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Original Article

Impact of acute and chronic stress hormone on male albino rat brain

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Abstract: The present investigation aimed to evaluate the acute and chronic effect of stress (stress hormone) in male albino rat brain. Nor-epinephrine was used for the treatment and saline used for the control. Nor-epinephrine was dissolved in the saline and administered orally to the rats. Following nor-epinephrine administration, the brain was removed surgically at 6 h, 12 h and 45 days. Alanine tansaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were significantly altered in the rats. Lipid peroxidation was measured as malondialdehyde (MDA), showed altered lipid peroxidation. Hematological markers such as packed cell volume (PCV), white blood cells (WBC), neutrophil, lymphocytes and hemoglobin were significantly altered compared to controls. Altered serum biochemical and hematological markers, lipid peroxidation and enzyme activities leads to adverse effect in the cellular metabolism and physiological activities of rats.

Keywords: Rat, brain, enzymes, lipid peroxidation, WBC

Introduction

Catecholamines regulate a several cellular and biochemical functions in the mammalian cells [1]. Generally, catecholamines act on target cells by binding to the cell surface receptors [2]. Identification of catecholamine action sites within the kidney is vital to distinguish direct effects from indirect adrenergic effects on epithelial transport and metabolism. Nor-epinephrine is a hormone secreted from medulla of the adrenal glands [3] and useful drug for several emergency medical conditions. The investigation of the nor-epinephrine action on brain cell metabolism could be useful in the biochemistry and pharmacology field. The increased nor-epinephrine level is found in phaeochromocytoma, hypoglycemia, myocardial infarction and certain essential familial tremor [4]. Palpitations, tachycardia, arrhythmia, anxiety, panic attack, headache, tremor, hypertension and acute pulmonary edema are the adverse effects of nor-epinephrine.

Usually, nor-epinephrine level is measured in blood to identify the causative agent in a potential poisoning victim. In resting adults, it is less than 10 ng/L, but may increase by 10-fold during exercise and by more than 50-fold during stress. Nor-epinephrine tremendously increased 1000-10000 ng/L in Pheochromocytoma patients. Even, 10,000 to 100,000 ng/L of nor-epinephrine increased during parenteral administration in acute-care cardiac patients [5]. The increased level of nor-epinephrine in the blood is removed by the oxidative breakdown in the liver and kidney. The increased oxidative reaction could produce high level of superoxide radicals, which in turn may affect the normal cell physiology and metabolism.

Acute and sub-acute toxicity of nor-epinephrine were studied in male albino rats. The acute (up to 3 g, orally and intraperitoneally) and chronic (15, 45, 90 and 180 mg/kg, intraperitoneally) toxicity were evaluated for 2 and 21 days, respectively. In chronic toxicity, changes in weight and amount of food intake as well as biochemical, hematological and pathological tests were studied in rats after 21 days. High oral and intraperitoneal doses of nor-epinephrine (3 g/kg) did not cause death within 2 days of study.

Table 1. Glucose, toltal protein, total cholesterol, triglycerides and reduced glutathione levels at 6 h, 12 h and 45 days following the nor-epinephrine (100 mg/kg bwt) oral administration in male albino rats

Parameters	Time intervals (h)	Control (mg/dl)	Treated (mg/dl)
Glucose	6	133.22±2.1	138.82±3.0
	12	128.63±2.6	142.84±1.6*
	24	130.72±1.7	146.214±1.7*
Total protein	6	5.972±0.6	5.522±0.4
	12	6.121±0.7	5.495±0.4*
	24	6.361±0.1	5.695±0.7
Total cholesterol	6	243.63±2.7	259.82±5.8
	12	251.62±2.9	265.92±3.7
	24	240.73±4.2	285.53±2.1*
TG	6	254.12±7.1	283.71±10.7*
	12	255.52± 5.2	279.97±7.5
	24	249.71±9.5	286.621±6.6*
Reduced glutathione	6	0.012±0.00	0.014±0.00
	12	0.011±0.00	0.014±0.00
	24	0.0121±0.00	0.016±0.00*

*P<0.05, n=6.

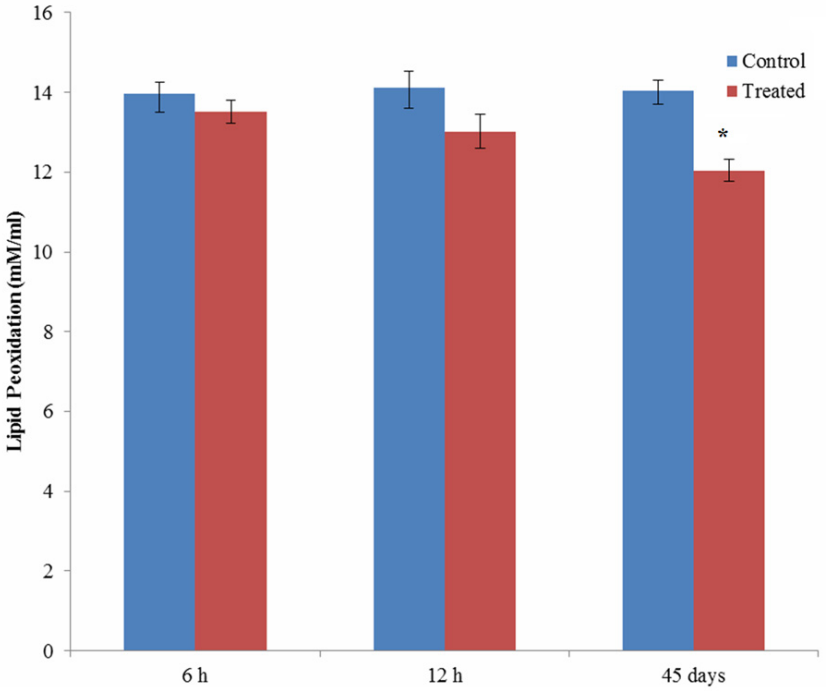

Figure 1. Lipid peroxidation level at 6 h, 12 h and 45 days following the nor-epinephrine (100 mg/kg bwt) oral administration in male albino rats. *P<0.05, n=6.

A dose 180 g/kg of nor-epinephrine in sub-acute study increased platelets and creatinin levels. The lower doses of the substance decreased albumin and ALP, and raised the LDL level dose independently. The main target of the present investigation is to determine the effect of nor-epinephrine on male albino rat brain.

Materials and methods

Animals and treatments

Healthy male albino rats were purchased from the animal house, Shangai Hospital, weighing (160-180 g) were selected for present study. They were kept in polypropylene cages, at temperature 25±0.5°C , relative humidity 60±5% and photoperiod of 12 h/day. Male albino rats were grouped into two groups of six rats each: Nor-epinephrine (100 mg/kg bwt) dissolved in saline and administered orally to the rats. Control rats were administered saline (4 ml/kg). Brain was removed surgically at 6 h, 12 h and 45 days of control and treated rats. Brain samples were homogenized and centrifuged at 3000 rpm for 15 minutes, and supernatant was carefully transferred to sterilized plain glass vials for the future investigation.

Biochemical parameters

Glucose was estimated by the Asatoor and King method [6]. Total protein was measured by Lowry et al. [7]. Total cholesterol was estimated by Zaks et al. [8]. TG was estimated by the standard method [9]. Reduced glutathione (GSH) was estimated by standard method [10].

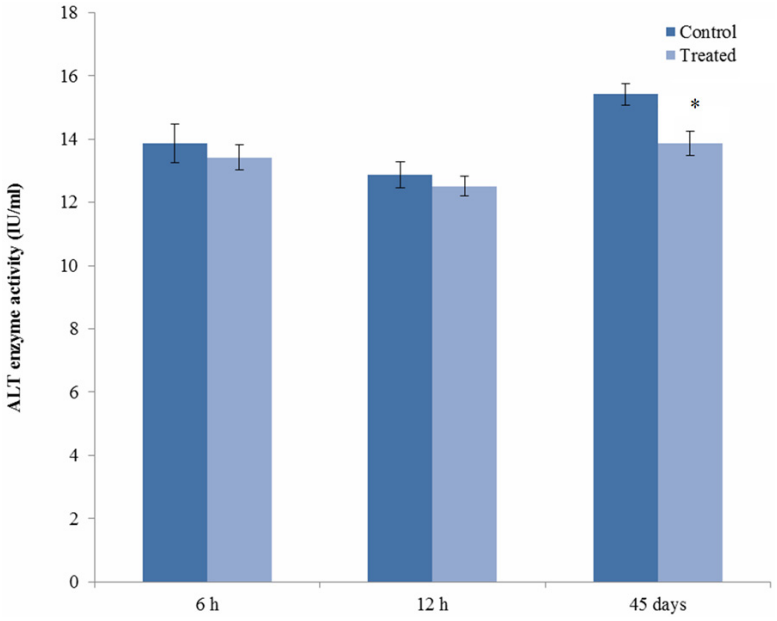

Figure 2. ALT enzyme activity at 6 h, 12 h and 45 days following the nor-epinephrine (100 mg/kg bwt) oral administration in male albino rats. *P<0.05, n=6.

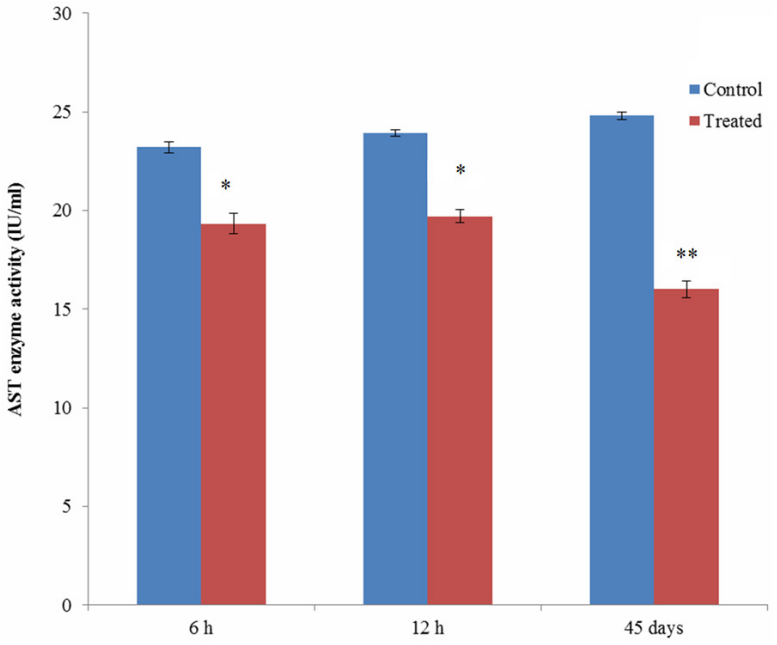

Figure 3. AST enzyme activity at 6 h, 12 h and 45 days following the nor-epinephrine (100 mg/kg bwt) oral administration in male albino rat brain. *P<0.05 and **P<0.01, n=6.

Enzymes

ALT and AST was estimated by the Reitman and Franklin method [11]. ALP was estimated by the standard method [12].

Lipid peroxidation

Lipid peroxidation was estimated by the TBARS method [13].

Hematological parameters

Haemoglobin was estimated by the acid-hematin method [14] employing Sahli's hemoglobinometer. Sahli's method is a visual comparison method [14]. The leukocyte count was estimated by the haemocytometer method [14]. The leukocyte differential count was determined by the Leishman Staining method. The packed cell volume was determined by centrifuging heparinized blood in a capillary tube at 10,000 RPM for five minutes [15].

Results

Biochemical markers

Glucose, TG and total cholesterol levels were augmented, while serum total protein content was inhibited compared with control. Glucose percentage altered upto 11.85% after 45 days of crocin administration in the male rats. Similarly, TG and total cholesterol were increased upto 14.78% and 18.61% respectively. Serum total protein content decreased by time dependent maner such as 7.53%, 10.22% and 10.47% respectively. GSH content increased 16.7, 27.3 and 33.3% at 6 h, 12 h and 45 days respectively (**Table 1**).

Enzymes

Oral administration of crocin significantly altered ALT, AST and ALP enzyme acitivity in male albino rat. ALT enzyme activity slightly reduced (10.1%) at 45 days whereas at 6 and 12 h, no significant changes was observed

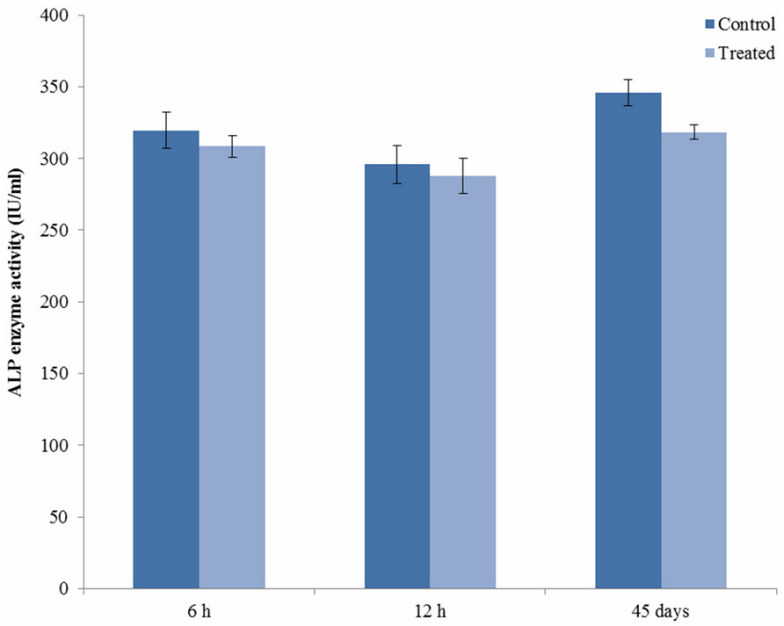

Figure 4. ALP enzyme activity at 6 h, 12 h and 45 days following the nor-epinephrine (100 mg/kg bwt) oral administration in male albino rat brain. N=6.

Table 2. Hemoglobin, WBC, lymphocytes, neutrophils and packed cell volume levels at 6 h, 12 h and 45 days following the nor-epinephrine (100 mg/kg bwt) oral administration in male albino rats

Parameters	Time intervals (h)	Control	Treated
Hemoglobin (g)	6	14.02±0.21	14.24±0.33
	12	13.93±0.19	14.19±0.25
	24	14.11±0.41	14.33±0.38
White Blood cells (10^3 mm^{-3})	6	6300.72±7.1	6174.2±10.72
	12	6424.52±10.7	5931.7±12.7*
	24	6382.81±16.3	5923.7±14.4*
Lymphocytes (%)	6	17.22±0.45	22.74±0.32*
	12	16.74±0.39	23.64±0.28*
	24	18.35±0.44	24.72±0.45*
Neutrophils (%)	6	78.73±3.3	75.22±3.5
	12	79.74±3.9	74.52±4.5
	24	80.74±4.1	72.24±4.7
Packed cell volume (%)	6	38.31±2.63	36.71±1.15
	12	39.61±3.11	35.78±0.75*
	24	39.72±1.42	36.22±1.63

*P<0.05, n=6.

(**Figure 1**). AST enzyme activity significantly reduced 16.7, 17.7 and 35.5% at 6 h, 12 h and 45 days respectively (**Figure 2**). ALP enzyme activity was insignificantly reduced compared to their respective controls (**Figure 3**).

Malondialdehyde determination (MDA)

Rate of lipid peroxidation was slightly altered following crocin administration. Lipid peroxidation was reduced less significantly 3.2, 7.8 and 14.2% at 6 h, 12 h and 45 days respectively (**Figure 4**, *P<0.05).

Hematological markers

Nor-epinephrine administration significantly altered hematological markers. Hemoglobin content was slightly increased in male albino rats following crocin administration. Lymphocyte content was increased 32.1, 41.2 and 34.7% at 6 h, 12 h and 45 days respectively. WBC content was reduced 2, 7.6 and 7.2% at 6 h, 12 h and 45 days respectively. Neutrophil content was reduced 4.5, 10.5 and 10.5% at 6 h, 12 h and 45 days respectively, in male albino rats following crocin administration. PCV content also reduced such as 4.2, 9.7, 8.8% at 6 h, 12 h and 45 days respectively (**Table 2**).

Discussion

Oral administration of norepinephrine into the male albino rats, showed increase in serum glucose level, which leads to hyperglycemic and diabetic conditions. Diabetes is clinical syndrome characterized by a loss of glucose homeostasis. The loss of glucose homeostasis is due defects in insulin secretion and action, both resulting in impaired glucose, fat and protein metabolism [16, 17]. Similarly increased content of TG and total cholesterol leads to hyperlipidimia in the blood, which

induces obesity. Hyperlipidemia is characterized by excess cholesterol and fats in the blood. Hyperlipidemia is a risk factor for the cardiac disease [18, 19]. Serum total protein content was decreased due to the accelerated proteolysis and transamination in the blood.

Administration of nor-epinephrine increased the reduced glutathione level in the heart tissue [20]. Administration of nor-epinephrine decreased the lipid peroxidation in the heart tissue. In our present study, administration of nor-epinephrine also increased the reduced glutathione content and reduced the lipid peroxidation. Hence, our results is consistent with the above findings.

Orally administered nor-epinephrine was effective in decreasing the elevated levels of ALP, AST, ALT. Nor-epinephrine administration could not markedly affect the serum ALT and AST activities. Nor-epinephrine treatments significantly reduced elevated ALP, AST and ALP. In our present study, administration of nor-epinephrine also redduced the serum hepatic enzymes. Hence, our results is consistent with the above findings.

Oral administration of nor-epinephrine into the male rat showed the increase in hemoglobin content, which leads to erythrocytosis and hypoxia. Increased lymphocytes crowd the bone marrow, and interfere with normal blood cell production which causes anaemia. Nor-epinephrine might have induced the metabolic rate, with the resultant increase in the generation of free radicals with the attendant cellular damage. The immune system responds to this damages caused by production of oxidants under stress condition. In this circumstances, free radicals are produced by the neutrophils, the first-responders to inflammatory cells. Neutrophils quickly congregate at a focus of infection, attracted by cytokines and expressed by activated endothelium, mast cells and macrophages [21]. The PCV was lower in nor-epinephrine treated rats compared to their controls. The observed decrease in PCV is believed to be as a result of the decreased RBC which is consistent with the findings of Eyong et al. [22]. This may possibly explain the low values of haematological parameters recorded in nor-epinephrine treated rats in this study, suppress the immune system and disruption/suspension of haematopoiesis.

In conclusion, hyperglycemic and lipidemia effects were caused by the oral administration of nor-epinephrine. Nor-epinephrine administration enhances the antioxidant defense and reduces the lipid peroxidation in brain tissue. In addition, nor-epinephrine reduces the hepatic marker enzymes. Hematologically, hypoxia, erythrocytosis and anemia are caused by the oral administration of nor-epinephrine, which lead to dysfunction of the immune system and disruption of haematopoiesis. In summary, our study confirms that acute and chronic exposure of stress (nor-epinephrine) seems to be toxic to rat brain.

Disclosure of conflict of interest

None.

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