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Impact of Some Experimental Procedures on Behavioral Patterns and Physiological Traits of Rats

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Abstract**—**Welfare may be considered to be a subjective experience; it has a biological function that is related to the fitness and survival of the animal accordingly, researches have suggested that welfare is compromised when the animal's evolutionary fitness is reduced. This study was carried out to explain the effect of some managerial stressors as handling and restraint on behavioral patterns and biochemical parameters of rats. A total of 24 (12 males & 12 females) Sprague-Dawley rats (12 months & 150-180g) were allotted into 3 groups, handled group (4 male & 4 female), restrained group (4 male & 4 female) and control group (4 males & 4 females).

The obtained results revealed that time spent feeding, drinking, movement and cage exploration frequencies increased significantly in handled rats than other groups, while lying time and licking increased significantly in restrained rats than handled and controls. Moreover, social behavior decreased in both stressed groups than control.

Triglycerides were significantly increased in handled rats than other groups, while total lipid, total protein and globulin significantly increased in both treated groups than control. Corticosterone increased in restrained and handled rats than control ones. Moreover, there was an increment in packed cell volume significantly in restrained rats than others. These deducted that if we want to study the effect of stress on animal welfare it is necessary to study the effect of such stressors on animal's behavior and physiological responses.

Keywords**—**Behavior, handling, restraint, rat, welfare.

I. Introduction

HUMANS have been using animals for a very long time, initially for food, transport and companion ship. The use of animals in experimental research parallels the development of medicine which had its roots in ancient Greece. The development of Cartesian philosophy in the 17th century meant that experiments on animals could be performed with little ethical concern. The discovery of anesthetics and Darwin's publication on the origin of species in 1859 emphasizing the biological similarities between humans and animals contributed to an increase in animal experimentation [8].

Researchers have suggested that welfare is compromised when animal's evolutionary fitness is reduced [7].Moreover, motivated behavior together with physiological data may provide a useful indicator of animal priorities, physical health and of the effects of environment, husbandry and experimental procedures performed on the animal [29].

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As animal welfare is a prerequisite for reliable experimental results, it is essential to seek for methods and procedures that will improve animal's well-being. Animal welfare and good science are inextricably connected. The environment of an animal however consists of a wide range of stimuli, including social environment of conspecific, contra specifics and humans as well as the physical environment of the cage and its contents. Housing and husbandry have a major impact on the laboratory animal throughout its life not only during but also before and after the experiment [57].

Furthermore, [25] reported that exposure to chronic mild stress decreased sucrose intake in rats. However, [42] found that chronic stress promoted palatable feeding which reduced the stress signs; they hypothesized that chronically restrained rats would exhibit a relative increase in comfort food ingestion. While [51] reported that long chronic stress procedure resulted in a strong decrease in sucrose preference. On contrary, [60] reported that stress increases the consumption of food.

Reference [12] studied the sexually dimorphic effects of prenatal stress on cognition, hormonal responses and central neurotransmitters and stated that in animal model disparate effects between males and females have been reported in the context of response to maternal deprivation, prenatal stress and restraint stress. Moreover, [14] reported that restraint stress elicited a reduction in general activity in male rats whereas female rats displayed prolonged increased activity following the repeated restraint stress exposure.

The objectives of the present study were to investigate the effect of repeated handling and restraint stress on behavior, serum glucocorticoid, hematological and biochemical parameters in rats.

II. Materials & Methods

A. Animals

A total number of 24 (12 males & 12 females) Sprague-Dawley rats (12 months & 150-180g) were allotted into 3 groups, handled group 8 rats (4 males & 4 females), control group 8 rats (4 males &4 females) and restrained group 8 rats (4 males & 4 females). They were housed in wire mesh cage with space allowance 225cm^2/rat (4 rats/cage).

B. Management

Rats were housed in wire mesh cages (30x50cm); with removable solid metal debris tray under cage floor. Animals fed diet contains 16.3% crude protein, 6.8% fat and 3.5% crude fiber. They were kept under natural light-dark cycle without artificial lighting. Temporary marking were carried

out by using stains but remarking would be necessarily at interval according to the disappearance of stain.

C. Handling Procedures

Rats were picked up by placing hand firmly over the back and rib cage and restraining head with thumb and forefinger just behind mandibles according to [19]. Each rat was handled (2 min) every day for 10 successive days during morning according to [21].

D. Restraint Stress

Each rat was restrained by inserting into strictly closed wire-mesh cylinder. The restraint period was 1 hr every day for 10 successive days [56].

E. Behavioral Observation

Focal sample observation was carried out according to [39], the pattern observed were ingestive, body care, resting, locomotor, investigatory and social behavior.

F. Blood Sampling

Blood samples were obtained from orbital venous plexus of ether anaesthetized rats according to [19] at 10th day of experiment. Two blood samples were collected from each rat. The 1st blood sample was for hematological examination and in tube containing EDTA, while, the 2nd blood sample was for subsequent analysis of corticosterone according to [43] and was left for 3 hrs at 4^{o}C then centrifuged at 3000 rpm for 15 minutes to obtain serum that was kept at -20^{o}C.

G. Hematological Measures

Determination of Hematocrit value (PCV) to detect the effect of handling and restraint stress on body fluid by using microhaematocrit technique [48].

H. Biochemical Measures

Blood serum was analyzed for determination of Glucose [55] mg/dl, Cholesterol [52] mg/dl, Total lipid [37] mg/dl, Triglycerides [47] mg/dl, Total protein [28] mg/dl, and Serum albumin [26] mg/dl, Globulins calculated by subtraction of albumin from total protein concentration and Albumin/ globulin ratio.

I. Statistical Analysis

Behavioral, hematological, and biochemical data were analyzed by two way analysis of variance ANOVA by SAS (Statistical Analysis system, version 6, 4th Edition, SAS Institute, Cary, NC. USA) proc GLM. Data was expressed as means±S.E.M. and P values <0.05 were considered significant in all tests, unless stated otherwise. Analyses of significant main effects of experimental treatment were performed using multiple range comparisons with Duncan multiple range test.

III. Results & Discussion

A. Behavioral Patterns

1. Ingestive, Resting, and Body Care Behavior

Motivated behavior together with physiological data may provide a useful indicator of animal priorities, physical health environmental effect, husbandry and experimental procedures performed on the animal [29].

The data presented in Table I showed that feeding time and drinking frequency increased significantly in handled rats than restrained and control groups (35.42±1.29 min/hr & 10.75±0.62 freq/hr vs. 10.41±0.88 min/hr & 2.95±0.31 freq/hr and 20.18±1.66min/hr & 5.15±0.53 freq/hr) respectively. However, standing time increased in restrained rats than handled ones (2.35±0.28 vs. 1.15±0.22min/hr).

Lying time increased in restrained rats than other groups (39.95±1.13 vs. 21.11±1.04 & 34.85±1.78min/hr). Furthermore, licking frequency increased in restrained rats than other groups (22.09±1.57 vs. 13.83±1.05 & 13.78±0.96freq/hr).

The obtained data showed that male rats exhibited longer feeding time, standing time and more drinking frequency than female rats. On contrary they spent short lying time than female. In addition, there was a non-significant effect of sex on licking and scratching frequencies. Similarly, [22] found that in rodent models effects of chronic stress are of far less magnitude in females than males.

Handling female rat significantly increased feeding time, drinking frequency and reduce lying time, while time spent feeding decreased in restrained male than other groups, moreover, drinking frequency increased in handled male than others significantly. On the other hand, restrained female exhibited significantly longer standing time and more licking frequency with less scratching frequency than handled and controls, furthermore, lying time and licking and scratching frequencies increased significantly in restrained male than handled and controls.

This increment in feeding time could be attributed to activation of CRH (corticotrophin releasing hormone) which lead to release of cortisol which increase the appetite [31]. Also, longer drinking bouts of handled rats could be attributed to the increased feeding bouts [5].

However, under stress animals engaged in more coping behavior which more important for an organism than eating [32] which explained the increase in resting behavior and the decrease in ingestive one of restrained rats. Moreover, intensity of stress-induced anorexia appeared to be related to the intensity of the stressful situation [35].

On the other hand, rats following chronic restraint stress procedure shift more rapidly from one vigilance state to another indicating that they have a less stable sleep-wake pattern [33] than before treatment. Moreover, restraint stress induces changes in a variety of factors that in turn are known to affect sleep, one upstream mediator of the restraint stress effects on sleep may be the corticotrophin releasing hormone a key regulator of the integrated stress response [27]. Exposures to restraint stress suppress food intake and body weight gain in rats [6], [30], [62].

Furthermore, [15] observed decreased REM sleep latency after chronic mild stress which returned close to baseline after 5wks of stress. Stress can release ACTH (adrenocorticotrophic hormone) which elicits grooming response as it is a displacement activity and stress related behavior [61].

Moreover, these results are in good agreement with [23] who reported that adrenocorticotrophic hormone (ACTH) induced body care behaviors in form of vibration, face washing, body grooming, anogenital, body shake, tail sniffing and combination of scratching and licking of paw. Moreover, adrenocorticotrophic hormones (ACTH) prolong self-grooming duration. Similarly, [32] observed that chronic stress increased the duration of self-grooming and comfort behavior.

TABLE I
LEAST SQUARE MEANS AND THEIR STANDARD ERRORS TO THE EFFECT OF HANDLING AND RESTRAINT OF BOTH SEXES ON BEHAVIOR OF RATS

Item		Ingestive behavior		Resting behavior		Body care behavior	
		Feeding Min/hr	Drinking Freq/hr	Standing Min/hr	Lying Min/hr	Licking Freq/hr	Scratching Freq/hr
Treatment							
Control		20.18±1.66^{b}	5.15±0.53^{b}	1.84±0.59^{ab}	34.85±1.78^{b}	13.78±0.96^{b}	10.94±0.82^{a}
Handled		35.42±1.29^{a}	10.75±0.62^{a}	1.15±0.22^{b}	21.11±1.04^{c}	13.83±1.05^{b}	11.75±1.14^{a}
Restrained		10.41±0.88^{c}	2.95±0.31^{c}	2.35±0.28^{a}	39.95±1.13^{a}	22.09±1.57^{a}	11.27±1.08^{a}
Sex							
Female		19.93±1.75^{b}	5.33±0.48^{b}	0.76±0.14^{b}	38.05±1.36^{a}	15.26±0.69^{a}	11.33±0.77^{a}
Male		24.32±1.10^{a}	7.26±0.53^{a}	2.82±0.43^{a}	26.82±1.10^{b}	17.75±1.30^{a}	11.30±0.89^{a}
Treatment*Sex							
	Control	11.18±1.13^{d}	2.60±0.34^{cd}	0.15±0.06^{c}	43.83±1.31^{a}	13.25±0.98^{c}	12.23±1.04^{abc}
Female	Handled	41.18±1.80^{a}	11.58±0.61^{a}	0.28±0.11^{c}	19.16±1.60^{d}	13.43±1.16^{c}	13.76±1.72^{ab}
	Restrained	4.92±0.95^{e}	1.80±0.26^{d}	1.85±0.34^{b}	47.40±0.95^{a}	19.36±1.23^{b}	8.05±1.02^{c}
	Control	27.79±2.29^{b}	7.77±0.84^{b}	3.53±1.12^{a}	25.39±2.63^{c}	14.34±1.68^{c}	9.62±1.26^{bc}
Male	Handled	29.67±1.31^{b}	9.90±1.09^{a}	2.05±0.39^{ab}	22.72±1.32^{cd}	14.21±1.73^{c}	9.79±1.45^{bc}
	Restrained	15.49±0.96^{c}	4.13±0.50^{c}	2.87±0.43^{ab}	32.31±1.14^{b}	24.62±2.76^{a}	14.75±1.79^{a}

Means within the same column carry different superscripts are significantly different.

2. Movement Activities, Social and Investigatory Behavior

The results presented in Table II showed that movement activities increased in handled rats than other groups (10.54±0.71 vs. 5.62±0.43&6.45±0.60 freq/hr) respectively, investigation of trough increased in handled rats than restrained ones while it is non-significant from control group, investigation of cage increased in handled rats than other groups (11.27±0.97 vs. 3.90±0.33 & 7.14±0.63freq/hr) respectively, while it decreased in restrained rats than others (3.90±0.33 vs. 11.27±0.97 & 7.14±0.63 freq/hr). Moreover, social behavior decreased in both treated groups than control ones (1.29±0.19 & 0.59±0.08 vs. 3.28±0.93freq/hr).

Movement, cage exploration and social frequencies were significantly higher in males than females while, others investigation was significantly less in males than females. This could be attributed to level of testosterone hormone in males which it may leads to more aggressive acts and increase muscularity of males than females. Moreover, males and females differ in their response to stress, in their sensitivity to steroid and in several behavior as well as emotional states, these differences result from sexual differentiation of the brain also are heavily influenced by the gonadal steroid environment during adulthood [2].

Movement and trough exploration frequencies increased significantly in handled female than others, while, movement and cage exploration frequencies were higher in handled male than restrained and controls significantly. However, social frequencies decreased in both male stressed groups than control. The frequency of cage exploration were less in restrained female than other groups, they exhibited much others exploration frequency. Restrained male exhibited less others exploration frequency. High CRH (corticotrophin releasing hormone) leads to activation of the sympathetic nervous system that increases the general activity [39]. Which may leads to increased movement activities in our study, similarly, acute stressors resulted in an increase in locomotor activity of laboratory rats [38].

Exposure of dominant rats to a severe stressor (18 hours immobilization) reduced aggressive behavior and inverted transiently dominant submissive relationship of pairs but exposure of submissive rats to the sever stressor resulted in only minor reductions of aggressive behavior in such animals [34]. This could be attributed to increment of fear related hormones which leads to depression [58].

The increment of exploration due to handling could be attributed to time spent investigating objects in a novel environment which has been found to be sensitive to prior exposure to stressors [9]. Similar finding was reported by [41] who showed that exposure of the animal to different durations of crowding and noise stress resulted in infrequent exploratory behavior.

On the other hand, decrement in exploratory activity in restrained rats could be explained by the fact that when a new stimulus is perceived by the animal a state of heightened awareness is induced, more information is gathered and an addition to the animal's experience occurs.

Some activities involve interactions with inanimate environment using species specific procedures which involve environmental testing and investigation [54]. These finding also stated by [53] who reported that a single or repeated sessions of immobilization reduced exploration in laboratory rodents.

TABLE II
LEAST SQUARE MEANS AND THEIR STANDARD ERRORS TO THE EFFECT OF HANDLING AND RESTRAINT OF BOTH SEXES ON BEHAVIOR OF RATS

Item		Movement Activities Freq/hr	Social Behavior Freq/hr	Investigatory Behavior		
				Cage Freq/hr	Others Freq/hr	Trough Freq/hr
Treatment						
Control		6.45±0.60^{b}	3.28±0.93^{a}	7.14±0.63^{b}	0.91±0.17^{a}	0.16±0.05^{ab}
Handled		10.54±0.71^{a}	1.29±0.19^{b}	11.27±0.97^{a}	0.93±0.15^{a}	0.23±0.06^{a}
Restrained		5.62±0.43^{b}	0.59±0.08^{b}	3.90±0.33^{c}	1.01±0.16^{a}	0.05±0.02^{b}
Sex						
Female		6.43±0.38^{b}	0.78±0.12^{b}	6.10±0.42^{b}	1.23±0.14^{a}	0.17±0.04^{a}
Male		8.64±0.62^{a}	2.66±0.63^{a}	8.95±0.78^{a}	0.68±0.11^{b}	0.13±0.04^{a}
Treatment*Sex						
	Control	4.88±0.54^{d}	0.10±0.05^{b}	6.41±0.78^{bc}	0.80±0.19^{bc}	0.10±0.05^{b}
Female	Handled	9.95±0.56^{ab}	1.70±0.28^{b}	8.08±0.67^{b}	1.20±0.24^{ab}	0.33±0.10^{a}
	Restrained	4.51±0.45^{d}	0.53±0.11^{b}	3.49±0.44^{d}	1.69±0.26^{a}	0.08±0.04^{b}
	Control	8.03±1.01^{bc}	6.45±1.72^{a}	7.87±0.99^{b}	1.03±0.28^{b}	0.23±0.10^{ab}
Male	Handled	11.13±1.30^{a}	0.88±0.26^{b}	14.73±1.7^{a}	0.65±0.15^{bc}	0.13±0.06^{ab}
	Restrained	6.72±0.69^{cd}	0.65±0.13^{b}	4.30±0.47^{cd}	0.35±0.09^{c}	0.03±0.02^{b}

Means within the same column carry different superscripts are significantly different

B. Biochemical and Hematological Measures

The findings presented in Table III revealed that Triglycerides level increased in handled rats (270.05±34.72vs. 161.83±13.83& 173.04±23.82mg/dl). Moreover, Total lipid level was significantly higher in both stressed groups (968.56±54.11 & 951.27±73.04vs. 804.72±41.70mg/dl). However, Total protein and Globulin levels increased significantly in handled than controls (10.60±1.07 & 6.70±1.40 vs. 7.70±0.60 & 4.82±0.73g/dl), while it was non-significant in restrained ones than controls.

On the other hand, in Table IV corticosterone level increased significantly in restrained and handled rats than control ones (816.00±188.4 & 530.00±32.56 vs. 233.33±32.93ng/ml) with highest in restrained rats. Moreover, the packed cell volume increased in restrained rats than other groups (33.75±1.49 vs. 30.25±0.80 & 27.50±1.12%). Furthermore, when studying the effect of sex it showed that male had higher levels for cholesterol and glucose, due to use of male for cholesterol in steroidogenesis and formation of testosterone along the life while the female use cholesterol in the synthesis of steroids at cycles only but the female also used the cholesterol in the synthesis of corticosterone as the cholesterol is a precursor for corticosterone synthesis. Moreover, the corticosterone level increased in male than female which could be explained by [22] who found that in rodent models effects of chronic stress are of far less magnitude in females than males. While there was a non-significant difference in levels of triglycerides, total lipid, total protein, globulin, albumin, A/g ratio and packed cell volume.

Handling male rats increased cholesterol, triglycerides, A/g ratio and albumin, the increment in cholesterol level could be attributed to concomitant haemoconcentration and sympathetic activation leading to release of catecholamines which increase synthesis of cholesterol [3] while, changes in triglycerides could be attributed to increase in the activity of lipoprotein lipase enzyme by stress [45]. Also, this increase in A/g ratio could be explained by increased level of albumin and decreased globulins level in handled male than other groups.

On contrary, total lipid, total protein and globulin increased in handled female than restrained and controls. However, restrained male showed higher levels of total lipid, corticosterone and packed cell volume than other groups. On the other hand, handled male showed lower level of globulin. Moreover, glucose level was higher in restrained female than handled and controls and decreased in restrained male than others. Furthermore, handled females showed a higher level of corticosterone than restrained and control ones. While, restrained male had an increased level of corticosterone than other groups which was the highest one.

During stress extra energy is supplied to blood in the form of metabolic fuels –namely, fatty acids and glucose. Catecholamines stimulate lipolysis in adipose tissue through activation of hormone sensitive lipase leading to the breakdown of triglycerols into fatty acids and glycerol. This effect is sensitized by cortisol [13]. The changes in their concentration, following stress, reflects increase in lipid biosynthesis or release into circulation or may be secondary to reductions in plasma volume. This leading to increased concentration of circulating proteins, after the changes in haemoconcentration have been recovered, the increase in lipid levels are no longer significant [4]. Moreover, it is measured in case of stressful condition by colorimetric method to estimate the lipolytic effect of stress.

It was supposed that higher level of triglycerides after handling stress could be attributed to the catecholamines effect which activate lipolysis in adipose tissue and increase the free fatty acid flow to the liver where increased triglycerides synthesis and secretion occur [47]. Similarly, [46] reported that in rats stressed by clamping of their hind limbs triglycerides level was found to be increased. Furthermore, [1] showed that immobilization stress not significantly alter triglycerides. On the other hand, the higher total lipid level in both stressors may be due to the statement of [18] who suggested that the increased sympathoadrenergic

activity is the underlying mechanism for stress-mediated increase in serum lipids.

Moreover, [24] stated that hepatic lipids accumulated in animals which exposed to a prolonged immobilization state due to decreased lipolysis and/or suppressed lipoprotein mobilization from the liver into the blood stream. However, the increase in total protein of handled rats could be explained due to the stress mediated decrease in plasma volume which leads to a haemoconcentration and increased plasma proteins [40]. On the other hand, [26] reported that there was a positive correlation between behavioral activity and the albumin conformational properties in rat groups subjected to acute immobilization stress. Moreover, albumin was increased in the highly active rats and decreased in low active ones.

A more possible mechanism for the current increase in globulin level of handled rats would be the indirect effect on immune function via changes in neuroendocrine status, although still in its infancy, that different stressors alter the levels of various neuroendocrine factors that in turn interact the immune system to disrupt function [50].

TABLE III
LEAST SQUARE MEANS AND THEIR STANDARD ERRORS TO THE EFFECT OF HANDLING AND RESTRAINT OF BOTH SEXES ON BIOCHEMICAL MEASURES OF RATS

Item		Cholesterol mg/dl	Triglycerides mg/dl	Total lipid mg/dl	Total protein g/dl	Globulin g/dl	Albumin/ Globulin Ratio	Albumin g/dl
Treatment								
Control		296.03±50.69^{a}	173.04±23.82^{b}	804.72±41.70^{b}	7.70±0.60^{b}	4.82±0.73^{b}	0.75±0.22^{a}	2.88±0.54^{a}
Handled		390.03±122.83^{a}	270.05±34.72^{a}	968.56±54.11^{a}	10.60±1.07^{a}	6.70±1.40^{a}	0.89±0.33^{a}	3.76±0.51^{a}
Restrained		247.35±16.98^{a}	161.83±13.83^{b}	951.27±73.04^{a}	8.95±0.39^{ab}	6.38±0.65^{ab}	0.44±0.08^{a}	2.64±0.24^{a}
Sex								
Female		205.13±17.19^{b}	194.68±24.92^{a}	882.08±45.29^{a}	8.91±0.82^{a}	5.78±0.90^{a}	0.70±0.15^{a}	3.05±0.28^{a}
Male		431.94±69.98^{a}	213.04±29.50^{a}	950.47±59.92^{a}	9.12±0.55^{a}	6.05±0.68^{a}	0.70±0.24^{a}	3.17±0.47^{a}
Treatment*Sex								
	Control	202.14±46.15^{b}	186.96±33.75^{ab}	866.35±29.37^{bc}	6.96±0.63^{b}	3.42±0.25^{c}	1.09±0.29^{ab}	3.54±0.79^{ab}
Female	Handled	190.31±25.38^{b}	249.27±59.42^{ab}	981.14±115.29^{ab}	12.18±0.95^{a}	9.38±1.23^{a}	0.32±0.10^{b}	2.78±0.34^{b}
	Restrained	219.23±11.49^{b}	147.82±12.88^{b}	798.75±44.29^{bc}	8.23±0.38^{b}	5.32±0.49^{bc}	0.56±0.07^{b}	2.83±0.16^{b}
	Control	421.22±3.02^{ab}	152.17±39.13^{b}	712.27±23.59^{c}	8.68±0.94^{b}	6.69±0.73^{b}	0.30±0.05^{b}	2.00±0.37^{b}
Male	Handled	589.74±186.82^{a}	290.82±45.44^{a}	955.97±34.48^{ab}	9.02±1.52^{b}	4.02±1.03^{c}	1.46±0.46^{a}	4.75±0.66^{a}
	Restrained	284.84±22.87^{b}	175.84±24.38^{ab}	1103.78±38.13^{a}	9.67±0.34^{ab}	7.43±0.85^{ab}	0.33±0.12^{b}	2.46±0.46^{b}

Means within the same column carry different superscripts are significantly different.

These are in agreement with [11] who reported that total protein and globulin levels were significantly elevated during stress period and for next 16 days. Similarly, immobilization of rats has no change in plasma glucose [20].

TABLE IV
LEAST SQUARE MEANS AND THEIR STANDARD ERRORS TO THE EFFECT OF HANDLING AND RESTRAINT OF BOTH SEXES ON BIOCHEMICAL MEASURES OF RATS

Item		Glucose mg/dl	Corticosterone ng/ml	PCV %
Treatment				
Control		84.81±14.96^{a}	233.33±32.93^{c}	27.50±1.12^{b}
Handled		93.43±19.14^{a}	530.00±32.56^{b}	30.25±0.80^{b}
Restrained		79.27±5.41^{a}	816.00±188.4^{a}	33.75±1.49^{a}
Sex				
Female		60.00±6.78^{b}	395.00±52.06^{b}	29.50±1.05^{a}
Male		112.63±8.84^{a}	611.11±137.16^{a}	31.50±1.28^{a}
Treatment*Sex				
	Control	66.49±13.78^{cd}	273.33±46.67^{cd}	27.50±1.89^{b}
Female	Handled	40.23±4.36^{d}	540.00±60.00^{b}	28.50±0.50^{b}
	Restrained	73.28±6.21^{c}	360.00±60.00^{c}	32.50±1.89^{ab}
	Control	112.29±20.25^{ab}	193.33±40.55^{d}	27.50±1.50^{b}
Male	Handled	133.34±5.87^{a}	520.00±40.00^{b}	32.00±0.82^{ab}
	Restrained	85.25±8.47^{bc}	1120.00±40.00^{a}	35.00±2.38^{a}

Means within the same column carry different superscripts are significantly different.

The increment of glucose level of restrained female could be attributed to the release of catecholamines which induce liver glycogenolysis and also to the gluconeogenesis activity of corticosteroids [47].These reported also by [17]who found that acute immobilization stress for none previously immobilized animals significantly increased blood glucose. Furthermore, [44] found that application of single immobilization stress significantly increased glucose level while chronic stress did not change blood glucose. On the other hand, [49] stated that decreased food intake along with increased insulin sensitivity in chronic stress might underline the observed hypoglycemia in our results of restrained male.

In naïve rats plasma corticosterone concentrations was found to be increased after acute stress exposure (handling, restraint and immobilization). Although it is not easy to determine stressor intensity which seems to be determinant in the level of the induced stress reactions, they themselves resulted in short and long-lasting alterations [16]. On the other hand, the mechanism of increasing corticosterone level in response to acute stress could be interpreted according to [36] who stated that stress stimulates hypothalamus to release corticotrophin releasing hormone which stimulate pituitary gland to release adrenocorticotrophic hormone which enhance the adrenal cortex to secret glucocorticoids. Moreover, these results are similar to those reported by [59] who stated that immobilization stress for 30 minutes per day 7-13 day postnatal induced high corticosterone levels and did not return to baseline until at least 120 minutes after the termination of

stress. Furthermore, [10] found high basal corticosterone level in rats which exposed to an unpredictable (variable) stress paradigm.

These increase in packed cell volume of restrained rats than others could be attributed to the stress mediated decrease in plasma volume which leads to a haemoconcentration [40].

IV. Conclusion

It could be concluded that there was behavioral, hematological and biochemical changes due to handling and restraint stress.

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