

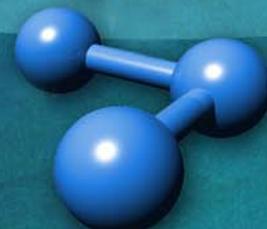
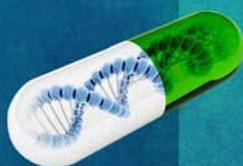
ISSN 2278 - 5221

Vol. 1, No. 2, October 2012



# International Journal of Pharma Medicine and Biological Sciences

IJPMBS



WWW.IJPMBS.COM

editorijpmbs@gmail.com or editor@ijpmbs.com



Research Paper

## LONGITUDINAL STUDY ON THE EFFECT OF RESTRAINT STRESS DURING PREWEANING PERIOD ON POSTNATAL GROWTH OF BODY AND CONSTITUENT PARTS IN ALBINO MICE

Prakash Billakanti Babu<sup>1\*</sup>, Ramachandra Bhat K<sup>1</sup> and Muddanna S Rao<sup>1</sup>

\*Corresponding Author: **Prakash Billakanti Babu**, ✉ [billakantibabu@yahoo.co.in](mailto:billakantibabu@yahoo.co.in)

Stress is the phenomenal; any stimulus which displaces the state of normal physiological function can cause stress and has its effect in various forms. Somatic growth and development are also affected by stress affecting growth releasing hormone. This study was conducted to observe growth pattern in normal and stressed condition in growing mice. Experiments were conducted to investigate the effect of restraint stress applied at different times of the light-dark cycle on growth rates (body and tail length and body weight of rats). BALB/ C strain of Albino rats were restrained for 6 hours daily from birth to end of three weeks (21 days) 6 hourly from birth till postnatal day 21 (21 days stress) and from postnatal day 16 to 21 day (5 day stress) in a restraining device, which consisted of a wooden platform to which a wire-mesh was attached. Body weight (BW), tail length (TL) and body length (BL) was recorded at the beginning and at the end of stress both in control and experimental groups. Recorded parameters were subjected to statistical analysis between the groups. Results of the study revealed that stress affects body weight significantly and longitudinal growth of body and tail lengths of albino mice.

**Keywords:** Albino mice, Restraint stress, Body measurements

### INTRODUCTION

Stress is a highly individualized response of an organism to external or internal challenges which individual cannot control or can control with difficulty. Any stimulus that displaces the state of normal physiological function can cause 'stress'. Brief early life experiences, pre or postnatal, can cause significant changes in the stress response

system and emotionality that persist into adulthood (e.g., susceptibility to disease, Barker, 1996; depression, Phillips, 2002; anxiety-type behavior, Heim and Nemeroff, 2001; hyper-responsiveness to stressors, Ladd *et al.*, 2000). The current interest in early life experience stems from the observation that specific early events can apparently programme the 'set-point'

<sup>1</sup> Anatomy Department, Kasturba Medical College, Manipal University, Manipal, Karnataka-576104, India.

of the hypothalamic–pituitary–adrenal (HPA) axis by altering the effective feedback. Ganong (1995) defines “stress” as those stimuli which increases ACTH is an intricate chain which begins with the inputs at the higher centre, that triggers the hypothalamus. In response to stress full stimuli hypothalamus releases corticotrophin releasing hormone (CRH) to stimulate the pituitary, which in turn releases ACTH. Thus, adrenal cortex is stimulated for release of cortisol and androgen precursors. ACTH and cortisol are secreted in episodic manner in response to stress.

Observations at birth and thereafter, day by day progress in growth of body, head and tail in the postnatal life of man and animals are amazing experience. Enormous studies on birth and growth phenomenon in the postnatal life of animals have been compiled over the years (Aremario *et al.*, 1984; Alario *et al.*, 1987; Avishai-Ephner *et al.* 1995; Bruno *et al.*, 1990). However, most of the investigators followed the traditional cross sectional method of study in which groups of animals generally mice or rats were sacrificed periodically (Tanner, 1951; and Tanner, 1962). Different strains of laboratory rats have been used for various types of investigations (Asling and Frank, 1963; Harel, 1995).

Over the decades, many longitudinal studies were reported, e.g. Saxton and Silberg (1947), Hughes and Tanner (1970) and Williams *et al.* (1974). Any such studies were related to development and growth (Morgan and Naismith, 1982). Specifically related with postnatal development and growth, some researchers have shown proclivity to study certain skeletal elements (Groot, 1963; Harkness and Trotter, 1980). Growth and development manifest by increment in size of the different parts of the body like head length, body length and tail length (Hughes, 1970).

In the present study two parameters were considered for investigation, (i) growth of normal albino mice, (ii) growth of albino mice reared under stress. As far for the stress is concerned restraint stress was inflicted.

Restraint stress has been proposed as an animal model of depression and anorexia nervosa, as many investigators have shown that stress suppresses food intake and body weight gain in rats (Ganong WF 1995, Kennett GA *et al.*, 1986). The stress-induced reduction in food intake has been demonstrated both as maintained decrease in 24-h food intake during and after repeated daily restraint stress, (Ganong, 1995; and Kratin *et al.*, 1990) and as an acute response in stress has ended, restraint rats fail to return to the body weight of control animals (Ganong 1995, Kennett *et al.*, 1986, Kratin *et al.*, 1990). In the rat, repeated separation from the mother for 3 h. has been shown to have potentially deleterious effects. Separated rat pups showed reduced hippocampal glucocorticoid receptors (GR), elevated basal plasma glucocorticoids (Plotsky and Meaney, 1993), and became hyper responsive to stressors during behavioral development (Ladd *et al.* 2000). There are reports that maternal separation of 3 hrs. can lead to adult rats having reduced cognitive performance (Ladd *et al.*, 2000). The mechanism underlying this hyper-responsiveness appears to be a reduction in the negative feedback to the HPA axis provided by the hippocampal brain region. Separated rat pups have a reduced density of glucocorticoid receptors in the hippocampus and consequently reduced capacity to inhibit the responsiveness of the HPA axis. In contrast, rat pups exposed to a positive post-natal experience (i.e., born to mothers that shows higher levels of maternal grooming) shows positive effects of this

experience. Pups born to more maternal mothers have increased hippocampal GR expression and reduced hypothalamic corticotropin-releasing hormone messenger ribo-nucleic acid (CRH mRNA) expression, suggesting that these pups had a less responsive HPA axis (Liu *et al* 1997). These pups also showed changes in the neural circuitry controlling fear behavior (Caldji *et al* 1998), suggesting that they should be less behaviorally and physiologically responsive to fear and stress-inducing stimuli. Other work where pups were removed from the mother for a short period (15 min) and then returned (a procedure now known to cause an increase in maternal grooming (Macri *et al.*, 2003), resulted in pups which were more easily handled, and showed reduced ACTH and glucocorticoid responses to an open-field test (Nunez *et al.*, 1996).

Since the parameters are precise but extensive, literature analyzed were substantial (Armario *et al.*, 1985a and b; Alario *et al.*, 1987; Hennessy *et al.*, 1989; Avishai-Ephner *et al.*, 1995). Thus the present work consists of growth of body length, tail length, day of eye opening of albino mice observed from birth to 3 weeks postnatal age and from postnatal age 16 to postnatal day 21, the study was conducted in normal health of growing albino rats and stress like restraint stress. To the best of our knowledge and endeavor we could not come across any single study which describes the effect of chronic restraint stress on albino rats.

## **MATERIALS AND METHODS**

### **Animals**

In the present study albino mice of BALB/C strain of both sexes were used. Control and

experimental of both sexes consisting of twelve albino mice in each group were formed randomly.

### **Environment**

The mice were maintained in well-ventilated room. Temperature ranged between  $27 \pm 3^\circ\text{C}$ . Doors and windows were closed during morning, evening and night hour to prevent them from colds. They were kept in natural source of light which was 12:12 hour L: D cycle. Size of the cages for housing the mice were 40 X 25 X 16cm. Cages contained paddy husk (Sterilized), which were changed on every 2nd day.

### **Diet Regimen**

The animals were fed with synthetic food pellets and tap water ad libitum. The food pellets contained mainly what and 22.05 crude proteins, 3.99% crude oil, 2.62% crude fibre, 1.34% sand silica and 7.81% ash. Drinking water was acidified with hydrochloric acid to give a pH of 2.0 -2.5 this was achieved by adding 2 ml of hydrochloric acid to 3 liters of tap water. The purpose of adding acid to water is to prevent massive bacterial proliferation in the water bottle.

### **Stress Regimen**

The new born pups ( $P_0$ ) were divided into two subgroups (a) Control (C) (b) Restraint stress (RS)

#### **Control Group**

New born pups in this group remained undisturbed with their mothers till the postnatal day 21.

#### **b) Restraint Stress Group (RS)**

Pups in this group were grouped into two sub groups

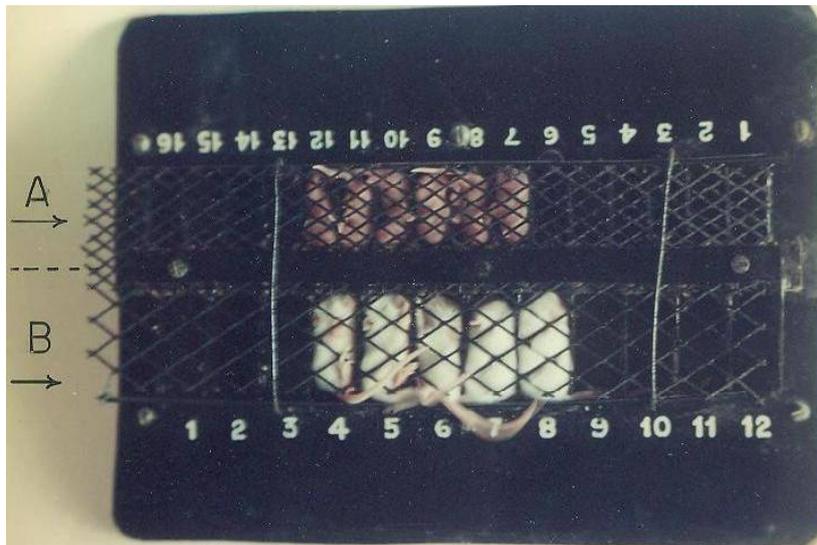
(i) 5 days stress group: Pups were stressed in a wire mesh restrainer, for 5 days (6h/day) from

$P_{16}$ - $P_{21}$  (postnatal day 16-21) in restrainer No-3 having dimensions 4.8 cm (L) x 2.2 cm (B) x 2.4 cm (H).

(ii) 21 days stress group: pups in this subgroup were stressed 6h/day for 21 days in the restrainer

No-1 ( $P_0$ - $P_7$ ) having dimensions (2.5cm (L) x 1.4cm (B) x 1.1cm (H), (Figure 1) restrainer No-2 ( $P_8$ - $P_{14}$ ) having dimensions (3.5 cm (L) X1.6 cm (B) x 1.2 cm (H) Figure 1 and restrainer No-3 ( $P_{15}$ - $P_{21}$ ) having dimensions (4.8 cm (L) x 2.2 cm (B) x 2.4 cm (H) (Figure 2).

**Figure 1: Restrainers With Mice A. Restrainer No. 1-Used to Stress the Mice Below 7 Days Of Age, B. Restrainer No. 2- Used to Stress the Mice Between 8 and 14 Days Of Age**



**Figure 2: Restrainers With Mice A. Restrainer No. 3-Used to Stress the Mice Between 15 and 30 Days of Age**



All the pups in the restraint stress group remained with their mother except when they were subjected to stress. Control mice pups remained unseparated from the mothers. The food and water were withdrawn from experimental group during stress presentation. After stress presentation mice were returned to their respective cages.

As the animals were allowed to grow and subjected to stress their growth was assessed including parameters like (i) Body weight gain, (ii) Tail length gain, (iii) Body length gain and (iv) Eye opening day. At the time of birth and at postnatal day all the growth parameters were observed and recorded.

## DATA COLLECTION

**Body Weight:** The body weights were measured using an electronic balance at the commencement of stress exposure and at the end of stress exposure. Body weight gain is defined as difference in pre-stress and post-stress body weights. In case of controls, body weights were measured at an age corresponding to beginning and end of stress exposure in stressed groups.

**Body Length:** The body length, i.e., from tip of snout to base of tail was measured at birth ( $P_0$ ) or at 16<sup>th</sup> postnatal day and at postnatal day 21 ( $P_{21}$ ) in the preweaning mice. The body length gain i.e., body length at  $P_{21}$  - body length at  $P_0/P_{16}$  was calculated for each mice.

**Tail length:** In growing preweaning mice tail length i.e., from base to tip, was measured using a measuring scale at the commencement and last day of stress exposure and also in corresponding age matched controls. Tail length gain i.e., tail length at  $P_{21}$  - tail length at  $P_0/P_{16}$  was calculated for each mice.

**Eye opening:** In the growing mice day of eye opening was taken as a growth parameter. Eye is said to be open when there appears visible slit between the upper and lower eyelids. Day of eye opening is noted in control and stressed mice.

**Data presentation:** The body weight gain in gram, tail length and body length in centimeter has been tabulated as mean and standard deviation. Unpaired 't' test was used for comparison of data between two groups and a 'p' value less than 0.05 was considered as significant.

## RESULTS

Results were made on the observations of growths of normal and stressed mice on body weight (BW), body length (BL) and tail length (TL).

### Growths of Mices Under Normal Condition

#### Body weight gain

The body weight gain was significantly decreased in 21 days restraint stressed (from birth to postnatal day 21) mice compared to controls. ( $6.32 \pm 1.74$  g in control vs  $4.14 \pm 0.68$  g in restraint stressed,  $P < 0.001$ ). There was no change in 5 days stress group (Table 1).

#### Tail length gain

The tail length also significantly decreased in 21 days restraint stressed mice compared to controls ( $4.45 \pm 0.40$  cm in control vs  $3.37 \pm 0.45$  cm in restraint stressed,  $P < 0.001$ ). There was no change in 5 days group (Table 2).

#### Growth in body length

Like body weight and tail length gain, body length also significantly decreased in 21 days restraint stressed mice compared to control ( $3.08 \pm 0.52$  cms in control vs  $2.62 \pm 0.36$  cms).

**Table 1: Body Weight (Mean ± SD, N = 12 rat/group) of Albino Mice in Control and Under Restraint Stress**

Groups	Body Weight Gain (gm)	
	Stress Duration	
	5 days	21 days
Control (C) (n)	6.32 ± 1.74 (12)	6.32 ± 1.74 (12)
Restraint Stress (RS)(n)	5.56 ± 0.59 (12)	4.14*** ± 0.68 (12)

Note:  $p < 0.001$  in comparison to control by students't' (two tailed), n=number of animals.

**Table 2: Tail Length (Mean± SD, N=12 rat/Group) in Control and Stress Mice**

Groups	Tail Length Gain (cm)	
	Stress Duration	
	5 days	21 days
Control (C) (n)	4.45 ± 0.40 (12)	4.45 ± 0.40 (12)
Restraint Stress (RS) (n)	4.40 ± 0.24 (12)	3.37*** ± 0.45 (12)

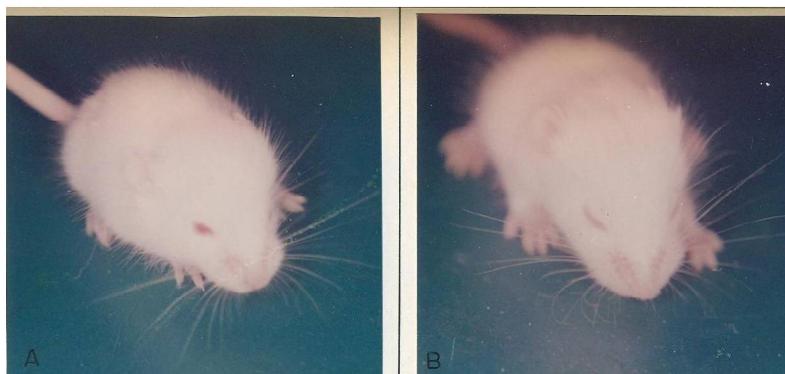
Note:  $p < 0.001$  in comparison to control by students't' (two tailed), n=number of animals.

Inrestraint stressed,  $P < 0.001$ ). There was no change in 5 days group (Table 3).

**iv. Eye opening Day**

Eye opening day was delayed in 21 days restraint stressed group but not in 5 days stressed group.

**Figure 3: photographs of mice.A.16 days old control mouse, B-16 days old mouse exposed to restraint stress from birth onwards. Note: Mouse in A opened its eyes on postnatal day13, whereas mouse in B, did not open its eyes even on postnatal day 16.**



**Table 3: Body Length (Mean  $\pm$  SD, N=12 rat/Group) in Control and Stress Mice**

Groups	Body Length Gain (cm)	
	Stress Duration	
	5 days	21 days
Control (C)(n)	3.08 $\pm$ 0.52 (12)	3.08 $\pm$ 0.52 (12)
Restraint Stress (RS)(n)	2.93 $\pm$ 0.46 (12)	2.62*** $\pm$ 0.36 (12)

Note:  $p < 0.001$  in comparison to control by students'  $t$ ' (two tailed), n=number of animals.

**Table 4: Prewaning age-Day of Eye Opening Each Value Represents Number of Pups Which Opened Their Eyes on that Particular Postnatal Day, N=Number of Animals**

Groups	n	5 days stress					
		12 <sup>th</sup> d	13 <sup>th</sup> d	14 <sup>th</sup> d	15 <sup>th</sup> d	16 <sup>th</sup> d	17 <sup>th</sup> d
Control	12	–	4	8	–	–	–
Restraint stress	12	–	6	6	–	–	–
Groups	n	21 days stress					
		12 <sup>th</sup> d	13 <sup>th</sup> d	14 <sup>th</sup> d	15 <sup>th</sup> d	16 <sup>th</sup> d	17 <sup>th</sup> d
Control	12	–	4	8	–	–	–
Restraint stress	12	–	–	1	6	2	3

Control mice opened their eyes on 13(n=4) or 14<sup>th</sup> (n=8) postnatal day (Figure 3) whereas mice restraint stressed from birth onwards opened their eyes between 15-17 postnatal day. (Figure 3) (Postnatal day 15: n=5, Postnatal day 16; n=5 and postnatal day 17=2) (Table 4).

## DISCUSSION

In life of an animal and man development and growth are almost symphonetic. Studies on rats often begin from before birth, i.e., prenatal development and followed to be observed in the postnatal life. altogether it makes voluminous literature and at the same time makes it beyond comprehension of the researcher. Therefore this paper was limited to postnatal period of albino

rats life from day zero to day 21 (3 weeks). It was well recognized for many years that the size of the litter is an important determinant of overall growth of the rats (Gates, 1925). Hughes and Tanner (1970) reported that somatic growth of the laboratory rat is sensitive to litter size. It is directly linked to demand and supply of milk. The more pups in the litter, the lesser milk available for the individual pups, resulting in slower growth rate. In the following year, Park and Nowsieiski (1971) emphasized the importance of genetic pattern and maternal environmental factors. Quality and quantity of food provided to the laboratory animal at any stage has important significance for growth of the animal (Moss, 1954;

Morgan, 1982; Lewis, 1989). In our earlier studies, Mukerjee (1987) and Shaligram (1998) we maintained only five pups kept with a healthy dame. It was common to observe that late in third week postnatal the young rat pups started to nibble the solid food provided to the dame; this can be taken as additional nutrient supplement for the young rat pups. Generally the rats are omnivorous, they prefer fresh food. They instinctively store excess food by covering them under the paddy husk provided on the floor of the cage. However, unless starvation is threatened, rats avoid stale food and water (Lane-Petter, 1976). Chronic exposure to stressors of certain severity cause anorexia and reduce body weight (Marti *et al.* 1994). In this study it has been observed that chronic stresses like maternal deprivation, restraint stress and electrical foot shock reduce food intake, increase water consumption and cause reduced physical activity by causing longer period of sleep. Rats in the stress group develop fear which is noticed by mere handling before stress presentation. Handling causes more urination and excrement.

In the present study there was a reduction in body weight gain, tail length gain and body length gain in mice undergone 21 days stress. Eye opening was also delayed in the above age group. Body weight gain was reduced in mice subjected to 21 days restraint stress. These results suggest growth retardation due to stress. Similar results were reported by Pullen (1977); Drago *et al.*, (1999); Smagin *et al.*, (1999) NagarajaHSand Jegannathan (1999) and Santos *et al.*, (2000). Retarded body growth may be due to (i) decreased food intake (ii) movement of corticotropin releasing factor (CRF) in the hypothalamic region (Delbende *et al.*, 1992). CRF receptors in the hypothalamus mediate acute

response to stress that can lead to permanent changes in hormonal or metabolic processes that determine the body weight and composition (Smagin *et al.*, 1999). Pathways projecting from limbic areas to hypothalamus could stimulate CRF secretion into pituitary-adrenal axis. Increased CRF could mediate this stress-induced suppression of food intake thereby reducing body weight (Delbende *et al.*, 1992) and also may reduce other growth parameters. It was reported that growth hormone gene expression in the brain, significantly suppressed by exposure to restraint stress (Yashizato *et al.*, 1998) which may also cause growth retardation. Circulating growth hormone level is shown to be decreased in animals exposed to stress during neonatal period (Kuhn *et al.*, 1978).

Tail length of the rat which at birth is shorter than the body length, increases rapidly due to addition of the newer vertebrae and the intervertebral discs by formation of sclerotomic and notochordal elements. In absence of substantial literature of the development of the vertebrae of albino rat knowledge of the development of vertebrae and the intervertebral discs in humans (Muller and O'Rahilly 1986; and O'Rahilly *et al.*, 1990) would explain the sequence of growth in the linear increment of the body size of Wistar rats.

## CONCLUSION

In the preweaning group 21 days stress reduced body weight gain, body length gain and tail length gain were reduced in mice to a greater extent than in 5 days stress. Eye opening day was also delayed in the above age group. These results suggest growth retardation due to stress, which may be due to suppression of growth hormone gene expression by stress, at a very early stage of development as reported in the literature.

## REFERENCES

1. Barker D J P (1996), "The Fetal Origins of Hypertension", *J Hypertens*, Vol.14, pp. S117-S120.
2. Phillips D I W (2002), "Endocrine Programming and Fetal Origins of Adult Disease", *Trends Endocrinol. Metabol*, Vol. 13, pp. 363-366.
3. Heim C, Nemeroff C B (2001), "The Role of Childhood Trauma in the Neurobiology of the Mood and Anxiety Disorders: Preclinical and Clinical Studies", *Soc. Biol. Psychiatry*, Vol. 49, pp. 1023-1039.
4. Ladd C O, Huot R L, Thirivikraman K V, Nemeroff C B, Meaney M J and Plotsky PM (2000), "Long-Term Behavioral and Neuroendocrine Adaptations to Adverse Early Experience", *Prog. Brain Res.*, Vol. 122, pp. 81-103.
5. Ganong W F (1995), *Review of Medical Physiology*, 17<sup>th</sup> Edition, *Prentice-Hall International Inc*, pp. 327-351.
6. Amario A, Ortiz R and Balash J (1984), "Effect of Crowding on Some Physiological and Behavioral Variables in Adult Male Rats", *Physiol. Behav.*, Vol. 32, pp. 35-37.
7. Alario P, Gamello A, Beato M J and Trancho G (1987), "Body Weight Gain, Food Intake and Adrenal Development in Chronic Noise Stressed Rats", *Physiol. Behav.*, Vol. 40, No. 1, pp. 29-32.
8. Avishal-Ephner S, Yi SJ, Newth CT, Baram T Z (1995), Effect of Maternal and Sibling Deprivation on Basal and Stress Induced Hypothalamic-Pituitary-Adrenal Components in the Infant Rat", *Neurosci Lett.*, Vol. 92, No. 1, pp. 49-52.
9. Bruno J F, Olchovsky D and White J D (1990), "Influence of Food Deprivation in Rat on Hypothalamic Expression of Growth Hormone Releasing Factor and Somatostatin", *Endocrinology*, Vol. 127, pp. 2111-2116.
10. Tanner J M and Whitehouse R H (1955), "A Caliper for Measuring X-Rays, Photographs and Drawings", *Nature*, Vol. 176, p. 180, London.
11. Saxton J A and Silberberg M (1947), "Skeletal Growth and Ageing in Rats Receiving Complete or Restricted Diets", *Am. J. Anat.*, Vol. 81, pp. 445-475.
12. Asling C W and Frank H R (1963), "Roentgen Cephalometric Studies in Skull Development in Rats I Normal and Hypophysectomized Females", *Am. J. Phys. Anthropol.*, Vol. 21, pp. 527-544.
13. Hare I Z and Tannenbaum G S (1995), "Long Term Alterations in Growth Hormone and Insulin Secretion After Temporary Dietary Protein Restriction in Early Life in the Rat", *Pediatr. Res.*, Vol. 38, pp.747-753.
14. Hughes P C R and Tanner J M (1970), "The Assessment of Skeletal Maturity in the Growing Rat", *J. Anat.*, Vol. 106, pp. 371-402.
15. Williams J P G, Tanner J M and Hughes P C R (1974), "Catchup Growth in Male Rats After Growth Retardation During the Suckling Period", *Pediatr. Res.*, Vol. 8, pp. 149-156.
16. Morgan B L G and Naismith D J (1982), "The Effect of Early Postnatal Under Nutrition of the Growth and Development of the Rat Brain", *Br. J. Nutr.*, Vol. 48, pp.15-23.

17. Groot CA de (1963), "Tail Growth In Thyroxin Treated Hypopysectomized Rats as a Sensitive Criterion for Growth Hormone Activity", *Actaendocr. Copenh.*, Vol. 42, pp. 423-431.
18. Harkness E M and Trotter W D (1980), "Growth Spurt in Rat Cranial Bases Transplanted in Adult Hosts", *J. Anat.*, Vol. 131, No. 1, pp. 39-56.
19. Kennett G A, Chauloff F, Marcou M and Curzon G (1986), "Female Rats are More Vulnerable than Males in an Animal Model of Depression: The Possible Role of Serotonin", *Brain Res.*, Vol. 382, pp. 416-421.
20. Kratin D D, Gosnell B A and Majchrzak M J (1990), "The Anorexic Effect of CRH and Restraint Stress Decrease with Repeated Exposures", *Biol. Psychiatry*, Vol. 27, pp. 1094-1102.
21. Plotsky P M and Meaney M J (1993), "Early Postnatal Experience Alters Hypothalamic Corticotropin-releasing Factor (CRF) Messenger-RNA, Median-eminence CRF Content and Stress-Induced Release in Adult-Rats", *Mol. Brain Res.*, Vol. 18, pp. 195-200.
23. Ladd C O, Huot R L, Thivikraman K V, Nemeroff C B, Meaney M J, Plotsky P M (2000), "Long-Term Behavioral and Neuroendocrine Adaptations to Adverse Early Experience", *Prog. Brain Res.*, Vol. 122, pp. 81-103.
24. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky P M and Meaney M J (1997), "Maternal Care, Hippocampal Glucocorticoids Receptors and hypothalamic-Pituitary-Adrenal Responses to Stress", *Science*, Vol. 277, pp. 1659-1662.
25. Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky P M and Meaney M J (1998), "Maternal Care During Infancy Regulates the Development of Neural Systems Mediating the Expression of Fearfulness in the Rat", *Proc. Natl. Acad. Sci. USA*, Vol. 95, pp. 5335-5340.
26. Macri S, Mason G P and Wurbel H (2003), "Effects of Postnatal Maternal Separations on Maternal Care and HPA-Responses to Stress in Rats", in Ferrante V (Ed.), *Proceedings of the 37<sup>th</sup> International Congress of the ISAE, Fondazione Iniziative Zooprofilattiche e Zootecniche, Italy*, p. 87.
27. Nunez J F, Ferre P, Escorihuela R M, Tobena A and Fernandez Teruel A (1996), "Effects of Postnatal Handling of Rats One Motional, HPA-axis, and Prolactin Reactivity to Novelty and Conflict", *Physiol. Behav.*, Vol. 60, pp. 1355-1359.
28. Kuhn C M, Butler Sr and Schanberg S M (1978), "Selective Depression of Serum Growth Hormone During Maternal Deprivation in Rats Pups", *Science*, Vol. 20, pp. 1034-1036.
29. Armario A, Castellanos J M and Balasch J (1985a), "Chronic Noise Stress and Insulin Secretion Inmale Rats", *Physiol Behav.*, Vol. 34, pp. 359-361.
30. Armario A, Restrepo C, Castellanos J M and Balasch J (1985b), "Dissociation Between Adrenocorticotropin and corticosterone Response to Restraint After Previous Chronic Exposure To Stress", *Life Sci.*, Vol. 36, No. 22, pp. 2085-2092.

31. Hennessy M B, Tamborski A, Schimi P and Lucot J (1989), "The Influence of Maternal Separation on Plasma Concentration of ACTH, Epinephrine and Norepinephrine in Guinea Pig Pups", *Physiol. Behav.*, Vol. 45, No. 6, pp. 1147-1152.
32. Gates W H (1925), "Littersize, Birth Weight and Early Growth Rate of Mice (*Mus musculus*)", *Anat. Rec.*, Vol. 29, pp. 183-193.
33. Mukerjee B (1987), "Effect of Stress on Hypophysigonadal Axis of Rat", Ph.D Thesis, Pondicherry University.
34. Shaligram Dhungel (1998), "Morphological Study of Epididymis in Growing Rat Subjected to Stress", M S (Anatomy) Dissertation, Pondicherry University.
35. Lane-Petter W (1976), "The Laboratory Rat", in *The UFAW Hand Book on the Care and Management of Laboratory Animal*, 5<sup>th</sup> Edition, Churchill Livingstone, pp.204-211, London: .
36. Marti O, Marti J and Armario A (1994), "Effect of Chronic Stress on Food Intake in Rats, Influence of Stressorintensity and Duration of Daily Exposure", *Physiol. Behav.*, Vol. 55 No. II, pp. 747-54.
37. Mullar F and O'Rahilly R (1994), "Occipitocervical Segmentation in Staged Hhuman Embryos", *J. Anat.*, Vol. 185, pp. 251-258.
38. O'Rahilly R, Muller F and Meyer D B (1990), "The Human Vertebral Column At The End of the Embryonic Period Proper. 4. Sacrococcygeal Region", *J. Anat.*, Vol.168, pp. 95-111.
39. Pullen A H (1976), "A Parametric Analysis of the Growing CFHB (Wistar) Rat", *Journal of Anatomy*, Vol. 121, No. 2, pp. 371-383.
40. Drago F, Di Leo F and Giardina L (1999), "Prenatal Stress Induces Body Weight Deficit and Behavioral Alterations in Rats: The Effect of Diazepam", *Eu. Neuro. Psycho. Pharmacol.*, Vol. 9, No. 3, pp. 239-245.
41. Smagin G N, Howell L A Redmann S Jr., Ryan D H and Harris R B (1999), "Prevention of Stress Induced Weight Loss by Third Ventricle CRF Receptor Antagonist", *Am. J. Physiol.*, Vol. 276 (5Ptz), pp. R1461-1468.
43. Nagaraja H S and Jegannathan P S (1999), "Effect of Short and Long Term Restraint Stress on Some Physiological and Biochemical Parameters in Rats", *Indian J. Med. Res.*, Vol. 109, pp. 76-80.
44. Santos J, Benjamin M, Yang P C, Prior T and Perude M H (2000), "Chronic Stress Impairs Rat Growth and Jejunal Epithelial Barrier Function; Role of Mast Cells", *Am. J. Physiol. Gastrointest Liver Physiol.*, Vol. 278, No. 6, pp. G847-854.
45. Delbende C, Delarue C, Lefebvre H, Bunel DT, Staferczy K A and Mocaer E (1992), "Glucocorticoids, Transmitters and Stress", *Br. J. Psychiatry*, Vol. 160, pp. 24-25.
46. Yoshizato H, Fujikawa I, Soya H, Tanaka M and Narkashima K (1998), "The Growth Hormone (GH) Gene is Expressed in the Lateral Hypothalamus: Enhancement by Gh-Releasing Hormone and Repression by Restraint Stress", *Endocrinology*, Vol. 139, No. 5, pp. 2445-2451.



**International Journal of Pharma Medicine and Biological Sciences**

**Hyderabad, INDIA. Ph: +91-09441351700, 09059645577**

**E-mail: editorijpmbs@gmail.com or editor@ijpmbs.com**

**Website: www.ijpmbs.com**

