

## Original Article

## Effect of stress induced by exposure to short and long term foot shock on liver, spleen and kidney in aged mice

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## ARTICLE INFO

## Article history:

Received 9 June 2018

Accepted 6 November 2018

Available online 13 November 2018

## Keywords:

Foot shock stress

Liver

Spleen

Kidney

Old age

## ABSTRACT

**Introduction:** Stress affects the central nervous system leading indirectly to modulation of the activity of steroid, catecholamine and opioid systems. It also affects behaviour, immune system, cardiovascular responses and gastrointestinal tract. In response to stress, a cascade of neurohumoral events chiefly at the level of hypothalamic-pituitary-adrenocortical (HPA) axis, is triggered, the result of which is the termination of stress reaction leading to normalization. During induction of stress hormone epinephrine concentration increases many times in the body.

**Methods:** In the present study short term (5 days) and long term (21 days) foot shock stress was given to Albino mice of old age of BALB C strain (481 days old) to see any the histological changes in liver, spleen and kidney.

**Results:** Mice subjected to long term stress showed in liver vacuolization, elongation of nucleus of hepatic cells and infiltration. While in kidney dilated Bowman's capsule, frequent vacuolization in renal cortex, degeneration of cells of proximal convoluted tubule, whereas in spleen there was increase in size of the follicles and red pulp morphology of parenchyma (increase in red pulp).

**Discussion:** Thus it is concluded from the entire study that long term stress causes degeneration in hepatic cells, infiltration in liver, degeneration of glomerulus, Bowman's capsule, convoluted tubules in kidney which finally leading to both hepato-toxicity and nephro-toxicity. In spleen long term stress alters the immune response in Old age Albino Mice by inducing morphology changes, explaining in part the indicating impaired immunity that develops in organisms that are exposed to chronic stress.

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## 1. Introduction

Stress refers to physical, mental or emotional factor which results in physical or psychological changes tension. Stress can result from some external factors (environmental, psychological or social situations) or internal situations i.e., illness or from a therapeutic procedure (Medicine Net.com Jan 2, 2016).<sup>1</sup> Biological changes and changes in emotion is a consequent response of stress which henceforth strengthens the subject (organism). Chronic stress induces reduction in weight gain, raised corticosterone level and changes in cognition, which affects both physiology and behavior and leads to development of psychological disorders (Brown et al.).<sup>2</sup> High rates of response of stroke was

characteristically seen in some mice subjected to chronic stress. Such animals were also prone to increased Liver metastasis, more susceptible to shock (endotoxic),<sup>3–5</sup> and impairment of antiviral immunity in animals that are wounded.<sup>6</sup> Though chronic stress brings in so many complications, still even episodes of short term stress may damage the organs. Stress insults for a short period (Acute stress) Aggressive behavior of male mice which tries to establish its dominance over the female, are some of the commonly used stress inducing factors in the laboratory. Matte<sup>7</sup> reported that there was an encountered increased levels of creatinine kinase in the serum in male animals subjected to stress and which has been fighting for about several hours. But there was a moderate increase in plasma lactate dehydrogenase and activity of transaminases.<sup>8</sup> Bing and Poulsen<sup>9</sup> described that renin levels in plasma is increased when aggression is seen. A review by Lupien et al.<sup>10</sup> reports that the brain is mainly sensitive to stress during early childhood and old age, as it undergoes vital changes in during these periods. Both Studies on human and animals studies have

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E-mail address: [billakantibabu@yahoo.co.in](mailto:billakantibabu@yahoo.co.in) (P. Babu B).

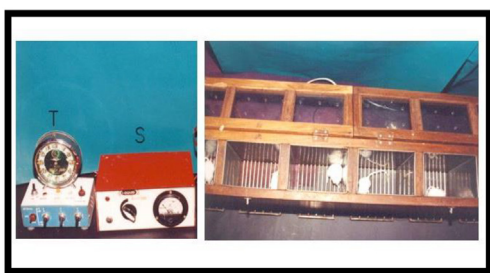
shown that exposure to early-life stress during early life, increases cognitive deficits in adulthood, and has an impact throughout the life-span. Old age is the host of neurocognitive changes as it is linked with many age related structural, chemical and functional alterations in organisms.<sup>11</sup> The mediators of the main axis of stress is (CRH) corticotrophin-releasing hormone secreted by the paraventricular nucleus located in the hypothalamus, (ACTH) Adrenocorticotrophic hormone secreted by the master and (Pituitary) and adrenal gland secretions (Glucocorticoids)

In order to maintain its normal homeostasis the glucocorticoids secreted by the adrenal maintains a negative feedback mechanism through its specific transducers (Receptors).<sup>12</sup> However, during chronic stress conditions an excess load is generated which would change its integrators and well as the neurotransmitters that aid in the transmission of impulses and changes in endocrine hormones,<sup>13,14</sup> and secondary lymphoid organs.<sup>15</sup> Clinical<sup>16</sup> studies have reported an increase in cortisol levels during depression.<sup>17</sup> However, there is no direct evidence of effect of acute and chronic stress on spleen liver and kidney and secondary lymphoid organ, the spleen. Thus, the aim of our study was to determine the effects of acute period (5 days) and chronic period (21 days) foot shock stress on histologically observed changes in liver, kidney and spleen in old age mice.

## 2. Materials and methods

The aim of the current study is to study the effect of acute (5 days) stress in mice and Chronic (21 days) stress in the histological changes in the spleen, Liver and kidney which is 481 days old. After last day of stress, P<sub>486</sub> (5 days group), P<sub>502</sub> (21 days group), mice were anesthetized using ether along with age matched controls (n=6). About 15 ml of 0.9% heparinized saline was perfused at the rate of 1 ml/min through left ventricle. This was followed by injection with 10% formalin (about 250 ml) at the same rate. The liver, spleen and kidneys were dissected out and were put in 10% formalin for a week.

### 2.1. Photograph of foot shock apparatus



**Foot-shock apparatus-Timer (T), Stimulator (S).**

Foot-shock apparatus-Timer (T), Stimulator (S).

### 2.2. Foot shock stress

In the foot-shock stress, mice were placed individually in the shock grid compartments. Intermittent foot shock was given of appropriate voltage 80–90 volts for 3 h daily at 5 min interval for 2–3 s, so that animals were subjected exposed to 12 foot shocks/hr. i.e. total of 36 foot shocks per day.

### 2.3. Tissue preparation for histological analysis

After one week in 10% formalin, Liver, spleen and kidneys, paraffin embedded, and are sectioned serially as (4–5 μm slices), and further processed for (H&E) Hematoxylin and Eosin staining.

## 3. Results

### 3.1. Effect of stress on histology of liver

Histological examination of the liver sections in the old age control groups (486 & 502 days old) showed prominent central vein in the hepatic lobule surrounded by the rows of hepatocytes with distinct nuclei and hepatic venous sinusoids between them (Fig. 1a&c). 5 days acute stress group showed very mild degeneration of hepatic cells of the liver. (486 days old) (Fig. 1b), but whereas 21 days chronic stress group showed degeneration of central vein, hepatic cell dilatation of the liver and congestion of the sinusoids (502 days old) (Fig. 1d).

### 3.2. Effect of stress on histology of spleen

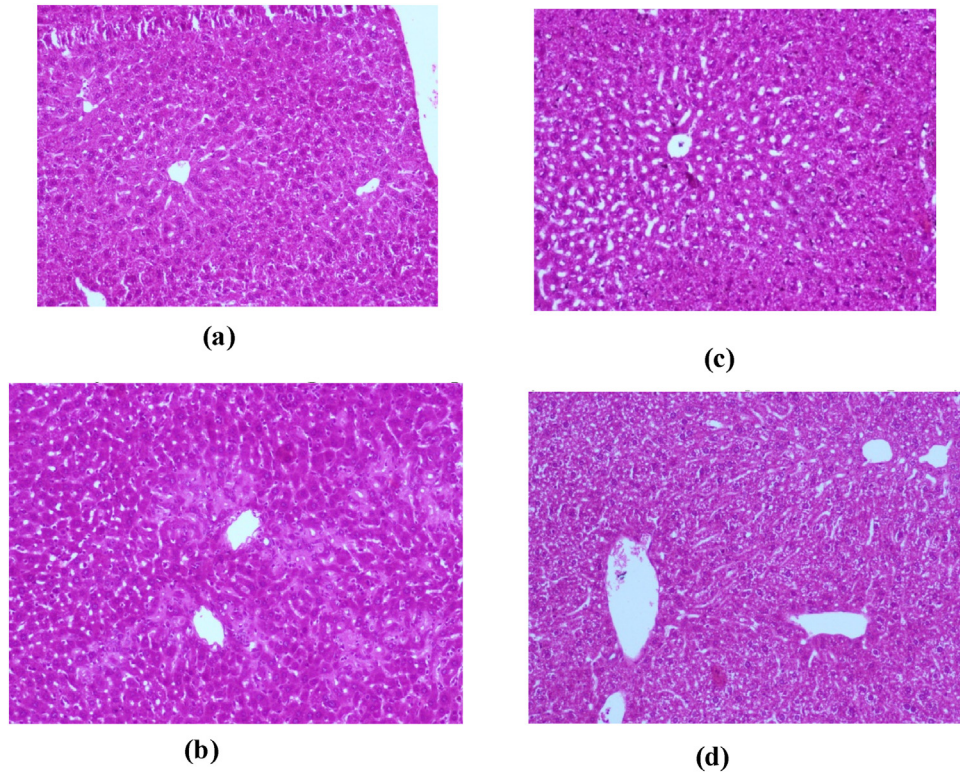
Histological examination of spleen of control groups (486 & 502 days old) showed large follicles in white pulp (Fig. 2a & c). Spleen of 5 days stressed group (Fig. 2b) was almost like controls (486 days old) and whereas in 21 days stress group (502 days old) there was an involution of the red pulp and expansion seen in the red pulp of spleen (Fig. 2d).

### 3.3. Effect of stress on histology of kidney

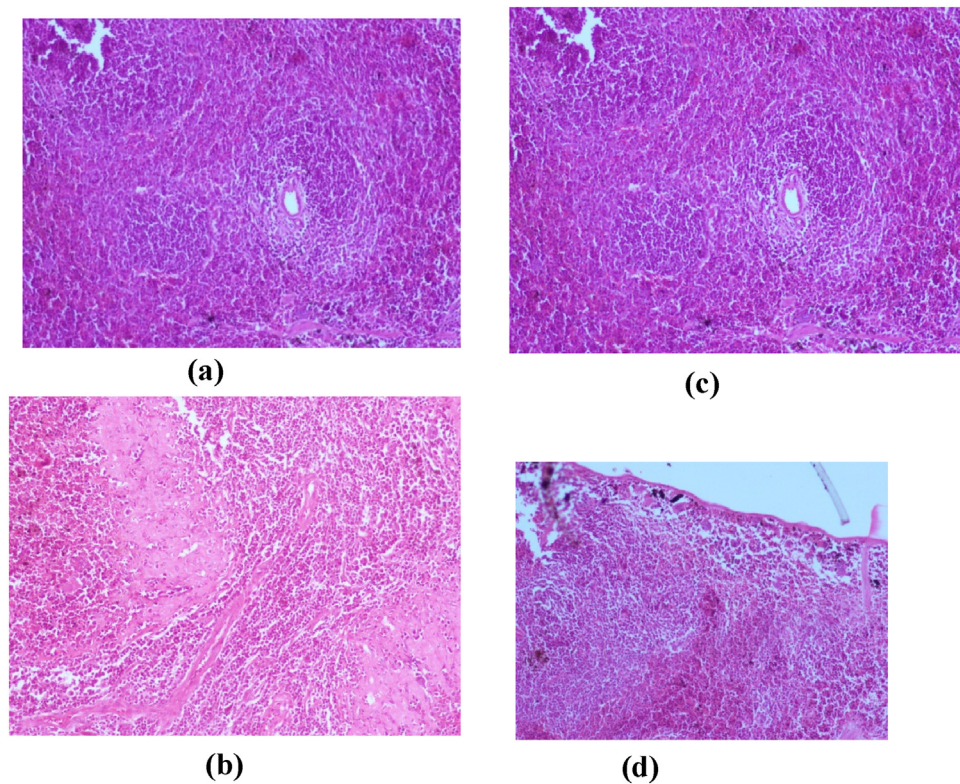
Histological examination kidney of control group (486 & 502 days old) showed a normal renal corpuscle and convoluted tubules (Fig. 3a&c). Further, the Glomerulus and Bowman's capsule were also observed. Mice of 5 days stress (486 days old) did not show any much histological changes (Fig. 3b). In 21 days stress group dilated Bowman's capsule, epithelial cells of PCT were observed in degenerated condition (Fig. 3d).

## 4. Discussion

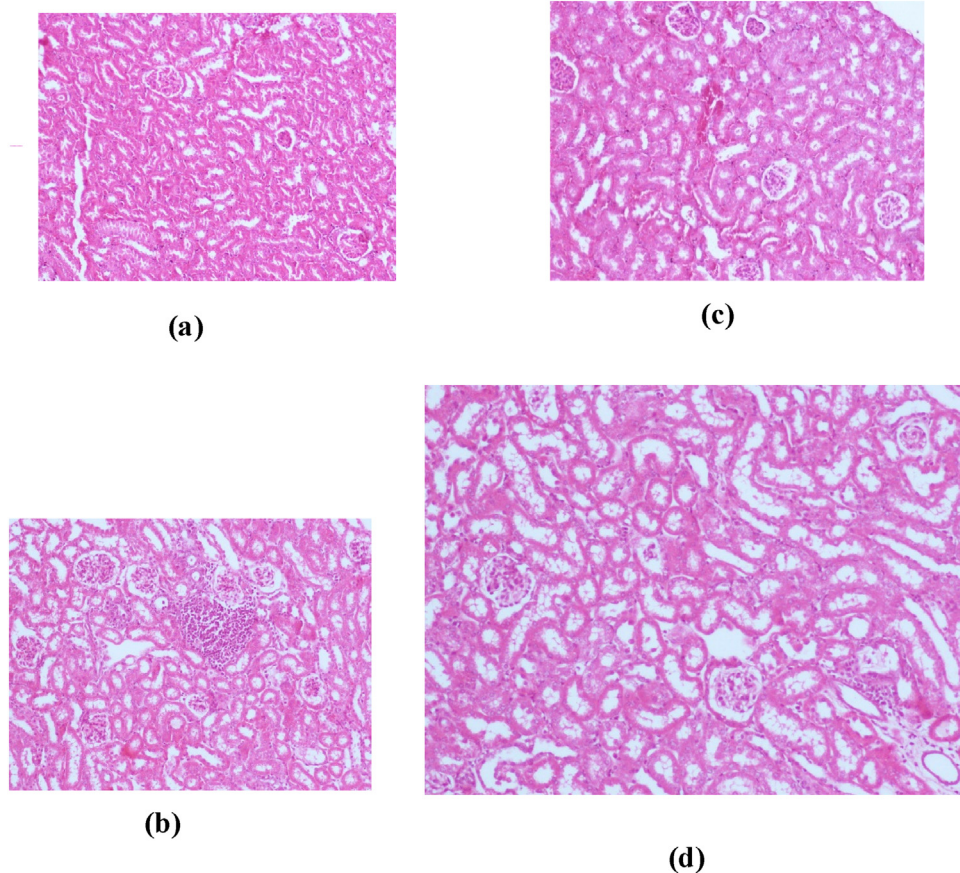
Glucocorticoids is a characteristic mediator that shows detrimental effects of such as immunosuppression during chronic periods of stress.<sup>18</sup> Maria EH et al.<sup>19</sup> examined the effects of stressors by estimating the corticosterone levels in the WKY rats. Mice of 9-week-old (male) was subjected for a period of 3 h of restraint stress every day, and chemical stress as given by (hydrocortisone treatment, 50 mg/Kg weight), mixed stress (restraint stress plus hydrocortisone), or control treatment (without stress) for 1, 4, and 7 weeks. After the study they observed that there was a decrease in the body weight as well as the weight of the suprarenal gland was reduced. Along with this a decrease in the levels of cortisol also was characteristically seen in these animals. Minimal changes were observed in animals subjected to immobilization group or a period after 7 days. The maximum effects in the corticosterone levels are attributed to the number of days exposed to stress and the stressors effect, which should have desensitized the HPA axis that might have been caused by impaired glucocorticoid receptor expression and function.<sup>20</sup> In depression patients similar mechanism of action is observed when treated with dexamethasone suppression test (DST).<sup>21,22</sup> The spleen is one of the largest secondary lymphoid organ and has two main compartments: red pulp and white pulp. For example, the CRH overexpressing transgenic mouse has high circulating levels of corticosterone, undergoes significant changes and fails to form germinal centers in the white pulp of the spleen.<sup>23</sup> Restraint stress can cause the spleen to involute,<sup>24</sup> impairing the host's ability to mount an immune response.<sup>25</sup> Male rats of 9-week old subjected to immobilization stress for 3 h. daily for 1, 4 and 7 weeks showed the follicles at white pulp differed in size in the spleen.<sup>19</sup>



**Fig. 1.** (a) Liver of 5 days control showing normal hepatocytes. (b) Liver of 5 days foot shock stress showing mild degeneration of hepatic cells. (c) Liver of old age 21 days control showing normal hepatocytes. (d) Liver of Old age 21 days foot shock stress showing congested sinusoids and neutrophil infiltration.



**Fig. 2.** (a) Spleen of old age 5 days control. (b) Spleen of old age mice subjected to 5 days foot shock stress showing white pulp. (c) Control Spleen of old age 501 days old. (d) Spleen of old age 501 days old subjected to 21 days foot shock stress showing involution of white pulp.



**Fig. 3.** (a) Kidney of old age 5 days control (486 days old) showing normal glomerulus and convoluted tubules. (b) Kidney of old age mice subjected to 5 days foot shock stress (486 days old) showing mild degeneration of glomerulus and convoluted tubules. (c) Kidney of old age mice (502 days old) showing normal glomerulus and convoluted tubules. (d) Kidney of old age mice subjected to 21 days foot shock stress (502 days old) showing degenerated convoluted tubules.

Acute and intense psychological stress inducing factors cause gastric ulceration and heart injury in rodents.<sup>26</sup> Several acute stressors also alter liver structure. Thus, stressors are different as restraint stress induce the formation of autophagic vacuoles among several ultra-structural modifications.<sup>27</sup> This is associated with DNA oxidative damage, lipid peroxidation,<sup>28</sup> protein oxidation.<sup>29</sup> All these changes may be caused by catecholamines. In recent years, it became clear that liver injury, caused by stress, is the consequence of an inflammatory response.<sup>30</sup> In present study frequent vacuolization, elongated nucleus, degenerated central vein were observed in liver hepatic cells in old age mice subjected to 21 days foot shock stress. Hepatic cells degeneration 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,<sup>31</sup> the redistribution of blood flow and changes in endocrine functions,<sup>32</sup> which can negatively affect the productive and reproductive performance of the animals. In mice subjected to 21 days FSS dilated Bowman's capsule was observed. Frequent vacuolization were also observed in cortex region. Epithelial cells of PCT were observed in degenerated condition. Degenerated podocytes were also observed in duct system. This finally leads to improper filtration and reabsorption and finally hampers urine formation.

## 5. Conclusion

Based on the function long term stress induces morphological changes in spleen that might alter cellular and humoral immune responses, degeneration of cytoplasm and nucleus in hepatic cells which leads to hepatotoxicity, degeneration of Glomerulus, bowmen's capsule, PCT and DCT to a greater extent which might cause abnormal filtration and reabsorption.

## Conflict of interest

The authors have no conflicts of interest to declare.

## Funding

None.

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