© Triveni Enterprises, Lucknow (India)
ISSN : 0254-8704
http : //www.jeb.co.in

J. Environ. Biol.
30(2), 253-258 (2009)
info@jeb.co.in

Levels of plasma sodium and potassium as well as alterations in adrenal cortex of *Rattus norvegicus* in response to sublethal heroin administration

S.R. Barai[1], S.A. Suryawanshi[2] and A.K. Pandey*[3]

[1]Department of Zoology, Udai Pratap Autonomous College, Varanasi-221 002, India
[2]Swami Ramanand Teerth Marathwada University, Nanded-431 606, India
[3]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, India

(Received: December 20, 2006; Revised received: July 19, 2007; Accepted: August 10, 2007)

Abstract: *In order to record the effects of sublethal heroin administration on plasma mineral metabolism, the drug was administered intramuscularly (16.4 mg kg^{-1} body weight day^{-1}; 0.75 LD_{50} dose) in Rattus norvegicus for 30 days. Plasma sodium and potassium levels of the control rats fluctuated between 153.14 ± 2.88-157.23 ± 2.16 meq l^{-1} and 5.04 ± 0.32-5.63 ± 0.41 meq l^{-1}, respectively. Plasma sodium level of the treated rats registered a progressive decline ($p<0.01$) at 24 hr with the minimum value (126.53 ± 2.68 meq l^{-1}) on day 30 whereas plasma potassium level registered a progressive increase during entire period of the treatment with peak (8.78 ± 0.23 meq l^{-1}) on day 30. Though sublethal herion adminstration for 30 days elcited cytoplasmic vacuolation in all the three zones of adrenal cortex, much of cytological alterations were observed in zona glomerulosa and zona fasciculata cells. In zona glomerulosa cells, degenerative changes in the organelles were more pronounced as evident by the loss of typical cristae in the mitochondria and hormone granules were rarely seen in these cells. Though rough endoplasmic reticula were scanty, many lipid granules encountered in zona glomerulosa cells of the treated rats.*

Key words: *Heroin, Plasma sodium, Plasma potassium, Adrenal cortex, Rattus norvegicus*
PDF of full length paper is available with author ***(*akpandey_cifa@yahoo.co.in; cifa@ori.nic.in; cifa@hun1.nic.in)***

Introduction

Heroin (Brown sugar) abuse is a burning problem of the society (Neri-Serneri and Modesti, 1991; Kringsholm *et al*., 1994). The drug (diacetylmorphine) metabolizes into morphine and 6-acetylmorphine in the human body (Goldberger *et al*., 1994; Jenkines *et al*., 1994). Nephrotoxic and hepatotoxic effects of the drug have been documented in human as well as rats (Weller *et al.,* 1984; Gomez Lechon *et al*., 1987; Kringsholm and Christoffersen, 1989; Barai *et al*., 2004a). Haematological as well as biochemical profiles of serum of mammals to exogenous drug administration have also been recorded (Hussain and Kumar, 1988; El Daly, 1994; Barai *et al*., 2004b). Though there exist few reports suggesting that chronic heroin addiction/administration modulates the pituitary-thyroid function as well as calcitonin secretion in mammals (Brambilla *et al.,* 1980; Sagaliaro *et al*., 1984; Kringsholm and Christoffersen, 1989; Barai *et al*., 2004c), information regarding the effects of heroin on adrenal cortex secretion of mammals are scanty (Glass *et al*., 1973; Pechnick, 1993; Spanagel, 1999; George *et al*., 2005). An attempt has, therefore, been made to record the effects of sublethal heroin administration on plasma sodium and potassium levels as well as adrenal cortex of *Rattus norvegius*.

Materials and Methods

Wild stock of healthy *Rattus norvegicus* (both sexes) weighing 200-250 g were procured from the Bombay Municipal Corporation, Mumbai. They were acclimatized under the ambient laboratory conditions (temparature 28±2°C; photoperiod 14 L:10 D) for 10 days, fed *ad libitum* on rat feed (Lipton, Bangalore) and clean water was provided for drinking. 50 rats were randomly selected and divided into two equal groups - experimental and control. The experimental group rats were given intramuscular injection of 0.75 LD_{50} dose (16.4 mg kg^{-1} body weight day^{-1}) of heroin (the drug was initially dissolved in small quantity of rectified (95%) alcohol and the desired dose was prepared in physiological saline) while the control rats received equal volume (0.2 ml kg^{-1} body weight day^{-1}) of the physiological saline. Animals from both the groups were dissected on day 1, 7, 15 and 30 of the treatment. Blood samples were collected in sterilized glass syringe from post-caval vein of the rats under mild ether anaesthesia and serum was separated by centrifugation at 3,500 rpm. Plasma sodium and potassium levels were estimated by the flame photometry method described by Wootton (1974). For estimation of sodium and potassium levels with this method, 0.1 ml of plasma was diluted up to 1.0 ml by adding 9.9 ml deionized water. The levels of both the electrolytes were estimated by digital flame photometer (Elico Model CL 22 D, Hyderabad). Values obtained for the control and experimental groups were evaluated for statistical significance using Students 't' test.

Both the adrenal 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 haematoxylin-eosin (H&E). For electron microscopic observations, the tissues were fixed in 3% glutaraldehyde maintained at 4°C. They were later transferred to Millaniq's buffer in which 3-4 changes were given. The small pieces of gland were treated with 1:1 mixture of osmium tetraoxide and Millaniq's buffer. After dehydration through the various grades of alcohol, they were transferred to propylene

Table - 1: Effects of sublethal heroin adminstration on plasma sodium and potassium levels (meq l^{-1}) of *Rattus norvegicus*

Group	Duration			
	24 hr	7 days	15 days	30 days
		Plasma sodium level		
Control	156.57 ± 2.30	155.89 ± 2.51	153.14 ± 2.88	157.23 ± 2.16
Heroin	149.64 ± 2.76*	141.38 ± 3.49**	135.47 ± 3.91**	126.53 ± 2.68**
	(-4)	(-9)	(-12)	(-20)
		Plasma potassium level		
Control	5.04 ± 0.92	5.19 ± 0.32	5.63 ± 0.81	5.12 ± 0.63
Heroin	5.72 ± 0.24	6.88 ± 0.67*	7.94 ± 0.31**	8.78 ± 0.46**
	(+14)	(+33)	(+41)	(+72)

Values are mean ± SD of 5 specimens. Values in parentheses indicate % increase (+) or decrease (-) over control. Significant response : *p<0.01; ** p<0.001

oxide for complete dehydration. For preparation of blocks, the tissues were embedded in plastic capsule KDB filled with araldite solution B. They were kept at 60°C for 48 hr for polymerization and hardening. The blocks were removed from the capsule and trimmed with a surgical blade under stereomicroscope. Semithin sections (1 μm) were cut using ultramicrotome, spread on glass slides and fixed by gentle heating. The sections were stained with toludine blue and examined under the light microscope. Ultrathin sections (600-800 A°) were cut from the selected area with glass knife and mounted on 400 mesh copper grides. The tissues were double stained with 10% alcoholic uranyl acetate for 20 minutes and Reynold's lead citrate for 10 minutes. Sections were scanned under electron microscope (Phillips 410).

Results and Discussion

Effects of heroin adminstration on plasma sodium and potassium levels of *Rattus norvegicus* have been summarized in Table 1. Plasma sodium level of control rats fluctuated between 153.14±2.88 and 157.23±2.16 meq l^{-1} while plasma potassium level varied between 5.04± 0.92 and 5.63± 0.81 meq l^{-1}. Plasma sodium level of the treated rats registered a significant decline (p<0.01) at 24 hr which progressed on day 7 and 15 and the minimum value (126.53±2.68 meq l^{-1}) was recorded on day 30 whereas plasma potassium level registered a progressive increase during the entire period with a peak (8.78±0.23 meq l^{-1}) on day 30 of the experiment. Though mild (but insignificant) hyponatremia was recorded, plasma potassium level was dangerously high (hyperkalemia) in a patient with heroin abuse (Pearce and Cox, 1980).

The adrenal glands of *Rattus norvegicus* are paired encapsulated structures lying in anterior region of each kidney. The gland is made up of two separate secretory tissues - the adrenal medulla, comprising chromaffin cells, is located in the centre while the surrounding adrenal cortex produces steroid hormones. It has been reported that the chromaffin cells initially arise from the neighbouring paraganglion cells of the neural crest complex and migrate to lie adjacent to the adrenocortical cell groups which are mesodermal in origin (Chester Jones, 1976; Balment *et al*., 1980; Chester Jones and Phillips, 1986). Like other mammals (Long, 1975; Idelman, 1978; Chester Jones and Phillips, 1986), the adrenal cortex of *Rattus norvegicus* is divided in three zones - zona glomerulosa, zona fasciculata and zona reticularis (Fig. 1). The glomerulosa is a thin zone consisted of 4-6 layers of ovoid or columnar cells immediately inside the outer connective tissue capsule. The nuclei vary in shape from sausage to oval with one or two nucleoli and cytoplasm contained large number of lipid droplets (liposomes) lying between nucleus and side of the cell which abuts the capillary (Fig. 3). Mitochondria are rod or thread-like, smaller in size and their number is quite large. Branching and anastomosing tubules of smooth-surfaced endoplasmic reticula are also seen. The Golgi complex is well developed lying adjacent to nucleus but has no articular orientation. Similar structures of the zona glomerulosa cells have been recorded in other mammals (Lentz, 1971; Chester Jones and Henderson, 1980; Chester Jones and Phillips, 1986). These cells are responsible for secretion of aldosterone, the mineralocorticoids which are involved with regulation of serum electrolytes (Chester Jones and Henderson, 1980).

The zona fasciculata is the widest zone of adrenal cortex. It comprises radial cords of polyhedral cells which are considerably larger, have round central nucleus with dense chromatin towards periphery, one or two nucleoli and cytoplasm packed with lipid droplets (Fig. 4, 5). Though branching and anastomosing tubules of smooth-surfaced endoplasmic reticula are extensive, the rough-surfaced endoplasmic reticulum is more abundant in this zone as compared to the cells of zona glomerulosa. The mitochondria are large, generally oval to spherical and Golgi apparatus well developed (Fig. 4). Lysosomes and lipofusin pigments are also encountered. The zona fasciculata cells secrete glucocorticoids (cortisol and corticosterone) which are involved in protein and carbohydrate metabolism (Chester Jones and Henderson, 1980).

The innermost zona reticularis, bordering the adrenal medulla of the rat, consists of anastomosing networks of reticular cell cords. The cells are smaller than those of the rest of the cortex with less lipid content. They possess smooth-surfaced endoplasmic reticula, stacks or whorls of rough-surfaced

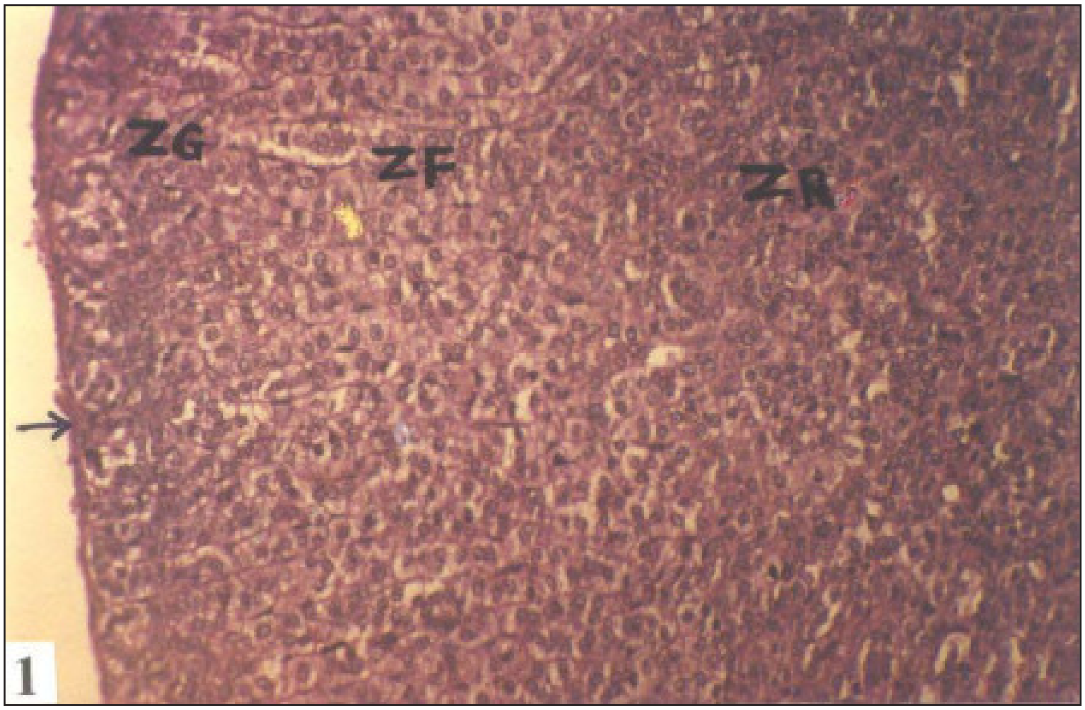


Fig. 1: Adrenal cortex of control *Rattus norvegicus* showing outer connective tissue capsule (arrow), zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). H&E x 100

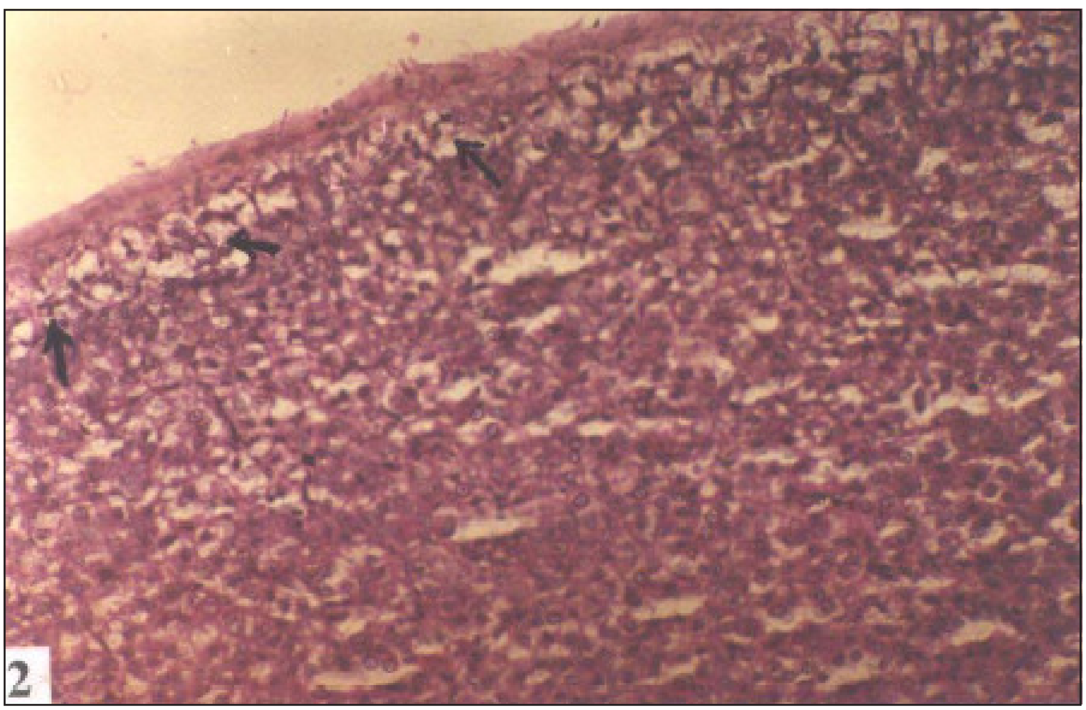


Fig. 2: Adrenal cortex of *Rattus norvegicus* on day 30 of heroin administration depicting degenerative changes in cells of all the three zones. Excessive degeneration in zona glomerulosa cells (arrow). H&E x 100

lamellae, cytoplasmic ribosomes, Golgi apparatus, lipid droplets and lysosomes. However, mitochondria are more elongated and lipofusin pigment granules are numerous in the cytoplasm (Fig. 6). Similar ultrastructures of the zona reticularis cells have also been observed in other mammalian species (Lentz, 1971; Chester Jones and Henderson, 1980) which are responsible for the secretion of sex steroids, particularly androgens (Chester Jones and Phillips, 1986).

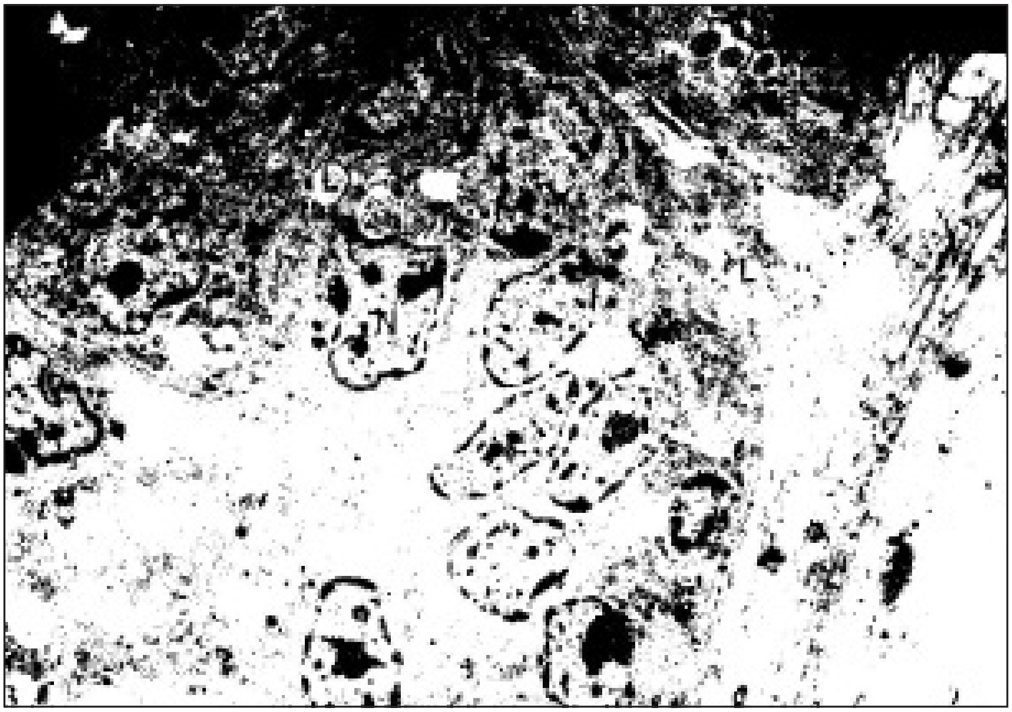
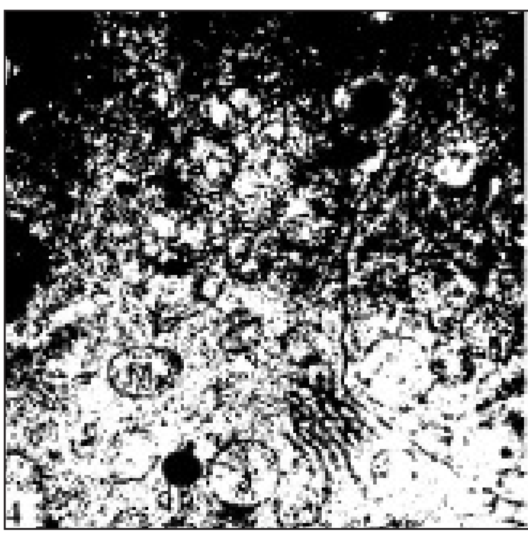


Fig. 3. Zona glomerulosa cells of control rat exhibiting abundant nuclei (N) and numerous lipid droplets (L). x 2,000



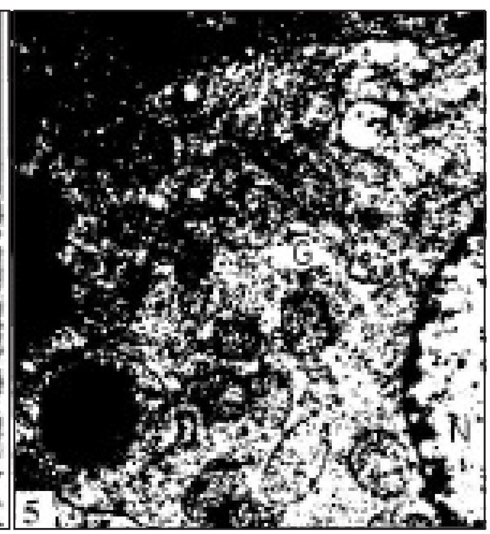
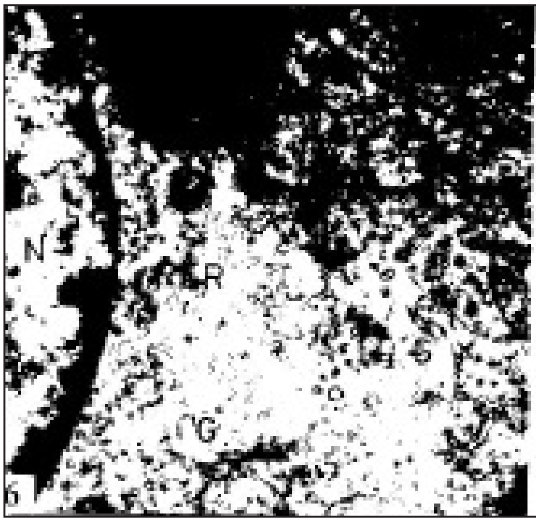


Fig. 4: Zona fasciculata cell of control rat showing mitochordria (M), rough endoplasmic reticulum (RER) and dense body (db). x 10, 000
Fig. 5: Zona fasciculata cell of control *Rattus norvegicus* depicting part of nucleus (N), numerous mitochodria (M), Golgi complex (G) and lipid droplets (L). x 10,000

Though sublethal herion adminstration for 30 days elcited varying degrees of vacuolization in the endocrine cells of all the three zones, cells of zona glomerulosa exhibited severe degenerative changes in cytoplasm and nuclei as compared to those of zona fasciculata and zona reticularis (Fig. 2). Ultrastructurally, degenerative changes were observed in various cell organelles. Mitochondira lost the typical cristae and hormone granules were rarely seen (Fig. 7). Though rough endoplasmic reticula were scanty, degenerative changes were also observed in these structures as well as in Golgi complex. Many lipid granules and vacuolization were also observed in zona glomerulosa as well as zona fasciculata cells of the treated rat (Fig. 8).

Several endocrine dysfunctions such as abnormal adrenal metabolism and insufficiency, abnormalities in circadian rhythm of corticoid secretion, increased levels of thyroxine (T_4) and triiodothyronine (T_3), thyroxine-binding globulin (TBG) and reduced T_3 level, abnormalities in insulin-glucose metabolism, reduction in testosterone level and abnormal FSH and LH levels have been recorded in the patients with chronic opiate misuse (Glass *et al.*, 1973; Pechnick, 1993; Spanagel, 1999; Sarnyai *et al.*, 2001; George



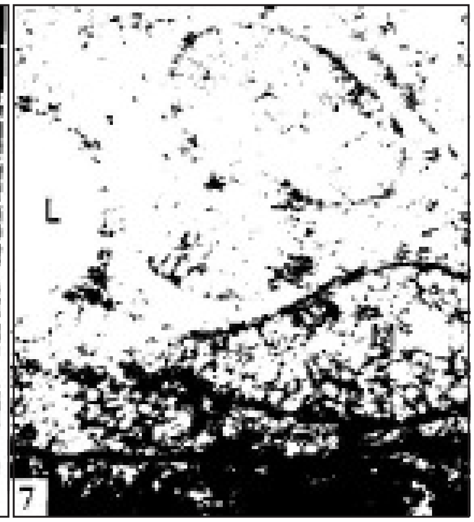


Fig. 6. Zona reticularis cell of control rat exhibiting part of nucleus (N), lipid droplet (L), rough endoplasmic reticulum (RER) and Golgi complex (G). x 15,000
Fig. 7. Zona glomerulosa cell of *Rattus norvegicus* treated with heroin showing many large mitochondria (M) with damaged cristae, desmosome (D) and lipid droplet (L). x 25,000

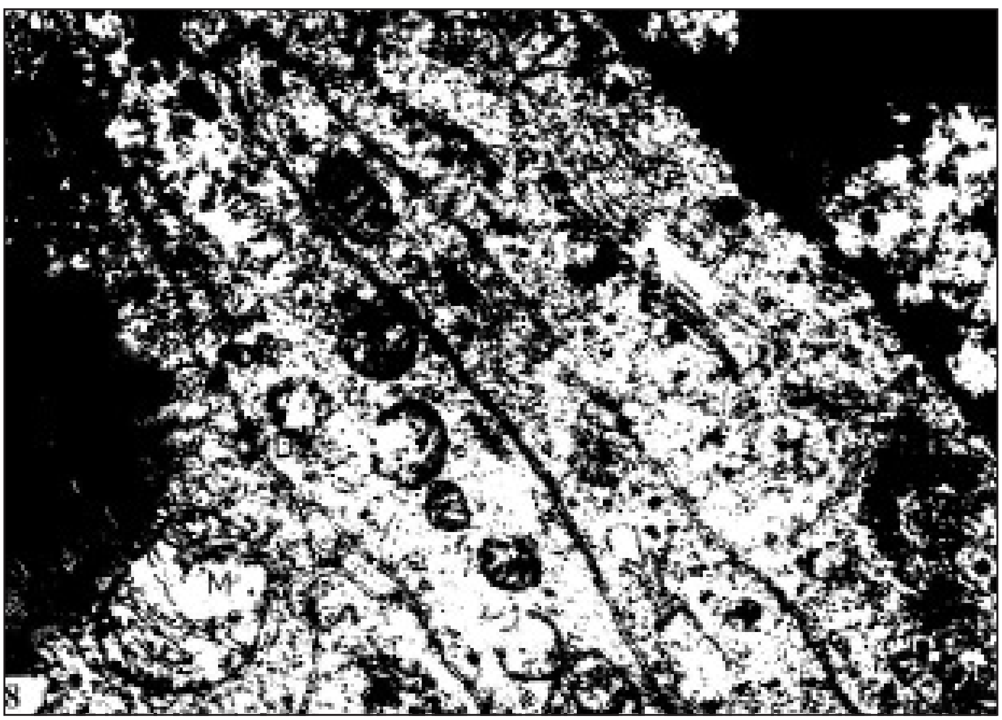


Fig. 8. Zona glomerulosa cell of *Rattus norvegicus* treated with heroin showing many large mitochondria (M) with damaged cristae, desmosome (D) and lipid droplet (L). Mark the degenerative changes in Golgi complex (G). x 25,000

et al., 2005). There exist reports that opioids affect the adrenal cortex function by involving hypothalamus-pituitary-adrenocortical (HPA) axis (Pechnick, 1993; Spanagel, 1999; Sarnyai *et al*., 2001; Laorden *et al*., 2002; George *et al*., 2005).

Though sublethal heroin administration for 30 days elicited cytoplasmic vacuolization in cells of all the three zones of adrenal cortex of *Rattus norvegicus,* degenerative changes were more marked in zona glomerulosa cells. Our study demonstrates that sublethal heroin administration induces alterations in plasma Na and K metabolism by affecting the zona glomerulosa cells of adrenal cortex.

Acknowledgments

One of us (SRB) is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi for the award of Fellowship to carry out this work. We are obliged to Hon'ble Justice Mrs. K.K. Baam, the then High Court Judge, Bombay for permitting us to work on heroin and to Mr. Rahul Rai Sur, the then Deputy Commissioner of Police, Narcotics Cell, Greater Mumbai for the procurement of the drug. We are thankful to Mr. Vijay Kate and Mr. Dilip Kanaskar of the Electron Microscopy Division, Jaslok Hospital, Mumbai for their assistance in this study.

References

Balment, R.J., I.W. Henderson and I. Chester Jones: The adrenal cortex and its homologues in vertebrates : Evolutionary considerations. *In*: General endocrinology and clinical endocrinology of the adrenal cortex. Vol. 3 (*Eds.:* I. Chester Jones and I.W. Henderson). Academic Press, New York. pp. 525-562 (1980).

Barai, S.R., S.A. Suryawanshi and A.K. Pandey: Effect of sublethal heroin administration on liver and kidney of *Rattus norvegicus. J. Natcon.,* **16**, 149-155 (2004a).

Barai, S.R., S.A. Suryawanshi and A.K. Pandey: Effect of sublethal heroin administration on acid phosphatase and alkanine phosphatase activities of *Rattus norvegicus. J. Exp. Zool. India,* **7**, 329-332 (2004b).

Barai, S.R., S.A. Suryawanshi and A.K. Pandey: Response of thyroid gland to sublethal heroin administration in *Rattus norvegicus. J. Appl. Zool. Res.*, **15**, 195-197 (2004c).

Brambilla, F., P. Nobile, A. Zanoboni, W. Muciaccia and P.L. Meroni: Effects of chronic heroin addiction on pituitary-thyroid function in man. *J. Endocrinol. Invest.*, **3**, 251-255 (1980).

Chester Jones, I.: Evolutionary aspects of the adrenal cortex and its homologues. The Dale Lecture. *J. Endocrinol.*, **71**, 1-31 (1976).

Chester Jones, I. and I.W. Henderson: General Endocrinology and Clinical Endocrinology of the Adrenal Cortex. Vol. 3. Academic Press, New York (1980).

Chester Jones, I. and J.G. Phillips: The adrenal and interrenal glands. *In:* Vertebrate endocrinology: Fundamental and biomedical implications. Vol. 1. Morphological considerations (*Eds.:* P.K.T. Pang and M.P. Schreibman). Academic Press, San Diego. pp. 319-50 (1986).

El Daly, E.S.: Effect of morphine and stadol on lipid content in liver of rat. *Life Sci.*, **55**, 1419-1426 (1994).

George, S., V. Murali and R. Pullickal: Review of neuroendocrine correlates of chronic opiate misuse : Dysfunctions and pathophysiological mechanisms. *Addict. Disord. Their Treat.*, **4**, 99-109 (2005).

Glass, L., B.K. Rajegowda, T.K. Mukherjee, M.M. Roth and H.C. Evans: Effect of heroin on corticosteroid production of pregnant addicts and their fetuses. *Am. J. Obstet. Gynecol.*, **117**, 416-418 (1973).

Goldberger, B.A., E.J. Cone, T.M. Grant, Y.H. Caplan, B.S. Levine and J.E. Smialek: Disposition of heroin and its metabolites in heroin-related deaths. *J. Anal. Toxicol.*, **18**, 22-28 (1994).

Gomez-Lechon, M.J., X. Ponsoda, R. Jover, R. Pabra, R. Trullenque and J.V. Castell: Hepatotoxicity of the opioids morphine, heroin, mepridine and methadone to cultured human hepatocytes. *Mol. Toxicol.*, **1**, 453-463 (1987).

Hussain. K. and A. Kumar: Physiological, haematological, biochemical and clinical effects of epidural morphine in dogs. *Ind. Vet. J.*, **65**, 491-495 (1988).

Idelman, S.: The structure of the mammalian adrenal cortex. *In*: General endocrinology and clinical endocrinology of the adrenal cortex. Vol. 2 (*Eds.:* I. Chester Jones and I.W. Henderson). Academic Press, New York. pp. 1-99 (1978).

Jenkines, A.J., R.M. Keenan, J.E. Henningfield and E.J. Cone: Pharmacokinetics and pharmacodynamics of smoked heroin. *J. Anal. Toxicol.*, **18**, 317-330 (1994).

Kringsholm, B. and P. Christoffersen: Morphological findings in fatal drug addiction. An investigation of injection marks, endocrine organs and kidneys. *Forensic Sci. Int.*, **40**, 15-24 (1989).

Kringsholm, B., E. Kaa, A. Steentoft, K. Worm and K.W. Simonsen: Deaths among drug addicts in Denmark in 1987-1991. *Forensic Sci. Int.*, **67**, 185-195 (1994).

Laorden, M.L., M.T. Castells and M.V. Milanes: Effect of morphine and morphine withdrawal on brainstem neurons innervating hypothalamic nuclei that controls the pituitary-adrenocortical axis in rat. *Br. J. Pharmacol.*, **136**, 67-75 (2002).

Lentz, T.L.: Cell Fine Structure. W.B. Saunders, Philadelphia, Pennsylvania (1971).

Long, J. A.: Zonation of the mammalian adrenal cortex. *In*: Handbook of physiology. Vol. VI (*Eds.:* H. Blaschko, G. Sayers and A.D. Smith). American Physiological Society, Washington. pp. 13-24 (1975).

Neri-Serneri, G.G. and P.A. Modesti: Medical complications connected with the use of drugs. *Ann. Ital. Med. Int.*, **6**, 313-324 (1991).

Pearce, C.J. and J.G.C. Cox: Heroin and hyperkalemia. *Lancet*, **2**, 923 (1980).

Pechnick, R.N.: Effects of opioids on the hypothalamo-pituitary-adrenal axis. *Ann. Rev. Pharmacol. Toxicol.*, **33**, 353-882 (1993).

Sagliaro, F., F. Capra, R. Dorizzi, G. Luisetto, A. Accordini, E. Renda and A. Parolin: High serum calcitonin levels in heroin addicts. *J. Endocrinol. Invest.,* **7**, 331-333 (1984).

Sarnyai, Z., Y. Shahan and S.C. Heinrichs: The role of corticotropin-releasing factor in drug addiction. *Pharmacol. Rev.*, **53**, 209-244 (2001).

Spanagel, R.: The hypothalamic-pituitary-adrenocortical system : A biological substrate of vulnerability to drug addiction. *Basic Clin. Sci. Subst. Rel. Disord.*, **168**, 1-6 (1999).

Weller, I.V., D. Cohen, A. Sierralta, M. Mitcheson, M.G. Ross, L. Mantano, P. Scheuer and H.C. Thomas: Clinical, biochemical, serological, histological and ultrastructural features of liver disease in drug abusers. *Gut*, **25**, 417-423 (1984).

Wootton, I.D.P.: Microanalysis in Medical Biochemistry. 5th Edn. Churchill Livingston, Edinburgh and London (1974).