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# EVALUATION OF EFFECT OF STRESS ON CHOLESTEROL AND INTESTINE OF MICE

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**ABSTRACT**

**Objective:** Stress is now a day's part of our lifestyle. Every individual faces stress in their day to day activity. When humans experience stress, as a survival mechanism, the body diverts energy, blood, enzymes and oxygen from the digestive organs to other areas of the body. In addition to mental and emotional stresses like fear, anger and worry, physical stresses including infections, trauma from injuries, surgery and environmental toxins can have a major effect on our digestive efficiency. In stress, proteins from the thymus and lymph glands are broken down into sugar for immediate energy. Also fat is mobilized from storage depots. Cortisol released in response to stress stimulates gastric-acid secretion. **Methods:** Mice were randomly divided into three groups (n=10 in each). One group served as control and epinephrine was administered intramuscularly to other two groups at the dose of 100nl/kg b.w. and 200nl/kg b.w. respectively. Animals were sacrificed after two weeks and four weeks of treatment, blood was collected for biochemical assay and tissues were fixed for histological study. **Results:** Cholesterol level and SGPT level in mice were increased after increased amount of stress. Stress also causes degeneration in muscular layer of intestine which adversely affects peristaltic movement of intestine. Goblet cells and endothelial cells were also degenerated leading to improper absorption of food. As level and duration of stress increases degeneration is also increases. **Conclusion:** Thus it is evident from study that stress adversely affects digestion and absorption of food in intestine and increased cholesterol may lead to cardiac arrest. **The significant finding (s) of the study:** Stress causes degeneration of villi in intestine due to increased epinephrine amount which finally causes improper absorption of digested food leading malnutrition in mice. Cholesterol and SGPT level were also increased after stress. **This study adds:** This is helpful to find digestive anomalies in stressed people, increased cholesterol was also related with increased number of heart patients now a days. If general individual know the role of stress and digestive system they may either take least stress or practice some anti stress therapy, which is helpful for them.

**Key words:** Stress, epinephrine, goblet cells, cholesterol and SGPT.

## INTRODUCTION

Stress, defined as an acute threat to the homeostasis of an organism,[1-3] be the real (physical) or perceived (psychological), and whether posed by events in the outside world or from within, evokes adaptive responses which serve to defend the stability of the internal environment and to assure the survival of the organism.[4-6] Now-a-days each and every people are suffering from a number of genetic as well as physiological disorders that are caused by long-term exposure to stress including hypertension, depression, high blood pressure, heart attack, cancer, asthma, obesity, infertility etc. Stress can manifest itself from several sources, be it bodily stress from sickness, injury, dehydration or poor nutrition, social stress from unpleasant events or disagreeable altercations with others, psychological stress from depression, worry, low self esteem or a lifestyle that includes alcohol or drug abuse and work related stress. Whatever influence causes our specific stress, it is invariably expressed in some form of physical or mental symptoms. Health care experts estimate that 75-90 percent of all visits to primary care physicians are due to stress related problems.

The secretion of stress hormones (glucagon, catecholamines, cortisol and GH) and especially cortisol increases during the acute stress and emotional stimuli.[7-12] Prolonged cortisol secretion (which may be due to chronic stress or the excessive secretion seen in Cushing's syndrome) results in significant physiological changes.[13] Cortisol counteracts insulin, contributes to hyperglycemia-causing hepatic gluconeogenesis and inhibits the peripheral utilization of glucose (insulin resistance) by decreasing the translocation of glucose transporters (especially GLUT4) to the cell membrane.[14,15] However, cortisol increases glycogen synthesis (glycogenesis) in the liver.[16] Cortisol's only direct effect on the hydrogen ion excretion of the kidneys is to stimulate the excretion of ammonium ions by deactivating the renal glutaminase enzyme.[17]
Thus the present work is designed to find out effect of stress on SGPT level, Cholesterol level and histology of intestine of mice.

## MATERIALS AND METHODS

**1. Chemical:** Epinephrine was used.

**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 three groups (n=10 in each). One group

served as control and epinephrine was administered intramuscularly to other two groups at the dose of 100nl/kg b.w. and 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 intestine 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 morphometrical under LM.

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

**RESULTS**

Cholesterol level in control group was 130 mg/dl. It was 144 mg/dl and 181 mg/dl after 2 weeks and 4 weeks of epinephrine 100 nl/kg b.w administration. While it were 162 mg/dl and 195 mg/dl after 2 weeks and 4 weeks of epinephrine 200 nl/kg b.w administration (Graph-1). SGPT level in control group was 18 IU/ml. It was 53 IU/ml and 82 IU/ml after 2 weeks and 4 weeks of epinephrine 100 nl/kg b.w administration. While it were 66 IU/ml and 109 IU/ml after 2 weeks and 4 weeks of epinephrine 200 nl/kg b.w administration (Graph-2).

Control group of intestine shows normal villi with well organized circular and longitudinal muscles i.e. muscularis externa. Distinct mucosa and sub-mucosa layers are also seen (Figure:1). Epinephrine 100 nl/kg b.w adminiatered for two weeks shows villi with degenerated goblet cells. Lumen is also not distinctly visible (Figure:2). Muscularis externa showing degeneration with some vacuolated spaces in submucosa layer. Villi also showing degeneration (Figure: 3). Epinephrine 100 nl/kg b.w adminiatered for four weeks shows degenerated muscularis externa with vacuolization in submucosa layer. Fragmented villi were also seen (Figure:4). Epinephrine 100 nl/kg b.w adminiatered for four weeks shows enlarged view of villi with clustered nuclei. Degenerated muscularis externa were also seen (Figure:5). Epinephrine 200 nl/kg b.w adminiatered for two weeks shows rudimentary muscularis externa with many vacuolated spaces. Villi with clustered nuclei were seen with many vacuolated spaces in submucosa layer (Figure: 6).

Epinephrine 200 nl/kg b.w adminiatered for two weeks shows enlarged view of degenerated muscularis externa with vacuolization in submucosa layer (Figure:7). Epinephrine 200 nl/kg b.w adminiatered for four weeks shows complete fragmentation of villi with clustered nuclei. Goblet cells were also degenerated (Figure:8). Epinephrine 200 nl/kg b.w adminiatered for four weeks shows enlarged view of muscularis externa with complete rupture of longitudinal muscle. Villi with complete degeneration of goblet cells were also seen. Degenerated cytoplasm and lacteals were also prominent (Figure: 9).

**DISCUSSION**

The association between psychological stress and small intestinal motility has been postulated for about 20 years. Some studies in experimental animals indicated contradictory results, which may be due to different stressors in part. Varied stressors can influence small intestinal motility via different mechanisms. Muelas et al.[18] demonstrated that restraint stress increased small intestinal motility both during fasting and after food.

Ditto et al.[19] reported that a prolonged active coping stressor with minimal motor requirements enhanced small intestinal transit. However, Tsukada et al.[20-22] demonstrated that the small intestinal transit was significantly inhibited by restraint stress but not by footshock stress. And footshock stimulus may cancel the inhibition of small intestinal motility by restraint stress. Leveau et al.[23] demonstrated that impairment in intestinal motility probably played a pathophysiological role in the development of bacterial overgrowth. We also find increased cholesterol in stressed mice, as level of stress increases cholesterol concentration in serum were also increases. Cortisol released in response to stress stimulates gastric-acid secretion. Net chloride secretion in the intestines is inversely decreased by cortisol *in vitro* (methylprednisolone).[24] Cortisol inhibits sodium loss through the small intestine of mammals.[25] It also reduces calcium absorption in the intestine.[26] in the study Serum Glutinine Pyruvate Test (SGPT) level were also increased with increased amount of stress.

Beaumont[27] reported that normal gut function was disturbed in a patient with a gastric fistula during periods of emotional stress. Selye introduced stress research in animal models and characterized induction of gastric ulcer as a classical response to stress[28]. In present study degeneration were observed in villi prominently. Mucosa and sub mucosa were rudimentary. Degeneration was also prominent in muscular layer of intestine with distinct vacuolization which finally causes improper peristaltic movement of intestine leading to indigestion of food as well as reduced hunger. Degenerated goblet cells were also prominent. Endothelial cells of intestine were also degenerated which adversely affect absorption in intestine. Clustered nuclei were also observed causes indigestion. Increased stress causes increased level of glucose while lipid peroxidation level also increases that leads to diabetes in mice.[29]

Thus it is concluded from study that stress causes increase in cholesterol level and SGPT level. Increased cholesterol with increased amount of stress may lead to cardiac arrest. It also causes degeneration in muscular layer of intestine which adversely affect peristaltic movement of intestine due to which food movement inside intestine is not properly occur, it causes indigestion of food. Mucosa and sub-mucosa layer were also degenerated which adversely affect assimilation of food. Goblet cells and endothelial cells were also degenerated leading to improper absorption of food. As level and duration of stress increases degeneration is also increases. Thus it is evident from study that stress adversely affects digestion and absorption of food in intestine.

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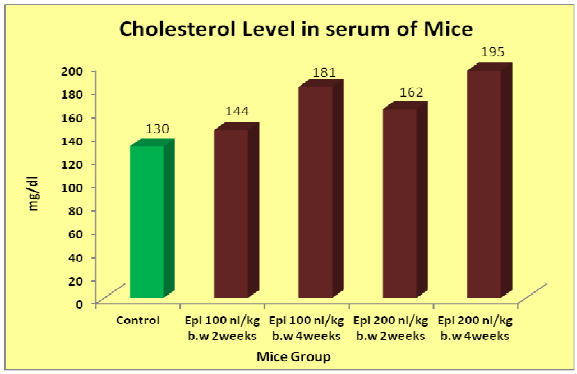
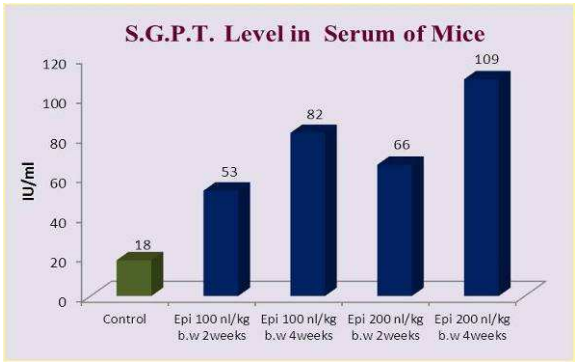
**Graph: 1**




**Graph: 2**



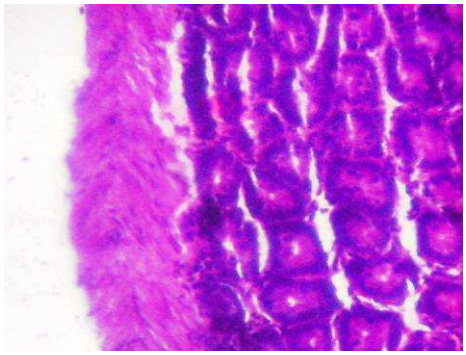



**Figure: 1 Control group of intestine showing T.S. of normal villi with well organized circular and longitudinal muscles i.e. muscularis externa. Distinct mucosa and sub-mucosa layers are also seen.**

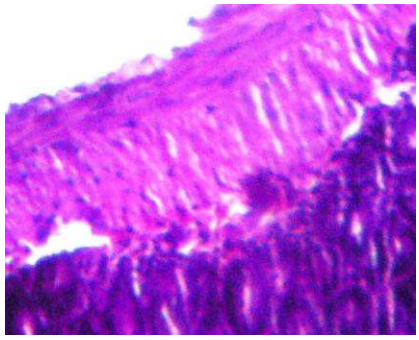


**Figure: 2 Epinephrine 100nl/kg b.w for 2 weeks administered group shows villi with degenerated goblet cells. Lumen was also not distinctly visible.**

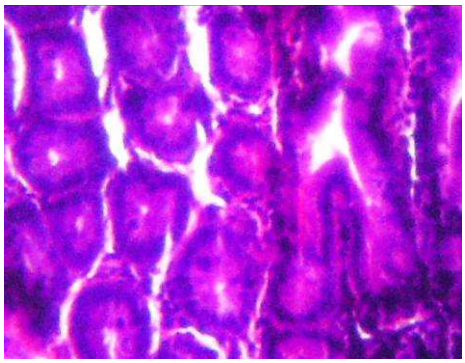


**Figure: 3 Epinephrine 100nl/kg b.w for 2 weeks administered group shows degeneration with some vacuolated spaces in submucosa layer. Villi also show degeneration.**

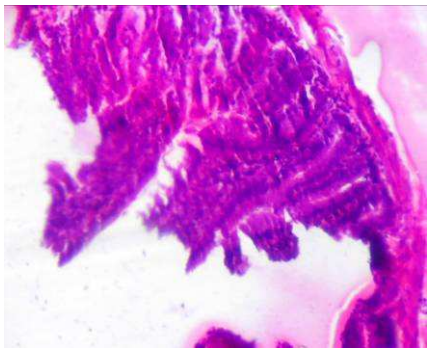


**Figure: 4 Epinephrine 100nl/kg b.w for 4 weeks administered group shows degenerated muscularis externa with vacuolization in submucosa layer. Fragmented villi were also seen.**

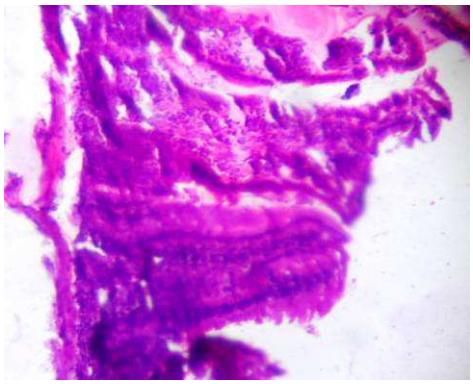


**Figure: 5, Epinephrine 100nl/kg b.w for 4 weeks administered group shows enlarged view of villi with clustered nuclei. Degenerated muscularis externa were also seen.**

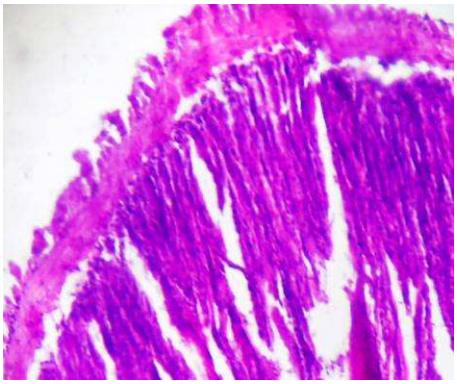


**Figure: 6, Epinephrine 200nl/kg b.w for 2 weeks administered group shows rudimentary muscularis externa. Villi with clustered nuclei were seen with degenerated submucosa layer.**

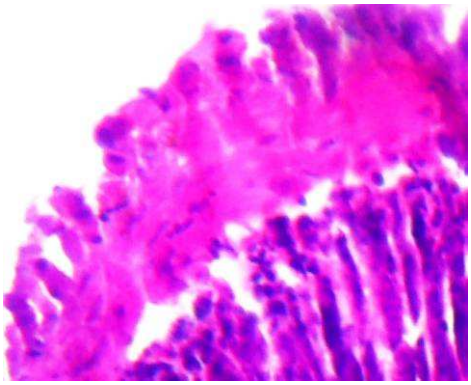


**Figure: 7, Epinephrine 200nl/kg b.w for 2 weeks administered group shows enlarged view of degenerated muscularis externa with vacuolization in submucosa layer.**

**Figure: 8, Epinephrine 200nl/kg b.w for 4 weeks administered group shows complete fragmentation of villi with clustered nuclei. Goblet cells were also degenerated.**

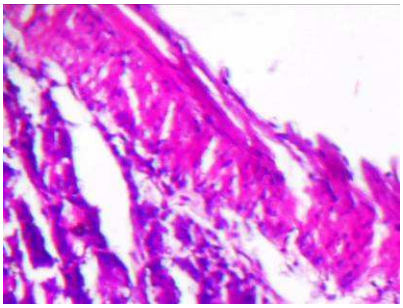


**Figure: 9 Epinephrine 200nl/kg b.w for 4 weeks administered group shows complete rupture of longitudinal muscle. Villi with complete degeneration of goblet cells were also seen. Degenerated cytoplasm and lacteals were also prominent.**

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