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IMPACT OF STRESS ON HISTOLOGY AND BIOCHEMICAL PARAMETERS OF LIVER AND KIDNEY OF MICE

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ABSTRACT

Epinephrine is a hormone and neurotransmitter. When produced in the body it increases heart rate, contracts blood vessels and dilates air passages and participates in the "fight or flight" response of the sympathetic nervous system. During stress concentration of epinephrine were increased many times. In present study epinephrine were administered to mice @ 200nl/k.g.bw to induce stress in animal, which were dissected after 2 and 4 weeks. SGOT and Urea level in serum were increased in epinephrine administered group. Liver and kidney tissue were fixed for light microscopy and serum were collected for SGOT and urea assay. While after administration of epinephrine frequent vacuolisation were observed. Elongated nucleus was also observed in hepatic cells. Degenerated hepatic vein were also observed. While in kidney dilated bowmen's capsule were observed. Frequent vacuolization were also observed in cortex region. Endothelial cells of PCT were observed in degenerated condition. Thus it is concluded from entire study that stress causes degeneration in hepatic cells, central vein, glomerulus, bowmens capsule, PCT and DCT which finally leading to both hepato-toxicity and nephro-toxicity.

INTRODUCTION

Stress is a response to physical, chemical, biological and emotional changes, consisting of a pattern of metabolic and behavioural reactions that helps in strenthing the organism[1].Stress has been defined as " the pattern of physiological reactions that prepares an organism for action"[2]. The relationship between stressful life and health is well documented [3]. Among the many consequences of chronic stress, it was shown that it is associated with exacerbated stroke outcome in mice [4], with increased liver metastasis [5], increased susceptibility to endotoxic shock [6], and impaired antiviral immunity in wounded animals [7]. Not only is chronic stress unhealthy, also short-term stress episodes may damage some organs.

Acute social stress insults, aggression between males to establish dominance, are a very common stressor in laboratory mice. Few studies addressed the consequences of such a behavior on organ integrity. Matte [8] reported a rise in plasma creatine kinase in regrouped male mice fighting for several hours; he could not reproduce such an increase, although he did observe a moderate increase in plasma lactate dehydrogenase and transaminase activities[9]. Since Bing and Poulsen[10] described that aggressive behavior in mice raises renin concentration in plasma, several laboratories have studied the influence of such behavior on other releasable submandibular salivary gland peptides.

Epinephrine is a hormone and neurotransmitter. When produced in the body it increases heart rate, contracts blood vessels and dilates air passages and participates in the "fight or flight" response of the sympathetic nervous system. It is a catecholamine, a sympathomimetic monoamine produced only by the adrenal glands from the amino acids phenylalanine and tyrosine. Japanese chemist Jokichi Takamine and his assistant Keizo Uenaka independently discovered adrenaline in 1900[11]**.** In 1901 Takamine successfully isolated and purified the hormone from the adrenal glands of sheep and oxen. Adrenaline was first synthesized by Friedrich Stolz and Henry Drysdale Dakin, independently, in 1904[12]**.**

Thus the present work is designed to study impact of epinephrine induced stress on S.G.O.T., Urea and histology of liver and kidney of mice.

MATERIALS AND METHODS

1. Chemical: Epinephrine was used to prepare stress model.

2. Experimental model: Reared sexually matured 6-8 weeks old age group male and female Swiss Albino mice (Mus musculus) weighing 25-35gm b.w. in the animal house section of Mahavir Cancer Institute and Research Centre, Patna, were selected as an experimental model in the present study. The animals were housed at controlled environmental conditions 22±2ºC, relative humidity

50±10%, and 12h dark-light cycle. Animals were housed and allowed to free access to food and water. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

3. Methodology:

a) Experimental protocol: Selected pathogen-free mice were randomly divided into two groups (n=6 in each). One group served as control and epinephrine was administered intramuscularly to other groups at the dose of 200nl/kg b.w. respectively. Animals were sacrificed after two weeks and four weeks of treatment with epinephrine in each group.

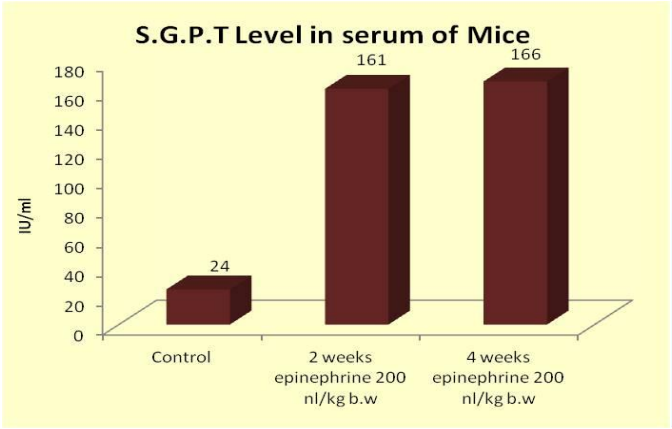
b) Histopathological Studies: The liver and kidney was dissected out and fixed in 10% neutral formalin solution and the tissue was processed. The slides were stained with Haematoxylene and Eosin and examined morphometrically under Light Microscope.

c) Biochemical Assessment: With the separated serum S.G.O.T. and Urea analysis were performed with standard kit (Coral) to establish the effects of epinephrine induced stress.

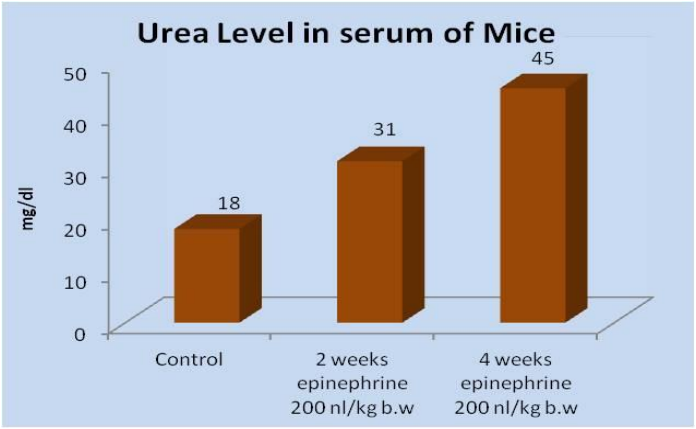
RESULTS

In the present study SGOT of control mice was 24 IU/ml. After two weeks administration of epinephrine (200nl/kg b.w) it was 161 IU/ml and after four weeks administration of epinephrine (200nl/kg b.w) it was 166 IU/ml (Graph: 1). Urea level of control mice was 18 mg/dl. After two weeks administration of epinephrine (200nl/kg b.w) it was 31 mg/dl and after four weeks administration of epinephrine (200nl/kg b.w) it was 45 mg/dl (Graph: 2).

Graph: 1



Graph: 2



Liver of control group of mice show normal hepatic cells, hepatic veins are also normal, central vein are prominent (Figure: 1). While after two weeks administration of epinephrine degeneration were observed in hepatic cells. Many vacuolated spaces were observed, clustered nuclei were also observed (Figure: 2). While after four weeks of administration of epinephrine frequent vacuolisation were observed. Elongated nucleus was also observed in hepatic cells. Degenerated hepatic vein were also observed (Figure: 3). Degeneration of hepatic cells were observed with rudimentary cytoplasm and clustered nuclei. Degenerated nuclei were also observed with frequent vacuolisation (Figure: 4).In control group of mice kidney show normal glomerulus. Bowmen's capsule was also normal in structure. Proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) were also observed in normal condition (Figure: 5). While after two weeks administration of epinephrine Clustered nuclei were observed in glomerulus with frequent degeneration of cytoplasm. Dilated bowmen's capsule were also observed (Figure: 6). While after four weeks of administration of epinephrine dilated bowmen's capsule were observed. Frequent vacuolization were also observed in cortex region. Endothelial cells of PCT were observed in degenerated condition (Figure: 7). Enlarged view of glomerulus show clustered nuclei and degenerated podocytes, degenerated cytoplasm were also observed in duct system (Figure: 8).

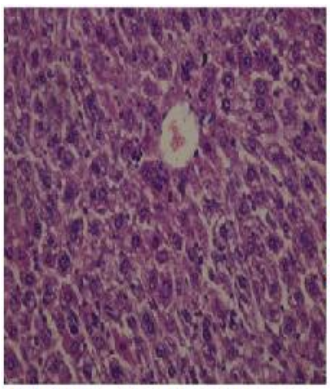


Figure: 1:- Liver of control group of mice show normal hepatic cells, hepatic veins are also normal, central vein are prominent.

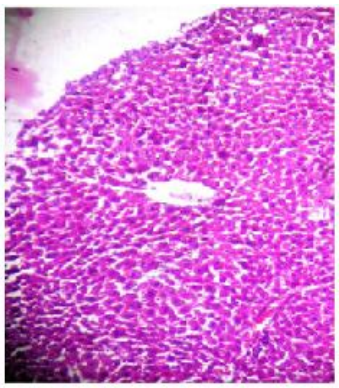


Figure: 2:- after 2 weeks administration of epinephrine degeneration were observed in hepatic cells. clustered nuclei were also observed.

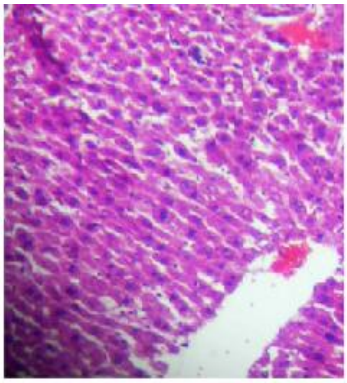


Figure: 3:- after 4 weeks of administration of epinephrine frequent vacuolisation were observed. Elongated nucleus in hepatic cells was also observed.

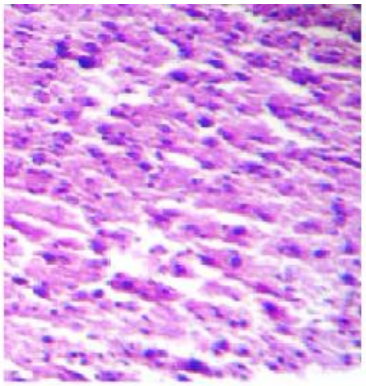


Figure: 4:- Degeneration of hepatic cells was observed with rudimentary cytoplasm and clustered nuclei. Degenerated nuclei were also observed.

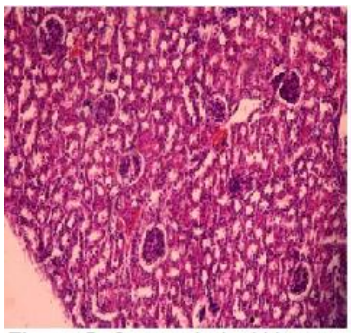


Figure: 5:- In control mice kidney show normal glomerulus and bowmen's capsule. PCT and DCT were also observed in normal condition.

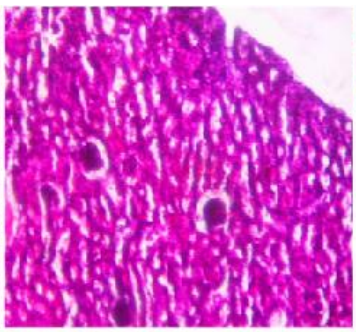


Figure: 6:- after 2 weeks administration of epinephrine Clustered nuclei were observed in glomerulus. Dilated bowmen's capsules were also observed.

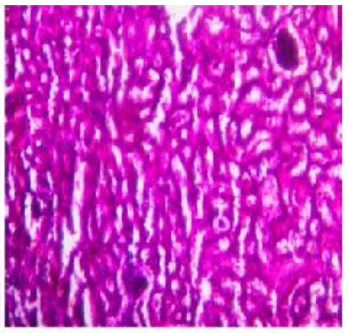


Figure: 7:- after 4 weeks of administration of epinephrine frequent vacuolization were also observed in cortex region. Endothelial cells of PCT were observed in degenerated condition.

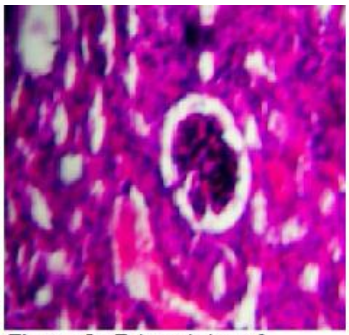


Figure: 8:- Enlarged view of glomerulus show clustered nuclei and degenerated podocytes, degenerated cytoplasm were also observed in duct system.

DISCUSSION

Acute and intense psychological stressors induce gastric ulceration and heart injury in rodents[13]. Several acute stressors also alter liver structure. Thus, stressors are different as restraint and forced exercise induce formation of autophagic vacuoles among several ultrastructural modifications [14]. This is associated with DNA oxidative damage [15], lipid peroxidation[16], protein oxidation[17], and, ultimately, the loss of hepatocyte integrity, as indicated by the rise in plasma transaminase activities[18]. All these alterations may be caused by catecholamines. Sustained elevation of plasma norepinephrine by means of miniosmotic pump implantation in peritoneal cavity causes hepatocyte injury and depresses liver function[19]. In recent years, it became clear that liver injury, caused by stress, is the consequence of an inflammatory response[20]. Both physical and psychological stressors elevate plasma IL-6 [21] and increase hepatic IL-6 expression[22]. In present study S.G.O.T level were increased to greater extent. Frequent vacuolisation were observed in hepatic cells. Elongated nucleus was also observed in hepatic cells. Degenerated hepatic vein were also observed. Degeneration of hepatic cells were observed with rudimentary cytoplasm and clustered nuclei causes improper function of liver.

Heat stress can negatively affect animal performance. Increase in body temperature and respiration rate are the most important signs of heat stress in farm animals. The increase in body temperature is associated with a marked reduction in food intake [23], the redistribution of blood flow and changes in endocrine functions [24], which can negatively affect the productive and reproductive performance of the animals. In present study urea level were gradually increased after increased duration. Dilated bowmen's capsule was observed in epinephrine administered group. Frequent vacuolization were also observed in cortex region. Endothelial cells of PCT were observed in degenerated condition. Degenerated podocytes were also observed in duct system. This finally leads to improper filtration and reabsorption. This finally hampers urine formation. The blood electrolyte balance is also altered during heat stress result in abnormal filtration of urine in kidney[25]. Additionally, environmental stress has been shown to cause an increase in the oxidative stress and an imbalance in the antioxidant status [26].

CONCLUSION

Thus it is concluded from above study that epinephrine induced stress causes increase in SGOT level and urea level in mice leading hepatotoxicity and nephrotoxicity. Stress also causes degeneration of nucleus in hepatic cells, cytoplasm were also degenerated which leads to hepatotoxicity. Glomerulus, bowmen's capsule, PCT and DCT were also degenerated to greater extent causes abnormal filtration and reabsorption.

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REFERENCES

1. Krupavaram, B.; Venkata Rao, N.; Nandakumar, K.; Gowda, T. S.; Shalam, M. D. and Shantakumar, S. M. (2007): A study on adaptogenic activity of root extracts of *Boerhaavia diffusa* (Linn). *Ind.Drugs.* 44 (4): 264-270.
2. Tortora, J.; Gerald and Anagnostakos, M. P. (1990): Principles of Anatomy and Physiology. (Harper and Row publishers, New York,). 533-537.
3. Baum A, Posluszny DM. Health psychology: mapping biobehavioral contributions to health and illness. *Annu Rev Psychol* 50: 137–163, 1999.
4. DeVries AC, Joh HD, Bernard O, Hattori K, Hurn PD, Traystman RJ, Alkayed NJ. Social stress exacerbates stroke outcome by suppressing Bcl-2 expression. *Proc Natl Acad Sci USA* 98: 11824–11828, 2001.
5. Wu WJ, Yamaura T, Murakami K, Murata J, Matsumoto K, Watanabe H, and Saiki I. Social isolation stress enhanced liver metastasis of murine colon 26–L5 carcinoma cells by suppressing immune responses in mice. *Life Sci* 66: 1827–1838, 2000
6. Quan N, Avitsur R, Stark JL, He LL, Shah M, Caligiuri M, Padgett DA, Marucha PT, Sheridan JF. Social stress increases the susceptibility to endotoxic shock. *J Neuroimmunol* 115: 36–45, 2001.
7. De Groot J, Boersma WJA, Scholten JW, Koolhaas JM. Social stress in male mice impairs long-term antiviral immunity selectively in wounded subjects. *Physiol Behav* 75: 277–285, 2002.
8. Matte AC. Effect of isolation induced aggression in mice on serum creatine kinase. *Psychopharmacology (Berl)* 42: 209–210, 1975.
9. Sánchez O, Arnau A, Pareja M, Poch E, Ramírez I, Soley M. Acute stress-induced tissue injury in mice: differences between emotional and social stress. *Cell Stress Chaperones* 7: 36–46, 2002.
10. Bing J, Poulsen K. In mice aggressive behavior provokes vast increase in plasma renin concentration,

causing only slight, if any, increase in blood pressure. *Acta Physiol Scand* 105: 64–72, 1979.

11. Yamashima, T. (2003): Jokichi Takamine (1854–1922), the samurai chemist, and his work on adrenalin. *J. Med. Biogr.* 11 (2): 95–102.
12. Bennett, M. (1999): One hundred years of adrenaline: the discovery of autoreceptors. *Clin. Auton. Res.* 9(3): 145–159.
13. Konturek SJ, Brzozowski T, Konturek PK, Majka J, Dembinski A. Role of salivary glands and epidermal growth factor (EGF) in gastric secretion and mucosal integrity in rats exposed to stress. *Regul Pept* 32: 203–215, 1991.
14. Salas M, Tuchweber B, Kourounakis P. Liver ultrastructure during acute stress. *Pathol Res Pract* 167: 217–233, 1980.
15. Adachi S, Kawamura K, Takemoto K. Oxidative damage of nuclear DNA in liver of rats exposed to psychological stress. *Cancer Res* 53: 4153–4155, 1993.
16. Kovacs P, Juranek I, Stankovicova T, Svec P. Lipid peroxidation during acute stress. *Pharmazie* 51: 51–53, 1996.
17. Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. *FASEB J* 10: 1532–1538, 1996.
18. Fernández G, Mena MP, Arnau A, Sánchez O, Soley M, and Ramírez I. Immobilization stress induces c-Fos accumulation in liver. *Cell Stress Chaperones* 5: 306–312, 2000.
19. Wang P, Tait SM, Chaudry IS. Sustained elevation of norepinephrine depresses hepatocellular function. *Biochim Biophys Acta* 1535: 36–64, 2000.
20. Swain MG. Stress and the gastrointestinal tract. I. Stress and hepatic inflammation. *Am J Physiol Gastrointest Liver Physiol* 279: G1135–G1138, 2000.
21. Zhou D, Kusnecov AW, Shurin MR, DePaoli M, Rabin BS. Exposure to physical and psychological stressors elevates plasma interleukin 6: relationship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinology* 133: 2523–2530, 1993.
22. Kitamura H, Konno A, Morimatsu M, Jung BD, Kimura K, Saito M. Immobilization stress increases hepatic IL-6 expression in mice. *Biochem Biophys Res Commun* 238: 707–711, 1997.
23. Rhoads ML, Rhoads RP, VanBaale MJ, Collier RJ, Sanders SR, Weber WJ, Crooker BA, Baumgard LH (2009). Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci., 92: 1986-1997.
24. Khodaei-Motlagh M, Zare Shahneh A, Masoumi R, Derensis F (2011). Alterations in reproductive hormones during heat stress in dairy cattle. Afr. J. Biotech., 10: 5552-5558.
25. Borges SA, Fischer da Silva AV, Majorka A, Hooge DM, Cummings KR (2004). Physiological Responses of Broiler Chickens to Heat Stress and Dietary Electrolyte Balance (Sodium Plus Potassium Minus Chloride, Milliequivalents Per Kilogram). Poult, Sci., 83: 1551–1558.
26. Sahin K, Sahin N, Onderci M, Yaralioglu S, Kucuk O (2001). Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. Vet. Med-Czech, 46:140–144.