 2006; Barai *et al*., 2009). The drug (diacetylmorphine) is initially metabolized to 6-acetylmorphine and subsequently to morphine in human body (Sawynok, 1986; Goldberger *et al*., 1994; Jenkines *et al*., 1994). Chronic abuse of heroin has diverse effects on various body systems due to widespread distribution of specific receptors in many tissues and organs (Martin, 1984; Sawynok, 1986; Hashemi *et al*., 2006). Nephrotoxic and hepatotoxic effects of the drug have been documented in mammals (Weller *et al.,* 1984; Gomez-Lechon *et al*., 1987; Kringsholm and Christoffersen, 1989, Barai *et al*., 2004a). Hematological as well as biochemical profiles of serum to exogenous drug administration have also been recorded (Hussain and Kumar, 1988; El-Daly, 1994; Barai *et al*., 2004b). Furthermore, there are few reports suggesting that the chronic heroin administration/addiction modulates hypothalamo-pituitary-gonadal (HPG) as well as hypothalamo-pituitary-adrenal (HPA) axes in mammals (Pechnick, 1993; Spanagel, 1999; Sarnyai *et al*., 2001; Laorden *et al*., 2002; George *et al*., 2005; Blesener *et al*., 2005; Brown *et al*., 2006). Though there are a few records on the altered serum calcitonin level and bone metabolism in heroin addicts (Tagliaro *et al*., 1984: Spagnolli *et al*., 1987, 1988; Pedrazzoni *et al*., 1993; Kim *et al*., 2006), the observations are highly contradictory (Tagliaro *et al*., 1992). An attempt has, therefore, been made to record the changes occurring in plasma Ca and Pi levels as well as parathyroid gland and calcitonin-producing C cells of rat, *Rattus novrvegicus,* in response to sub-lethal heroin administration.

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Materials and Methods

Healthy male rat, *R. norvegicus* weighing 150-200 g were procured from the Brihanmumbai Municipal Corporation, Mumbai. They were acclimatized under the ambient laboratory conditions (temperature 28±2°C; photoperiod 14L:10 D) for 10 days, fed *ad libitum* on rat feed (Lipton, Bangalore) and clean water was provided for drinking. 60 male rats were randomly selected and divided into three equal groups - two experimental and one control. Heroin was dissolved initially in small quantity of alcohol and diluted with physiological saline to prepare two test doses - 0.50 LD_{50} (10.9 mg kg^{-1}) and 0.75 LD_{50} (16.4 mg kg^{-1}). The drug was administered through intramuscular route to the experimental rats while the control rats received equal volume (0.2 ml kg^{-1} body weight) of the physiological saline. Animals from both the groups were killed on day 1, 7, 15 and 30 of the treatment. Blood samples were

Table - 1: Effects of heroin administration on plasma calcium and inorganic phosphate levels (mg 100 ml^{-1}) of rat, *Rattus norvegicus.*

Group	Duration (days)			
	1	7	15	30
		Plasma calcium		
Control	9.88±0.22	9.64±0.16	9.69±0.57	9.53±0.32
Heroin (0.50 LD_{50})	10.82±0.31^{a} (+9)	12.16±0.22^{c} (+26)	9.02±0.12 (-7)	7.84±0.38^{b} (-18)
Heroin (0.75 LD_{50})	10.93±0.28^{a} (+11)	12.71±0.28^{c} (+32)	9.24±0.35 (-5)	7.43±0.21^{c} (-22)
		Plasma inorganic phosphate		
Control	4.62±0.21	4.68±0.32	4.55±0.18	4.71±0.24
Heroin(0.50 LD_{50})	5.31±0.28 (+15)	5.68±0.28^{a} (+21)	6.25±0.21^{c} (+37)	7.36±0.39^{c} (+56)
Heroin (0.75 LD_{50})	5.24±0.36 (+13)	5.83±0.14^{b} (+25)	6.35±0.35^{b} (+40)	7.14±0.32^{c} (+52)

Values are mean ± SD of 5 specimens. Values in parentheses indicate % increase (+) or decrease (-) over control. Significance: ^{a} p <0.05, ^{b} p <0.01; ^{c} p <0.001

collected in sterilized glass syringe from post-caval vein of the rats under mild ether anesthesia and plasma was separated by centrifugation at 3,500 rpm. Plasma calcium (Ca) and inorganic phosphate (Pi) levels were estimated according to Trinder (1960) and Fiske and Subbarow (1925) methods, respectively. Values obtained for the control and experimental groups were evaluated for statistical significance using Students 't' test (Snedecor and Cochran, 1989).

Both thyroid and parathyroid glands were surgically removed and fixed immediately in Bouin's solution for light microscopic studies. After routine processing, sections were cut at 6 µm and stained with hematoxylin-eosin and lead-hematoxylin (Solcia *et al.,* 1969). The sections were also stained with toluidine blue and examined under the light microscope. For electron microscopic observations, the tissues were fixed in 3% glutaraldehyde maintained at 4°C. They were washed thoroughly with 0.1N cacodylate buffer to remove traces of glutaraldehyde. Ultrathin sections (600-800Å) were cut from the selected area with glass knife and mounted on 400 mesh copper grids. The tissues were double stained with 10% alcoholic uranyl acetate for 20 min and with Reynold's lead citrate for 10 minutes. Sections were scanned under Jeol-100 electron microscope.

Results and Discussion

Variations in plasma calcium (Ca) and inorganic phosphate (Pi) levels of *Rattus norvegicus* in response to sub-lethal heroin administration have been summarized in Table 1. Plasma Ca level of control rats ranged between 9.53±0.32 to 9.88±0.22 mg 100 ml^{-1} while Pi concentration fluctuated between 4.55±0.18 and 4.71±0.24 mg 100 ml^{-1}. Sub-lethal heroin administration elicited significant increase in plasma Ca level during the first seven days ($p<0.001$), thereafter the level registered a steady decline approaching normocalcemia on day 15 while by day 30, frank hypocalcemia was observed in both the experimental groups. However, plasma Pi level of the heroin-treated rats registered a progressive increase ($p <0.05$) by 24 hr and attained the peak ($p <0.001$) on day 30.

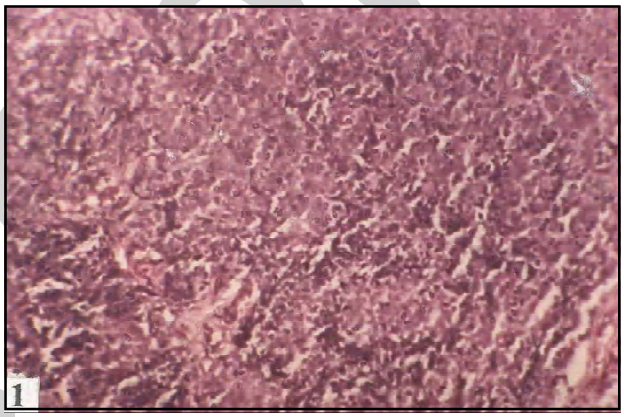

Fig. 1: Parathyroid gland of control rat, *Rattus norvegicus* showing distribution of the chief cells. H&E x 100

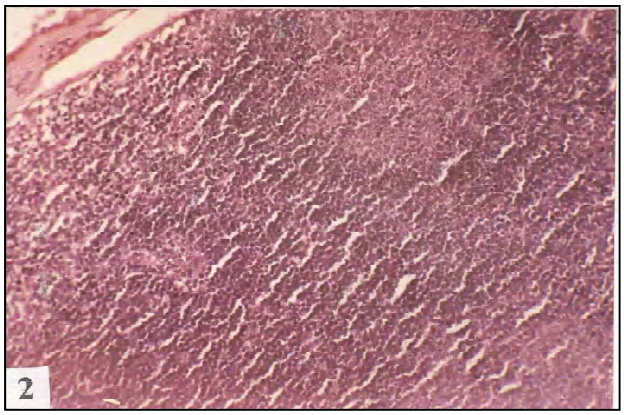

Fig. 2: Parathyroid gland of rat injected with heroin (0.75 LD_{50}) for 30 days exhibiting disintegrated and patchy areas of chief cells. H&E x 100

Parathyroid glands of control rat, consisted mainly of chief cells arranged in elongated and branching cords, separated by connective tissue stroma, capillaries and sinusoids (Fig.1). Ultrastructurally, they were bound by unit membranes and

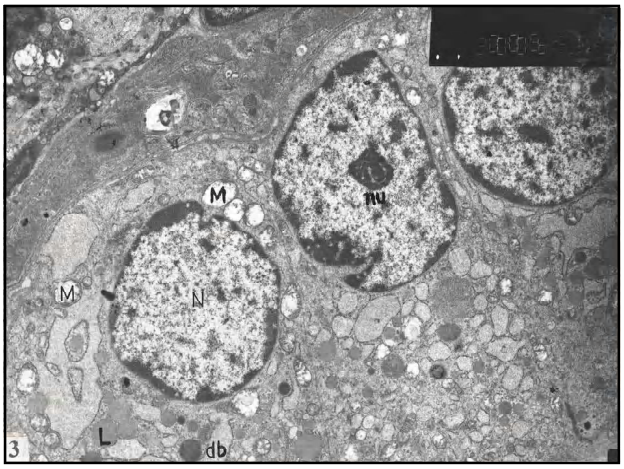

Fig. 3: Chief cells of control rat depicting nucleus (N), nucleolus (nu), mitochondria (M), lipid droplet (L), dense body (db) and rough endoplasmic reticulum x 3,000

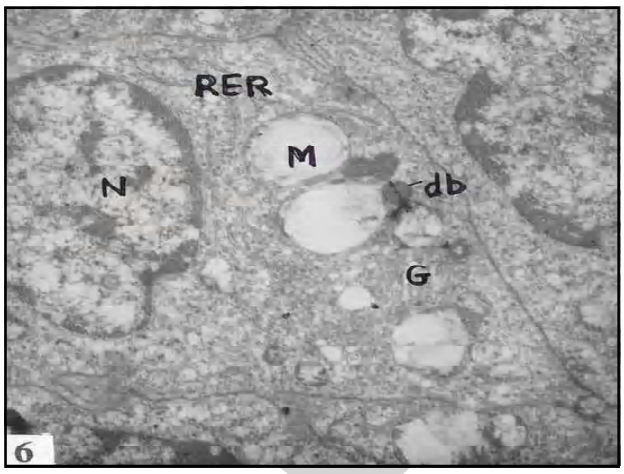

Fig. 6: Oxyphil cell of control rat depicting nucleus (N), dense body (db), mitochondria (M) and Golgi complex (G) x 8,000

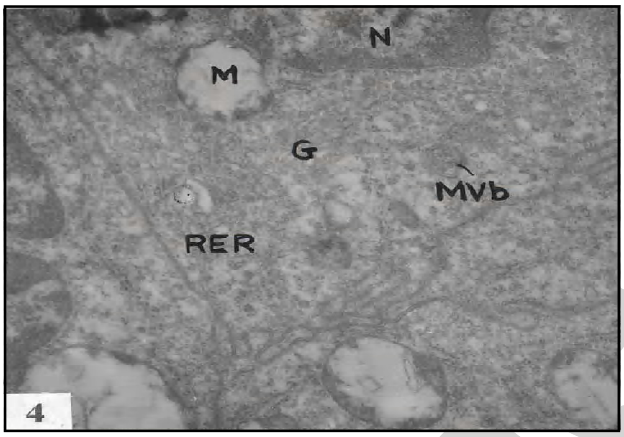

Fig. 4: Chief cell of control rat showing prominent nucleus (N), rough endoplasmic reticulum (RER), Golgi complex (G), mitochondria (M) and multi-vesicular body (MVb) x 15,000

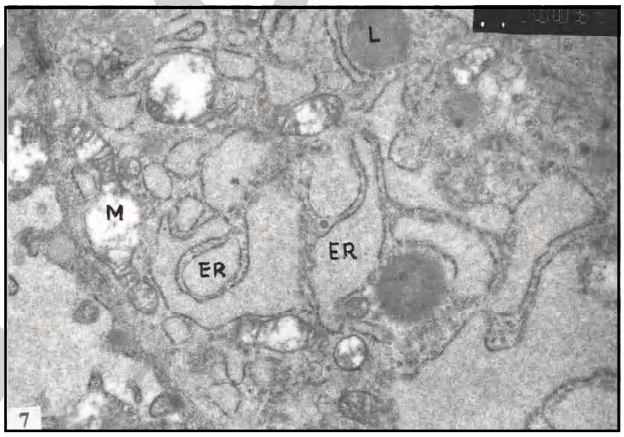

Fig. 7: Oxyphil cell of rat treated with heroin (0.75 LD_{50}) for 30 days showing mitochondria with damaged cristae (M), dilated endoplasmic reticulum (ER) and lipid body (L) x 10,000

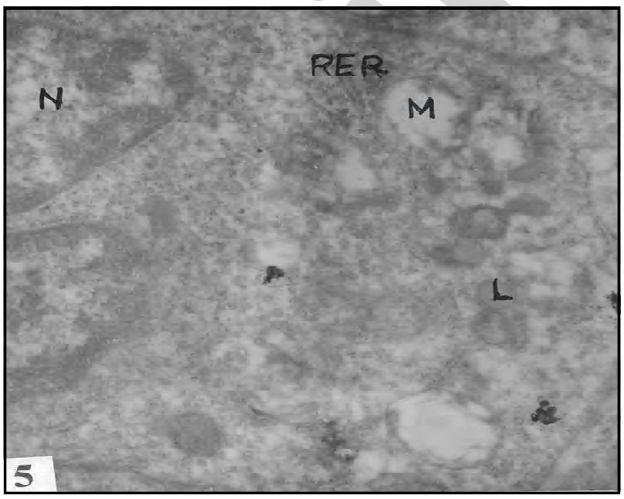

Fig. 5: Chief cell of rat treated with heroin (0.75 LD_{50}) for 30 days exhibiting part of nucleus (N), rough endoplasmic reticulum (RER), lipid droplet (L) and disintegrated mitochondria (M) x 15,000

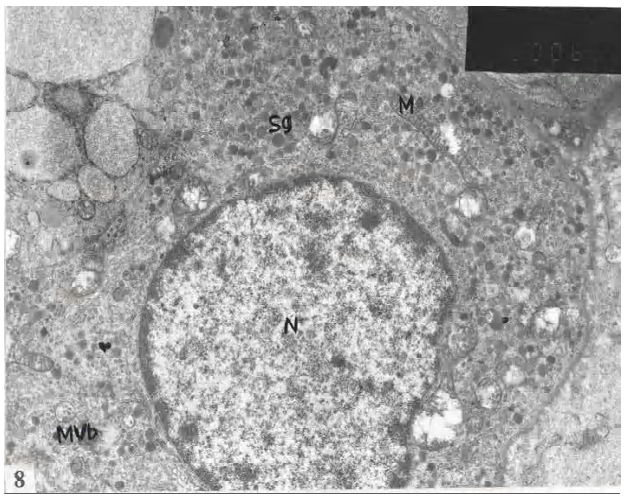

Fig. 8: C cell of control rat exhibiting prominent nucleus (N), mitochondria (M), multi-vesicular body (MVb) and large number of secretory granules (Sg) x 6,000

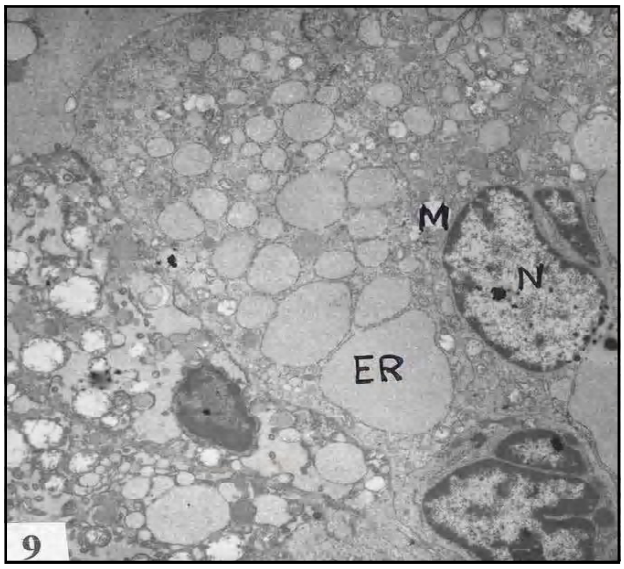

Fig. 9: C cell of rat treated with heroin (0.75 LD_{50}) for 30 days depicting nucleus (N), mitochondria (M), dilated endoplasmic reticulum (ER) and loss of secretory granules x 4,000

showed clear desmosomes and terminal bars joining the plasma membranes. The nucleus was large, spherical or oval structure containing many small granules concentrated more towards the periphery. The cytoplasm contained prominent rough endoplasmic reticulum and Golgi apparatus was composed of straight or curved stacks of membranes with small vesicles and granules. Mitochondria were distributed throughout the cytoplasm. Only a few electron dense secretory granules could be seen in cytoplasm. Besides these, lipid, glycogen and lysosomal bodies were also noticed in the chief cells (Fig. 3, 4). Sub-lethal heroin administration for 30 days induced degenerative changes in the parathyroid gland as evident by cytoplamic vacuolization, presence of more pycnotic nuclei and occurrence of patchy areas among the chief cells (Fig. 2). Degenerative changes were also noticed in cristae of mitochondria, Golgi complex and endoplasmic reticulum. There was decrease in chromatin material in nucleus and loss of hormone granules in cytoplasm of the chief cells (Fig. 5).

A few oxyphil cells, polygonal in shape and larger than the chief cells, were also encountered in the parathyroid gland. Their nuclei were smaller, irregular and denser than those of the chief cells. The abundant cytoplasmic area of the oxyphil cells was filled with numerous large mitochondria. The endoplasmic reticulum, Golgi apparatus and secretory granules were poorly developed in these cells (Fig. 6). Oxyphil cells of the heroin-treated rats depicted dilation of endoplasmic reticulum and damaged cristae of mitochondria on day 30 (Fig. 7).

Calcitonin-producing C cells of the rat were unevenly distributed in thyroid follicular cells. They were larger in size with more electronlucent cytoplasm as compared to those of thyroid follicular cells. Ultrastucturally, they possessed conspicuous endoplasmic reticulum, prominent Golgi apparatus, numerous mitochondria (both circular and elongated types) and large number of electron dense secretory granules in their cytoplasm (Fig. 8). In a few cells, desmosomes and terminal bars were also encountered. Sub-lethal heroin administration in the rat for 30 days induced dilation in endoplasmic reticulum and loss of secretory granules (Fig. 9). It is peretinent to remark that the degenerative changes in these glands were more pronounced in higher dose (0.75 LD_{50}) of heroin as compared to those given 0.50 LD_{50} dose.

Parathyroid glands made their first phylogenetic appearance only in tetrapods (Clark *et al*., 1986; Pandey, 1991, 1992), probably to protect against the development of hypocalcemia and to maintain skeletal integrity in terrestrial animals (Wendalaar Bonga and Pang, 1991; Pandey, 1992). Parathyroid hormone (PTH) is a predominant hypercalcemic and hypophosphatemic factor which controls the plasma Ca and Pi metabolism of mammals in concert with calcitonin (CT) and 1,25-dihydroxyvitamin D_3 (active metabolite of vitamin D_3). These hormones exert their control in an integrated manner through three main processes - (i) the balance between the rate of deposition and mobilization of Ca and Pi in the bone (reservoir), (ii) the urinary excretion of Ca and Pi and (iii) the absorption of Ca and Pi from the gastro-intestinal tract (Tayler, 1984; Pang and Schreibman, 1989; Wendelaar Bonga and Pang, 1991; Aurbach *et al*., 1992; Dacke *et al*., 1996; Dacke, 2000). There exist limited observations on the effects of opioid drugs on calcium-regulating hormones suggesting that the circulating levels of calcitonin may be higher in heroin addicts (Tagliaro *et al*., 1984, 1985; Spagnolli *et al*., 1987, 1988), however, Tagliaro *et al*. (1992) observed increase only in "calcitonin-like" material, but not immunoreactive calcitonin, in such subjects. The opioids may play a role in regulation of calcitonin secretion in rat (Gozariu *et al*., 1985). In the present study, sub-lethal heroin administration in rat for 30 days elicited degenerative changes in chief and oxyphil cells of the parathyroid gland as well as calcitonin-producing C cells. Conversely, Padrazzoni *et al*. (1993) recorded increased blood ionized and urinary calcium in heroin addicts. Though oxyphil cells made their first phyletic appearance in parathyroid gland of mammals, its function remains obscure except an increase in number with advancing age (Roth and Schiller, 1976; Setoguti, 1977; Clark *et al*., 1986). The observed hypocalcaemia and hyperphosphataemia in the rat due to prolonged sub-lethal heroin administration appears to be due to degenerative changes in the chief cells which are the source of parathyroid hormone (PTH) in mammals.

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