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Original Article

STRESS RESPONSES IN ALBINO RATS

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Since antiquity, people have observed a complex relationship between alcohol and stress. Apart from stress response dampening effect, long-term ethanol is known to produce remarkable morphological and neurochemical abnormalities in the central nervous system. The present study compared the effect of isolation stress and chronic ethanol administration in modulating various physiological and biochemical parameters in rats. Two groups of Wistar strain adult Albino rats were kept under stress by isolation method for a period of 7 days and 14 days, respectively. Another two groups of rats were treated with ethanol in two dosages, 1 g and 2g/Kg body weight for a period of 7 days and 14 days. The effects of stress and ethanol on body and organ weights, neutrophil and eosinophil counts, blood sugar, serum cholesterol, and serum transaminase levels were studied. Despite decreasing body weights, both isolated stress and ethanol treatment significantly increased liver, heart, kidney, and adrenal weights (expressed as percent of body weight). Blood sugar, serum cholesterol, neutrophils, and eosinophils significantly decreased in both isolation and ethanol groups. In addition, serum transaminase levels increased significantly in all the study groups. The present study indicates that isolation stress and chronic ethanol treatment induce similar physiological and biochemical responses in rats.

Key words: Stress, isolation, ethanol, organ weights, white blood cells

For centuries, people have used alcohol to relief stress, that is, the interpretation of event as signaling harm, loss, or threat. The organism usually responds to stress with a variety of behavioral, biological, and cognitive changes (Sayette, 1999). The perception of stress elicits a varied response that may involve wide range of behaviors (e.g., escape or avoidance behavior); biological responses; and in humans, subjective awareness of a distressed emotional state. Stress related biological responses include psychophysiological reactions, such as sweating, muscle tension, and cardiovascular responding (e.g. changes in heart rate) as well as changes in action of various brain regions. Stress is a highly individualized response of an organism. The notion that alcohol consumption can reduce stress is taken for granted by most people and this effect of alcohol is suggested as a mechanism underlying the overindulgence of alcohol, even in the phase of harmful consequence (Anisman and Merali, 1999).

Increased concentrations of hypothalamic-pituitary-adrenal (HPA) axis hormones, particularly corticosterone or cortisol are often used as an index of stress and any stimulus that causes an increase in

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HPA axis activity is identified as stressor. In many cases, the so-called stress-response is initially adaptive responses that enable organisms to respond to environmental challenge. The pathological consequence of stress occurs after prolonged exposure to the stressor or mediators of the stress response, including the hormones of HPA axis. Regardless of their degree of severity, however, stressors may promote physiological and behavioral disturbances, ranging from psychiatric disorders to immune system dysfunction. Stressful events may also profoundly influence the use of alcohol and other drugs (DeVries, 2002).

The relationship between ethanol and stress was inconsistent. Ethanol reduced stress in some studies, did not affect stress responses in other analyses (Chester et al., 2004), and exacerbated stress in still other investigations (Sayette, 1993). The present work was mainly designed to compare the effect of isolation stress and chronic ethanol administration on selected physiological and biochemical parameters in rats.

Materials and Methods

Healthy adult male albino rats of Wistar strain weighing approximately 150 – 200 g were selected for the experiment. The animals were housed two per cage under controlled conditions of a 12 h light/dark cycle, 50% of humidity and 24^{o}C, and minimum noise levels. Animals had free access to water and standard pellet diet. The animals were divided randomly into following groups for the study.

Control: This group consisted of 10 rats housed in the laboratory for two weeks (two per cage) and was not exposed to any kind of stress except for daily handling.

Saline control: Two sub-groups of rats consisting of 10 animals each received normal saline injection intraperitoneally. In a sub-group, the rats received saline injection for a period of 7 days and in the other sub-group for 14 days.

Isolation stress: Rats were divided into two sub-groups of 10 animals each and care was taken that each sub-group had similar average body weight. The animals were isolated and placed individually in a specially designed isolation cage (45x15x15cm) so that the animals could not see each other. Thus, the social contact of the animal is cut off totally for the stipulated duration of the study. The test period lasted for 7 days in one sub-group and for 14 days in another sub-group.

Ethanol treatment: Rats were divided into four sub-groups having 10 animals each. The animals were kept in polypropylene cages in groups of 3 or 4 rats per cage. Ethanol was administered intraperitoneally everyday between 10 AM to 11 AM for each rat. Rats received ethanol injection at a dose of 1g/Kg body weight for a period of 7 days in one sub-group and for 14 days in another sub-group. Other two sub-groups of rats received ethanol injection at a dosage of 2 g/Kg body weight for a period of 7 days and 14 days, respectively.

At the end of the experimental period, body weights of all animals were recorded, the animals were then sacrificed, and blood samples were collected in fluoride tubes. Blood was also collected in plain glass tubes, which was allowed to coagulate and then centrifuged to separate the serum. Laparotomy was done and different organs like liver, heart, kidneys, and adrenals were removed. The wet weights of the organs were determined and organ weight was expressed as grams per 100 g of body weight. From the blood samples, absolute neutrophil and eosinophil counts were done by standard laboratory methods. Blood sugar level, serum cholesterol, and serum transaminase levels (alanine transaminase, ALT and aspartate aminotransferase, AST) were spectrophotometrically determined. All the experimental procedures were in accordance with the ethical standards of animal experimentation and the study was approved by the Institutional Animal Care and Use Committee.

Statistical analysis was done by one-way analysis of variance (ANOVA), followed by least significance difference test (LSD). P-values less than 0.05 were considered statistically significant.

Table 1. Effect of isolation stress and ethanol treatment on body and organ weights

Parameter	Duration	Control (N=10)	Saline Control (N=10)	Isolation Stress (N=10)	Ethanol 1g/Kg B wt. (N=10)	Ethanol 2g/Kg B wt. (N=10)
Body Weight (g)	7 days	− 0.70 ± 0.001	−0.68 ± 0.002	−8.70 ± 0.02 ***♣♣♣	−10.27 ± 0.05 ***♣♣♣	− 9.12 ± 0.25 ***♣♣♣
	14 days	− 0.28 ± 0.001	−0.34 ± 0.001	−10.38 ± 0.17 ***♣♣♣	−11.57 ± 0.09 ***♣♣♣	−8.54 ± 0.31 ***◆♣♣♣
Liver	7 days	3.134 ± 0.005	3.254 ± 0.004 ◊	3.634 ± 0.046 ***♣♣♣	3.588 ± 0.042 ***♣♣♣	4.153 ± 0.076 ***◆◆♣♣♣
	14 days	3.192 ± 0.003	3.211 ± 0.002 ◊	3.862 ± 0.088 ***♣♣♣	3.746 ± 0.050 ***♣♣♣	4.241 ± 0.075 ***◆◆♣♣♣
Heart	7 days	0.316 ± 0.002	0.333±0.003 ◊◊	0.343 ± 0.012 **♣	0.348 ± 0.008 **♣♣	0.374 ± 0.010 ***◆♣♣♣
	14 days	0.318 ± 0.004	0.337±0.001 ◊	0.354 ± 0.002 **♣	0.364 ± 0.012 **♣♣	0.370 ± 0.011 ***◆♣♣
Kidney	7 days	0.348 ± 0.003	0.324±0.005 ◊	0.395 ± 0.005 **♣♣♣	0.374 ± 0.008 **♣♣♣	0.408 ± 0.007 ***◆◆♣♣♣
	14 days	0.331 ± 0.003	0.348±0.004 ◊	0.387 ± 0.007 **♣♣♣	0.381 ± 0.007 **♣♣	0.472 ± 0.009 ***◆◆♣♣♣
Adrenals	7 days	0.005 ± 0.0001	0.005 ± 0.0001	0.011 ± 0.0002 ***◆◆♣♣♣	0.010 ± 0.0003 **♣♣♣	0.012 ± 0.0002 ***◆♣♣♣
	14 days	0.005 ± 0.0001	0.007 ± 0.0002 ◊	0.010 ± 0.0003 **♣	0.012 ± 0.0002 ***♣♣♣	0.013 ± 0.0001 ***◆♣♣♣

Values are Mean ± SEM, N=Number of animals; − Decrease; ∗ P<0.05, ∗∗ P<0.01, ∗∗∗P<0.001 – Control versus Experimental groups; ♣ P<0.05, ♣♣ P<0.01, ♣♣♣P<0.001 – Saline control versus Experimental groups; ◆ P<0.05, ◆◆ P<0.01, ◆◆◆ P<0.001 – Ethanol 1g/Kg BW versus 2g/Kg B. wt; ◊ P<0.05, ◊◊ P<0.01, ◊◊◊P<0.001 – Control versus Saline control

Results

There was a significant decrease in the body weight (P<0.001) after 7 days and 14 days of isolation stress when compared to control groups. No significant change in body weight was seen between control and saline injected sub-groups. A significant increase in the weight of the liver was observed after 7 days of isolation stress. Liver weight increased significantly more in 14 days isolation stress sub-group (P<0.01). Weight of the kidney and heart also increased significantly after 7 and 14 days of isolation stress (P<0.001). Adrenal gland weight also increased significantly after 7 (P<0.001) and 14 days (P<0.01) of stress exposure. Similar changes in the organ weights were observed when saline control group was compared with isolation stress (Table 1).

Intraperitoneal injection of ethanol (1g/Kg body weight and 2g/Kg body weight) in unstressed rats for the same duration also significantly increased the weights of liver (P<0.001), heart (P<0.01), kidneys (P<0.01), and adrenal glands (P<0.001). Between the two doses, significant

increases in the organ weights were observed with 2g/Kg body weight of ethanol treatment (Liver - P<0.01; Heart - P<0.05; Kidney-P<0.01; Adrenals –P<0.05) (Table 1).

There was a significant decrease in absolute neutrophil and eosinophil counts after isolation stress for a period of 7 (P<0.001) and 14 days (P<0.001) (Fig. 1 and 2). A similar decline in these absolute counts following chronic ethanol administration was observed for a treatment period of 7 and 14 days in unstressed rats (P<0.001) when compared to control groups (Fig. 1 and 2). Administration of ethanol with a dosage of 2g/Kg body weight resulted a more significant decrease in absolute neutrophil (P<0.05) and absolute eosinophil counts (P<0.01 -7 days; P<0.05 -14 days). A comparison between saline control and the experimental groups indicated similar changes in the neutrophils and eosinophil counts (Fig. 1 and 2).

Blood sugar and serum cholesterol levels significantly decreased after isolation stress for a period of 7 and 14 days in rats (P<0.001) (Table 2). Similar decreases in these biochemical parameters were observed after 7 (P<0.001) and 14 days (P<0.001) of ethanol administration in unstressed animals. Blood sugar (P<0.001 -7 days; P<0.05 – 14 days) and serum cholesterol levels significantly decreased more after 2g/Kg body weight ethanol treatment compared to 1g/Kg body weight ethanol (P<0.01) (Table 2).

A significant increase in serum transaminases was observed after chronic isolation for a period of 7 days (P<0.001) and 14 days (P<0.001) (Table 2). When unstressed rats were treated

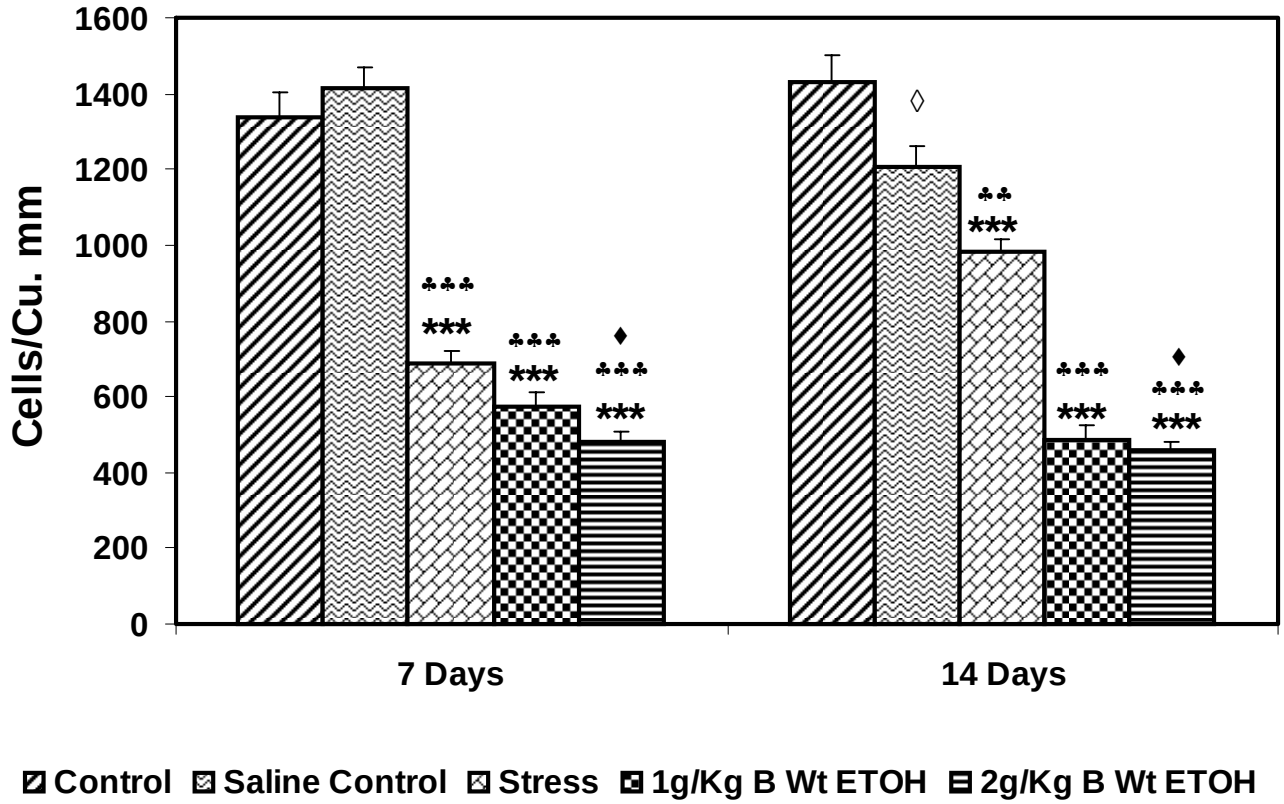

Figure 1. Effect of stress and ethanol treatment on neutrophil count; ∗ P<0.05, ∗∗ P<0.01, ∗∗∗P<0.001 – Control versus Experimental groups; ♣ P<0.05, ♣♣ P<0.01, ♣♣♣P<0.001 – Saline control versus Experimental groups; ♦ P<0.05, ♦♦ P<0.01, ♦♦♦ P<0.001 – Ethanol 1g/Kg BW versus 2g/Kg B. wt; ◊ P<0.05, ◊◊ P<0.01, ◊◊◊P<0.001 – Control versus Saline control

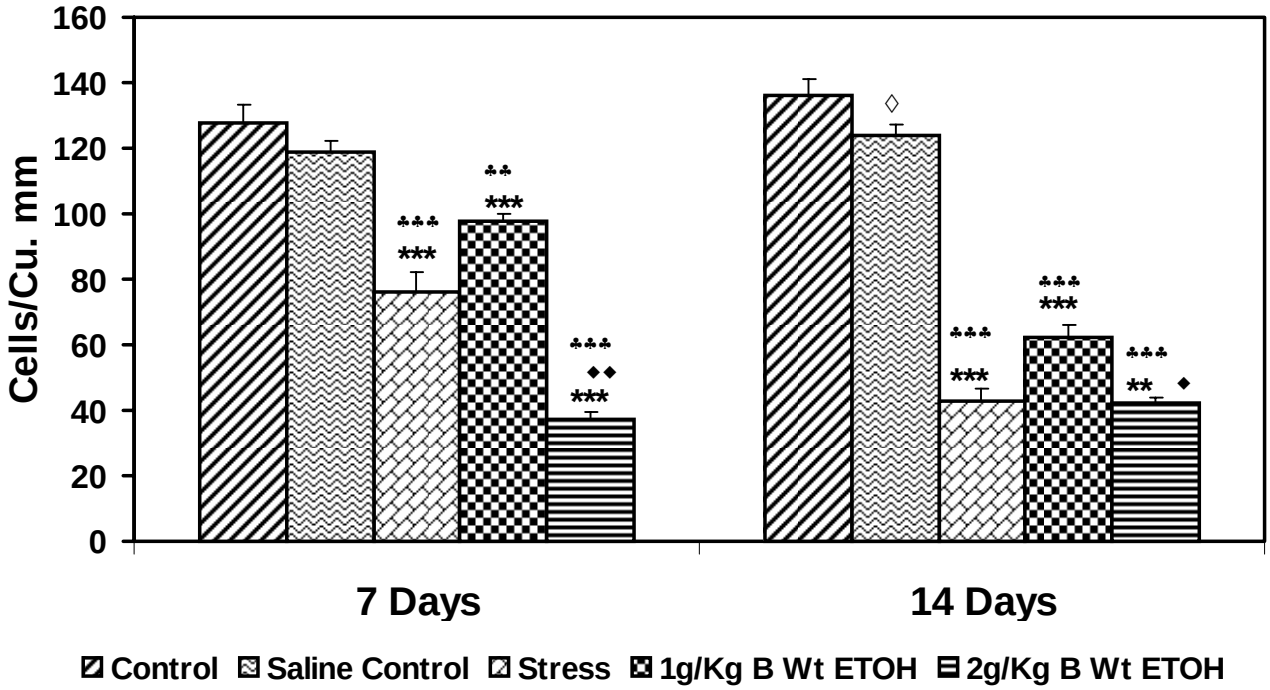

Figure 2. Effect of stress and ethanol treatment on eosinophil count; ∗ P<0.05, ∗∗ P<0.01, ∗∗∗P<0.001 – Control versus Experimental groups; ♣ P<0.05, ♣♣ P<0.01, ♣♣♣P<0.001 – Saline control versus Experimental groups; ◆ P<0.05, ◆◆ P<0.01, ◆◆◆ P<0.001 – Ethanol 1g/Kg BW versus 2g/Kg B. wt; ◊ P<0.05, ◊◊ P<0.01, ◊◊◊P<0.001 – Control versus Saline control

with ethanol (1g and 2g/Kg body weight), a similar increase in the transaminases were also observed (P<0.001). There was a significant rise in ALT level after 2g/Kg body weight ethanol in unstressed rats when compared to 1g/Kg body weight ethanol treatment (P<0.001) both in 7 days and 14 days sub-groups (Table 2). The biochemical changes observed in the saline control group was almost similar to the control group and a comparison with the stressed and ethanol groups indicated statistically significant changes in these parameters (Table 2).

Discussion

Exposure to isolation stress decreased body weight in the present study. The observed decrease in body weight could be due to the direct effect of stress on the food intake behavior of the rats (Marti et al., 1993). Stress might have increased the protein catabolism and hampered the utilization of food consumed during the stress period, thereby causing decrease in body weight. Increases in various organ weights after chronic isolation stress observed in this study confirm other previous reports (Sardessai et al., 1993). Increased secretion of the stress steroid, corticosterone is known to increase the metabolic activities and m-RNA levels in the hepatic cells. More amounts of proteins are required for repair of wear and tear caused by chronic stress and the metabolic changes are higher during stress. This might increase significantly the liver weight. An increase in the heart weight after stress might be a result of increasing workload on the heart during stress (Chang et al., 1995). Under the influence of stress, epinephrine and nor-epinephrine usually release more from the adrenal gland. These hormones increase the force of cardiac contraction that

gradually produces cardiac hypertrophy. Increased mass of kidneys after chronic stress may be a consequence of increased cardiac output and blood pressure during stress. The observed increase in

Table 2. Effect of isolation stress and ethanol treatment on biochemical parameters

Parameter	Duration	Control (N=10)	Saline Control (N=10)	Isolation Stress (N=10)	Ethanol 1g/Kg B wt. (N=10)	Ethanol 2g/Kg B wt. (N=10)
Blood Sugar (mg/d L)	7 days	96.26 ± 3.04	94.30±1.33	67.82±1.62 ***♣♣♣	87.17 ± 1.26 ***♣♣♣	62.83 ± 1.13 *** ♦♦♦♣♣♣
	14 days	94.36 ± 1.51	97.11±1.78	69.27 ± 1.59 ***♣♣♣	60.64 ± 0.68 ***♣♣♣	48.17 ± 1.12 *** ♦♣♣♣
Cholesterol (mg/d L)	7 days	98.25 ± 1.39	94.82±2.08	82.95 ± 0.72 ***♣♣♣	80.86 ± 1. 74 ***♣♣♣	76.14 ± 0.09 *** ♦♦♣♣♣
	14 days	96.37 ± 1.09	93.59±1.21	84.39 ± 1.95 ***♣♣♣	84.03 ± 1.43 ***♣♣♣	72.06 ± 1.05 *** ♦♦♣♣♣
AST (IU/L)	7 days	0.636 ± 0.014	0.653±0.039	5.659 ± 0.834 ***♣♣♣	6.611 ± 0.149 ***♣♣♣	6.989 ± 0.173 *** ♦♣♣♣
	14 days	0.620 ± 0.025	0.642±0.004	5.187 ± 0.438 ***♣♣♣	5.896 ± 0.125 ***♣♣♣	5.698 ± 0.174 ***♣♣♣
ALT (IU/L)	7 days	0.490± 0.013	0.511±0.029 ◊	6.453 ± 0.372 ***♣♣♣	3.820 ± 0.112 ***♣♣♣	5.853 ± 0.153 *** ♦♦♦♣♣♣
	14 days	0.505 ± 0.016	0.542±0.021 ◊	7.483 ± 0.326 ***♣♣♣	3.979 ± 0.136 ***♣♣♣	6.223 ± 0.261 *** ♦♦♦♣♣♣

Values are Mean ± SEM, N=Number of animals; ∗ P<0.05, ∗∗ P<0.01, ∗∗∗P<0.001 – Control versus Experimental groups; ♣ P<0.05, ♣♣ P<0.01, ♣♣♣P<0.001 – Saline control versus Experimental groups; ♦ P<0.05, ♦♦ P<0.01, ♦♦♦ P<0.001 – Ethanol 1g/Kg BW versus 2g/Kg B. wt; ◊ P<0.05, ◊◊ P<0.01, ◊◊◊P<0.001 – Control versus Saline control

kidney weight after stressful stimuli is in agreement with the reports by Chang et al. (1995). An increase in the adrenal gland weight is considered as one of the initial responses of the organism to various stresses. The increased adrenal gland weight after chronic isolation stress in the present study might be due to the chronic stimulation of the adrenal cortex by adrenocorticotropic hormone (ACTH) hypersecretion induced by stress. This is supported by the rise in corticosterone level of stressed rats. The increment is considered as an index of stress and its severity (Tuli et al., 1995).

Ethanol treatment at a dosage of 1g and 2g/Kg body weight in unstressed rats significantly increased liver, heart, kidney, and adrenal gland weights. Chronic ethanol administration is known to enhance the protein and fat accumulation in the liver. Ethanol and acetaldehyde is known to induce cell injury and necrosis by enhancing the production of reactive oxygen species by the process of lipid peroxidation. In addition, the cell injury forms protein products that activate the Kuppfer cells and fibroblasts (Kurose et al., 1996). Hypertrophy of the adrenal glands after ethanol treatment confirms other reports (Spencer and McEwen, 1990), which has been attributed to the excessive secretary activity of the adrenal glands. Because of the increased adrenal gland secretion

after ethanol treatment, there might be increased epinephrine and nor-epinephrine that increased cardiac contraction, leading to gradual cardiac hypertrophy. Increased secretion of these catecholamines due to ethanol administration could elevate blood pressure in rats, thereby increasing the workload on the kidneys. This could be the reason for increased kidney weight after 7 and 14 days of ethanol injections in unstressed rats.

There was a significant decrease in the absolute counts of neutrophil and eosinophil after chronic isolation stress for a period of 7 and 14 days. The observed decrease in neutrophil count may result from its abnormal distribution due to local chemotaxis that causing cell retention in several organs. Under the influence of stress, neutrophils might exit from the circulation because of increased capillary permeability due to local tissue damage. Eosinopenia after stress is a well-established phenomenon, which could be due to the increased secretion of stress steroids after chronic stress (Dhabhar et al., 1994).

Ethanol treatment in unstressed rats for a period of 7 and 14 days also significantly decreased absolute neutrophil and absolute eosinophil counts. Ethanol is known to depress the hemopoiesis in an organism and to produce vacuolation in the granulocyte precursors of the bone marrow. Neutropenia has been reported in patients consuming alcohol (Eichner and Hillman, 1971). Eosinopenia after ethanol treatment may be due to the direct effect of ethanol on adrenal gland secretion. Ethanol-induced corticosterone secretion might be the cause of decreased eosinophil count in the present study (Sipp et al., 1993).

Chronic isolation stress lasting for a period of 7 and 14 days produced a severe hypoglycemia rather than much anticipated hyperglycemia. The observation confirms the report by Rodnick et al. (1990). There are contradictory observations about blood sugar responses to chronic stress in rats. Several lines of evidence report hypoglycemia during stress that is similar to the effect of exercise. Physical training involving stress was found to increase the sensitivity of insulin to glucose in humans and rats. Decreased food intake along with increased insulin sensitivity (Stallknecht et al., 1990) in chronic stress might underline the observed hypoglycemia in this study.

Similar changes were observed in the biochemical parameters after administration of two different dosages of ethanol in unstressed rats. The fall in blood sugar level after ethanol has been very well documented and is a well-known phenomenon, called reactive hypoglycemia. Ethanol directly stimulates the beta cells of the pancreas and suppresses gluconeogenesis (Larue et al., 1990). Hepatocellular damage under the influence of ethanol would result in hyperinsulinemia due to diminished breakdown of insulin resulting in hypoglycemia (Shelmet et al., 1988).

The physiological mechanism of stress-induced changes in cholesterol remains unclear. Decreased serum cholesterol after isolation stress in the present study agrees with several other studies, but at the same time, contradicts a few (Malyszko et al., 1994). Chronic stress may increase cell damage and metabolism and then, circulating cholesterol is more taken up for the repair of cell membranes at various tissues. A persistent increase in serum transaminases (AST and ALT) after the stress duration of 7 and 14 days also support the possibility of cell membrane injury. In addition, increased activity of the HPA axis also contributes to the hypocholestrolemeia after chronic stress (Brennan et al., 1996).

A decrease in serum cholesterol after ethanol administration denotes higher esterification. This is attributed to a higher extraction of cholesterol from circulation for the contribution of esterification in the cells in alcoholics (Chari and Muddeshwar, 1991). Increased secretion of the steroidal hormones and the increased hepatocellular damage could be the reason for the significant elevation of serum transaminases with different doses of ethanol in unstressed rats.

In summary, the present study indicates that both isolation stress and chronic ethanol treatment alter physiologic and biochemical parameters in a similar manner. However, the degrees of changes depend on the stress duration, doses and duration of ethanol treatment.

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