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Increased epinephrine concentration during stress in relation to diabetes in mice

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ABSTRACT
Stress is a part and parcel of modern day life. Everyone remain in stress for either short period or long period depending on their life style. It is well known fact that during stress epinephrine concentration in body rises several times. Now a days every one remain in stress for either short or long period due to changed life style and secrete increase amount of epinephrine in body according to duration of stress. Experimental group receives epinephrine@100 ng/kg b.w and 200 ng/kg b.w on day 1 and 14th of experiments and sacrificed after 4th week. Serum were collected for biochemical assay and tissues for histological study. Glucose level were incresed after increase in epinephrine concentration in mice. Lipid peroxidation level were also increases in stressed group of mice. Hepatic cells and central vein were observed in degenerated condition. Beta cells degeneration were more in comparision to alpha cells in epinephrine administered group of mice. Thus it is concluded from entire study that increased stress causes increased level of glucose while lipid peroxidation level were also increased. Hepatic cells and beta cells of pancreas were degenerated which finally leads to diabetes in mice.

Introduction

Stress is a part and parcel of modern day life. A U.S. Public Health Survey estimated that 70%-80% of Americans experience at least "some stress" every two weeks and visit a physician each year for a stress-related disorder. Job-related stress costs U.S. businesses $ 60 million annually1. Stress is a feeling that's created when one reacts to particular events. It's the body's way of rising to a challenge and preparing to meet a tough situation with focus, strength, stamina, and heightened alertness. The events that provoke stress are called stressors which may be physical or mental2. Stress can be sub divided into following types which includes physical stress, conditional stress and chemical stress. It is estimated that over 24 million Americans are already caught with diabetes, from those 5.7 million people remain undiagnosed. Diabetes prevalence has increased steadily in the last half century and will continue rising among people of developing country also.

There is no evidence that stress causes diabetes. However, stress may sometimes unmask diabetes, by causing blood glucose levels to rise3. In people who have diabetes, the stress response does not work well. Insulin is not always able to let the extra energy into the cells, so glucose piles up in the blood4. These results in increase propensity of various diseases and diabetes may be an outcome of stress, which further sets in a vicious cycle of stress-diabetes relationship1.

Epinephrine is a hormone of catecholamine group which is synthesized in the chromaffin cells of the adrenal medulla5. It is responsible for the "fight or flight" reaction in mammals. It is one of the most powerful vasopressor and hence used as a drug in order to restore heart rhythm in certain cases of cardiac arrest. It acts as a bronchodilator in acute bronchial asthma. It is also used as a vasoconstrictor in anaphylactic shock. The secretion of stress hormones (glucagon, catecholamines, cortisol and GH) and especially cortisol increases during the acute stress and emotional stimuli6-13. ome of these hormones are diabetogenic and might be involved in the development of diabetes during the stress.10 For example, epinephrine inhibits the insulin secretion both in animals and humans14,15 .

It is well known fact that during stress epinephrine concentration in body rises several times. Now a days every one remain in stress for either short or long period due to changed life style and secrete increase amount of epinephrine in body according to duration of stress. Present study aims to illustrate effect of stress on induction of diabetes.

Materials and Methods

1. Chemical: Epinephrine was used.
2. Experimental model: Reared sexually matured 6 week old age group male and female Swiss Albino mice (Mus musculus) weighing 25-35gm b.w. in the laboratory animal resource 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).

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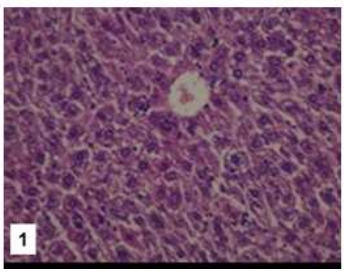

Fig 1. Showing liver of control mice with well organized hepatic cells. Central veins are also normal in structure. Cytoplasm and nucleus of hepatic cells are well organized

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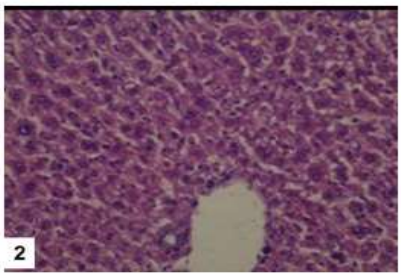

Fig 2. Showing well organized hepatic cells with prominent nucleus. Sinusoids are also normal in structure.

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 liver and pancreas 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 lipid peroxidation analysis were performed to establish the effects of Epinephrine induced stress leading to diabetes.

i. Lipid Peroxidation Levels: Lipid peroxidation test gives the amount of lipid oozes out from lipid biomembrane. It measures the levels of Melanaldehyde in blood sample. The LPO test was performed with separated serum of different groups of control, and treated mice according to Ohkawa et al.16

ii. Glucose analysis: it was measured by glucometer (Clever check – TD 4209)

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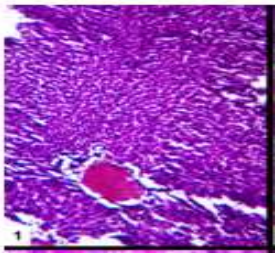


Fig 1. Microphotograph of liver of epinephrine (at the dose of 100nl/kg b.wt. for 2 weeks) administered mice showing degeneration in cytoplasmic material of hepatic cells. Rudimentary hepatic veins were also visible with many vacuolated spaces.

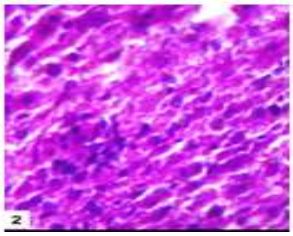


Fig 2. Microphotograph of liver of epinephrine (at the dose of 100nl/kg b.wt. for 4 weeks) administered mice showing many vacuolated spaces in hepatic cells. Degenerated cytoplasmic material was visible. Clustered nuclei were also observed. Fragmentation of nucleus is also visible.

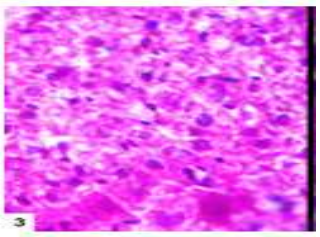


Fig 3. Microphotograph of liver of epinephrine (at the dose of 200nl/kg b.wt. for 2 weeks) administered mice showing degeneration of cytoplasmic material to greater extent. Vacuolated nuclei were observed in different shapes. Vacuolization were prominent in cytoplasm.

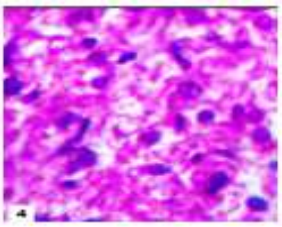
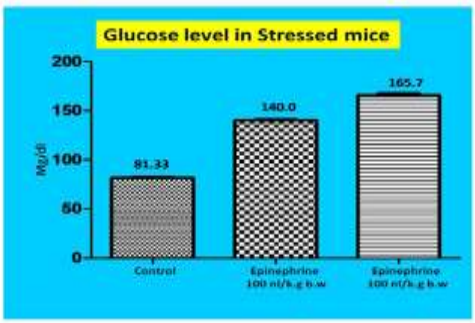


Fig 4. Microphotograph of liver of epinephrine (at the dose of 200nl/kg b.wt. for 4 weeks) administered mice showing many vacuolated spaces with degenerated cytoplasmic material. Elongated thread-like nuclei were visible. Hollow nuclei were also visible.

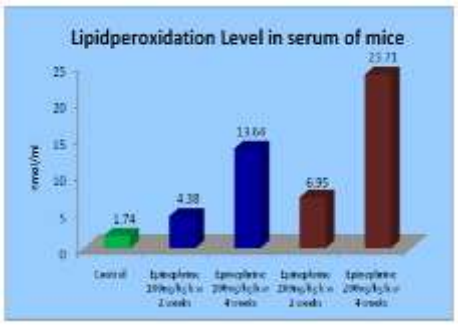
Observation

In control group of mice lipid peroxidation level was 1.74nmol/ml, while after 2 weeks of administration of single dose of epinephrine at the rate of 100nl/kg b.wt., it will became 4.38nmol/ml. It became increased after 4 weeks of administration of epinephrine i.e., 13.64 nmol/ml. Increased concentration of epinephrine causes increased level of lipid peroxidation in mice i.e., in mice group, which was exposed to epinephrine at the rate of 200nl/kg b.wt., after 2 weeks of administration lipid peroxidation level were 6.95nmol/ml. It became gradually increased after increased duration of time i.e., after 4 weeks of exposure it became 23.71nmol/ml (Text Figure: I) (Table: I).

Text Figure: I



Text Figure:II



In control group of mice fasting glucose level was 81.33 mg/dl, while after 4 weeks administration of 100 nl/kg b.w epinephrine fasting glucose level was 140.0 mg/dl. When mice were administered with 200 nl/kg b.w epinephrine for 4 weeks fasting glucose level was 165.7 mg/dl (Text Figure: II).

Liver of control mice showing well organized hepatic cells. Central veins are also normal in structure. Cytoplasm and nucleus of hepatic cells are well organized in control group of mice (Plate – I, Fig: 1). Control group of mice also shows well organized hepatic cells with prominent nucleus. Sinusoids are also normal in structure (Plate – I, Fig: 2). While epinephrine administered at the dose of 100nl/kg b.wt. for 2 weeks mice showing degeneration in cytoplasmic material of hepatic cells. Rudimentary hepatic veins were also visible with many vacuolated spaces (Plate – II, Fig: 1). When this group is left for four weeks showing many vacuolated spaces in hepatic cells. Degenerated cytoplasmic material was visible. Clustered nuclei were also observed. Fragmentation of nucleus is also visible (Plate – II, Fig: 2). When epinephrine is administered at the dose of 200 nl/kg b.wt. for 2 weeks it showed degenerated of cytoplasmic material to greater extent. Vacuolated nuclei were observed in different shapes. Vacuolization were prominent in cytoplasm (Plate – II, Fig: 3). While epinephrine administration at the dose of 200nl/kg b.wt. for 4 weeks showing many vacuolated spaces with degenerated cytoplasmic material. Elongated thread-like nuclei were visible. Hollow nuclei were also visible (Plate – II, Fig: 4).

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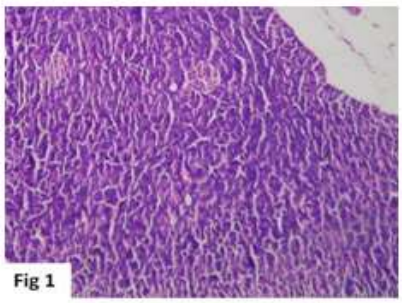

Fig 1. Showing pancreas of control mice with well organized acinar cells and islets of langerhans cells. Alpha and beta cells are normal in shape

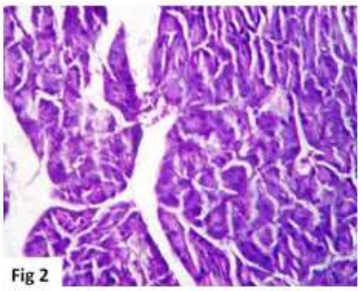

Fig 2. Microphotograph of pancreas of epinephrine (at the dose of 200nl/kg b.wt. for 4 weeks) administered mice showing degenerated acinar cells. Degeneration in beta cells were clearly observed. Alpha cells were observed with prominent nucleus.

Pancreas of control mice show well organized acinar cells and islets of langerhans cells. Alpha and beta cells are normal in shape (Plate – III, Fig: 1). Pancreas of epinephrine at the dose of 200nl/kg b.wt. for 4 weeks showing degenerated acinar cells. Degeneration in beta cells was clearly observed. Alpha cells were observed with prominent nucleus (Plate – III, Fig: 2).

Discussion

Now a day's stress is very common problem. Every one remains in stress for either short period or longer duration of time depending on their lifestyle. It is well established fact that epinephrine level were increases with stress. Role of stress on diabetes were evaluated in details by ADA4. Effects of stress on exacerbation of diabetes mellitus, serum glucose and cortisol levels and body weights in rats were reported by Maryam Radahmadi6. We also find elevated level of glucose in stressed mice. Glucose level were increases directly with increase in epinephrine level.

The temporal relationship between endogenously secreted stress hormones and metabolic decompensation in diabetic man were evaluated by Schade8. Wortsman9 also reported Adrenomedullary response to maximal stress in human. Lipidperoxidation level were also increases with increased degree of stress which directly indicate that stress causes depletion of lipid from biomembrane. Due to which cell secretion pathway were altered causes malformation of insulin leading diabetes in mice.

Barnes11 has reported effects of age on the plasma catecholamine response to mental stress in man. Morrow14 were reported effects of epinephrine on insulin secretion and action in human. Beta cells were degenerated after stress. Hepatic cells were also observed in degenerated condition and degree of degeneration was increases with increase in amount of epinephrine.

Thus it is concluded from entire study that increased stress causes increased level of glucose while lipid peroxidation level were also increased. Hepatic cells and beta cells of pancreas were degenerated which finally leads to diabetes in mice.

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Table: I (Fasting glucose level in mice)

Mice Group	Number	Glucose Level (Mean ± SEM)	P value
Control	6	81.33 ± 1.333	< 0.0001
Epinephrine 100 nl/kg b.w	6	140.0 ± 1.183	< 0.0001
Epinephrine 200 nl/kg b.w	6	165.7 ± 1.944	< 0.0001