

responsiveness to a changing environment and susceptibility for high blood pressure leading to hypertension in chronically stressed rats (4, 5, 6). Cardiac electrical abnormalities of different nature were documented on exposure to social stress in rats (7). Psychosocial stress affected cardiovascular and adrenal physiology significantly in five different types of rat strains (8). Exposure to various stressful stimuli trigger the activation of hypothalamo pituitary adrenal (HPA) axis and the sympathetic adrenomedullary system. There is considerable evidence showing that the HPA response is progressively reduced after repeated exposure to the same stressor. This phenomenon called habituation was shown to occur following exposure to various chronic intermittent stressors such as physical restraint, noise etc. (9, 10).

In the present work, it was aimed to study the effect of different physical and psychological stressors of both acute and chronic nature on the heart rate and electrophysiological changes in albino rats in order to compare the cardiovascular responses to a variety of stressors.

METHODS

Wistar strain, adult male albino rats with the body weight ranging between 150–250 g. were selected for the experimental study. Rats were housed under ideal laboratory conditions with food and water provided *ad libitum*. Animals were divided into six groups for the experiments.

Group I: Control group; Rats were kept in the standard laboratory conditions without being exposed to any kind of stress.

Group II: Isolation stress; Rats were divided into three subgroups. Animals were kept individually in a specially designed 'isolation cage' and isolated totally. Isolation stress was studied for a period of 7 days, 15 days and for 30 days in three different subgroups.

Group III: Immobilization stress; Rats were restrained in the prone position in the restraining wooden boards for a period of one hour daily. Immobilization stress study lasted for 7 days in one subgroup, for 15 days in the second subgroup and for 30 days in the third subgroup.

Group IV: Overcrowding stress; Group of animals were kept in small polypropylene cages (25 cm × 20 cm × 15 cm) in such a way that minimum movement was possible for the rats inside their cages. This overcrowding condition itself was stress for the rats. Overcrowding stress was studied in three subgroups for 7 days, 15 days and 30 days.

Group V: Fresh water swimming stress; In this type of stress, rats were forced to swim in a small plastic tub (height: 60 cm, diameter: 40 cm) containing water at room temperature (28°C). Water depth was always maintained at 30 cm. The forced swimming stress was studied for 15 days in one subgroup, for 7 days in another subgroup and rats were forced to swim only for 45 min on first day in another subgroup and this was taken as one day fresh water swimming subgroup.

Group VI: Cold water swimming stress; Rats were forced to swim in the cold water maintained at 10°C. Cold water swimming

stress was also studied for a period of 15 days, 7 days and for one day as in the case of fresh water swimming stress.

All the experiments were carried out between 10 AM to 12 Noon to minimise the circadian variability. At the end of the stress period the animals were anaesthetised by injecting sodium pentobarbitol (40 mg/kg, ip) and the electrocardiograms were recorded in limb lead II. Rats were sacrificed by decapitation and blood samples were collected and serum was separated. Laparotomy was done and different organs like heart and adrenals were removed. The wet weight of the organ was expressed per 100 g of the body weight. From the serum samples, serum transaminases were estimated by colorimetric method (11). Electrocardiograms were analysed; heart rate and P-R interval were calculated.

All the values were expressed as Mean \pm SEM. Statistical analysis was done by one way analysis of variance (ANOVA). The P value less than 0.05 was considered statistically significant.

RESULTS

Heart weight increased significantly after 7 days of isolation stress ($F=3.87$; $P<0.05$) and 7 and 30 days of overcrowding stress ($F=12.80$; $P<0.00001$) (Fig. 1). 15 days of isolation stress had significantly lower weight than 7 days of stress. In the immobilization stress, 7 days and 15 days stress subgroup showed a significant increase ($F=7.67$; $P<0.00001$) in the heart weight. 30 days immobilization was having significantly lower weight than 7 days of immobilization stress. Overcrowding stress

for 7 days was having significantly higher heart weight than 30 days ($F=21.66$; $P<0.00001$) and 15 days stress subgroups (Fig. 1). Heart weight increased significantly ($F=46.80$; $P<0.00001$) in both 7 days and 15 days of fresh water swimming and cold water swimming stress (Fig. 2). Among the different types of stress, cold water swimming stress for 7 days had significantly higher heart weight ($F=37.63$; $P<0.00001$) than all other stress groups. Isolation stress for 7 days had significantly lower heart weight than all other types of stress. After 15 days, fresh water swimming stress had significantly higher ($F=8.91$; $P<0.00001$) heart weight than other types of stress for the same duration, and isolation stress for 15 days resulted in significantly lower heart weight than all other types of stress groups.

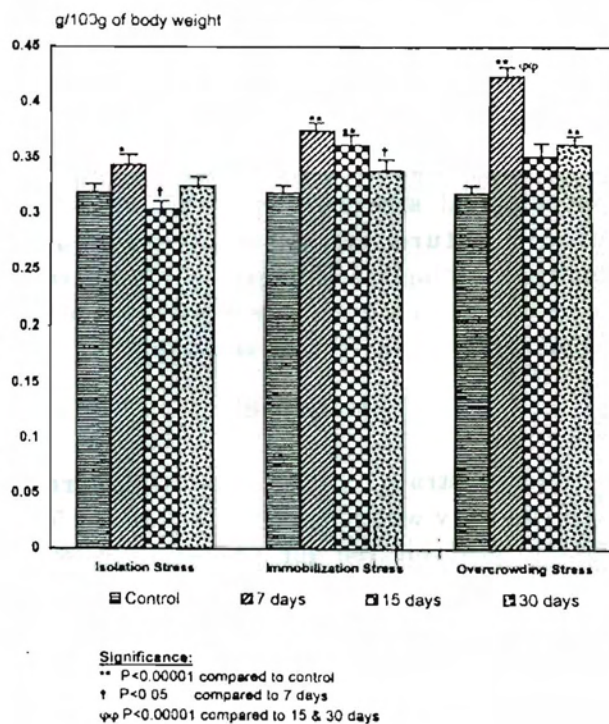
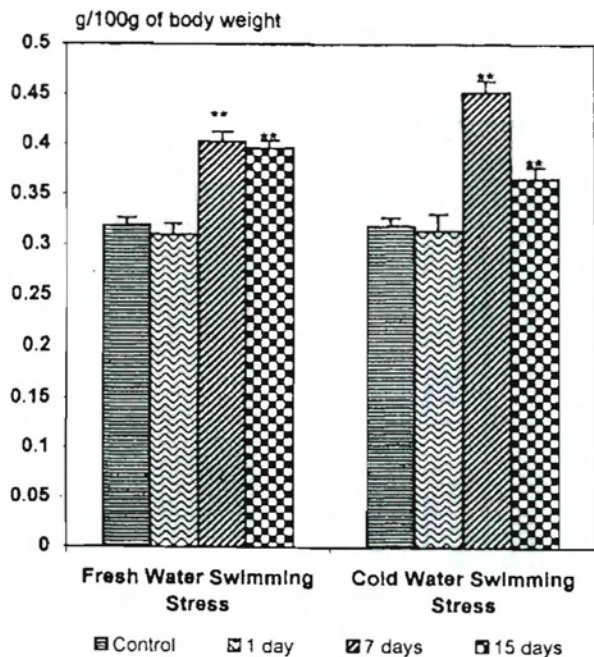


Fig. 1 : Heart weight in isolation, immobilization and overcrowding stress.

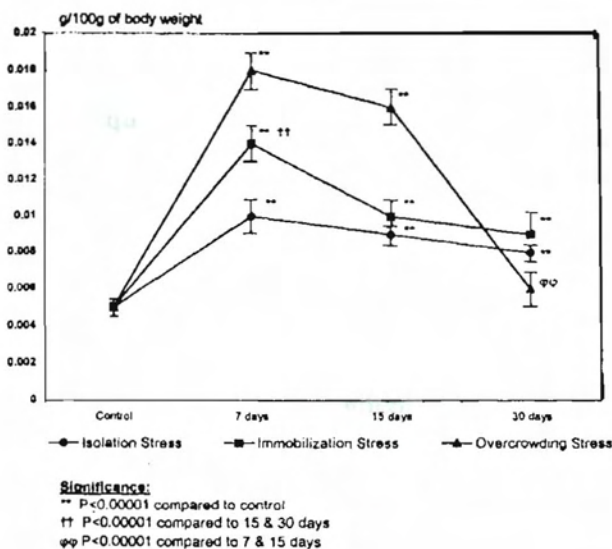


Significance:

** P<0.00001 compared to control

Fig. 2 :Heart weight in fresh water swimming and cold water swimming stress.

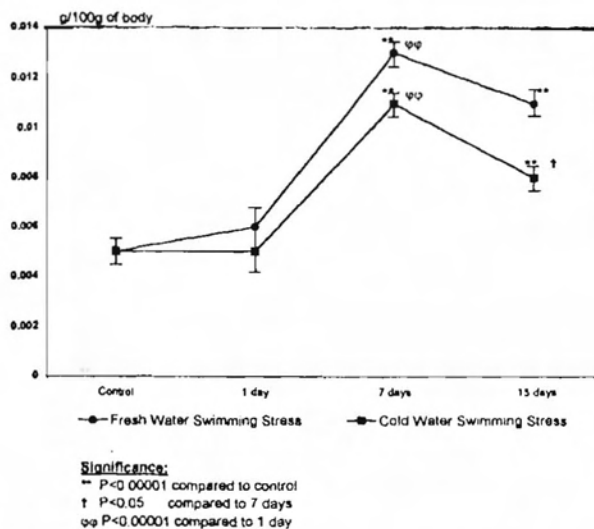
Adrenal gland weight increased significantly ($F=19.07$; $P<0.00001$) in all the three durations of isolation and immobilization stress (Fig. 3). 7 days subgroup was having significantly higher weight than 15 to 30 days subgroups ($F=19.55$; $P<0.00001$) in the case of immobilization stress. In the overcrowding stress, adrenal gland weight increased significantly after 7 days ($F=65.58$) and 15 days stress exposure ($P<0.00001$). 30 days overcrowding stress had significantly reduced adrenal weight ($F=4.12$; $P<0.00001$) than other two subgroups. Adrenal weight increased significantly ($F=10.85$; $P<0.00001$) in both 7 days and 15 days subgroup of fresh water swimming and cold water swimming stress (Fig. 4). 7 days stress was having significantly higher weight than one day stress exposure.



Significance:

** P<0.00001 compared to control
 †† P<0.00001 compared to 15 & 30 days
 ††† P<0.00001 compared to 7 & 15 days

Fig. 3 : Adrenal weight in isolation, immobilization and overcrowding stress.



Significance:

** P<0.00001 compared to control
 † P<0.05 compared to 7 days
 †† P<0.00001 compared to 1 day

Fig. 4 :Adrenal weight in fresh water swimming and cold water swimming stress.

Heart rate increased significantly ($F=2.85$; $P<0.05$) after 7 days of isolation stress. But compared to 7 days stress, heart rate decreased significantly after 15 days ($F=2.52$; $P<0.05$) and 30 days ($F=2.72$; $P<0.05$) of isolation stress (Table I). In the immobilization

stress also heart rate increased significantly after 7 days of stress ($F=9.51$; $P<0.00001$). 7 days subgroup was having significantly higher heart rate than 15 days and 30 days subgroups. After overcrowding stress, no significant change in the heart rate was observed in all the three subgroup (Table I). Increased heart rate was also observed after one day and 7 days of fresh water swimming stress ($F=8.51$; $P<0.00001$) and also after one day cold water swimming stress ($F=12.74$; $P<0.00001$) (Table II). 15 days subgroup was having significantly lower heart rate than one day and 7 days stress subgroup in both fresh water swimming and cold water swimming stress. There was a significant increase in the heart rate after one day fresh water swimming stress ($F=10.55$; $P<0.00001$) when compared to one day cold water swimming stress. After 7 days stress, immobilization showed significant increase in the heart rate than other types of stress. But after 15 days stress, there was no significant difference in the heart rate among different subgroups.

P-R interval increased significantly after one day fresh water swimming stress ($F=12.17$; $P<0.00001$). One day stress exposure was having significantly higher P-R interval than 7 days and 15 days stress exposure (Table II). But cold water swimming, isolation and immobilization stress did not show statistically significant change in the P-R interval in the different subgroups (Table I and Table II). In the case of overcrowding stress, P-R interval increased significantly ($F=5.99$; $P<0.00001$) after 30 days of overcrowding stress. 30 days of overcrowding stress resulted in significantly higher P-R interval than 7 days and 15 days stress subgroups.

There was no change in the pattern of ECG recorded from limb lead II in all the stress

TABLE I : Heart rate and P-R interval in Isolation, Immobilization and Overcrowding Stress.

Parameter	Control (n=10)	Isolation stress			Immobilization stress			Overcrowding stress		
		7 Days (n=10)	15 Days (n=10)	30 Days (n=10)	7 Days (n=10)	15 Days (n=10)	30 Days (n=10)	7 Days (n=7)	15 Days (n=7)	30 Days (n=7)
Heart Rate (per min)	362±8	397±17*▲	355±17	345±6	430±11**▲	345±17	392±9	379±12	389±19	379±12
P-R Interval (sec)	0.045±0.002	0.044±0.003	0.042±0.002	0.050±0.003	0.042±0.003	0.044±0.003	0.040±0.000	0.043±0.003	0.040±0.000	0.057±0.003**▲▲

Values are expressed as Mean ± SEM

Significance:

- * $P<0.05$ compared to control
- ** $P<0.00001$ compared to control
- ▲ $P<0.05$ compared to 15 & 30 days
- ▲▲ $P<0.00001$ compared to 7 & 15 days

TABLE II : Heart rate and P-R interval in Fresh Water Swimming and Cold Water Swimming Stress.

Parameter	Control (n=10)	Fresh water swimming stress			Cold water swimming stress		
		1 Day (n=9)	7 Days (n=10)	15 Days (n=10)	1 Day (n=10)	7 Days (n=10)	15 Days (n=10)
Heart Rate (per min)	362±8	411±9**	390±11**	348±8 [▲]	387±2**	376±1	344±2* [▲]
P-R Interval (sec)	0.045±0.002	0.056±0.003***♦	0.040±0.000	0.040±0.000	0.048±0.003	0.046±0.003	0.044±0.003

Values are expressed as Mean ± SEM

Significance :

- * P<0.05 compared to control
- ** P<0.00001 compared to control
- ▲ P<0.05 compared to 1 & 7 days
- ♦♦ P<0.00001 compared to 7 & 15 days

subgroups. No arrhythmia was detected, no extrasystole was found and mean electrical axis did not show any change in all the types of stress exposure.

Serum transaminases (SGOT and SGPT) increased significantly in all the types of stress studied in all the subgroups (Table III and Table IV). 15 days isolation stress showed more significant increase in the transaminase level than 7 days stress subgroup (F=95.45; F=76.70; P<0.00001). In the case of immobilization stress, 30 days stress exposure was having significantly more SGOT level than 7 days stress subgroup (F=56.45; P<0.00001) (Table III). 15 days of overcrowding stress was having significantly higher SGOT and SGPT level than 30 days stress subgroup (F=51.02; F=65.10; P<0.00001). One day fresh water swimming stress showed significantly higher transaminase level than 7 and 15 days stress (F=204.79; P<0.00001) (Table IV). After 15 and 30 days stress exposure, isolation stress was showing significantly higher SGOT level than other types of stressor. The SGPT level increased significantly more after isolation stress in 7 and 15 days subgroup, when

compared to other types of stress for the same duration.

DISCUSSION

The significant increase in the weight of the heart after the rats were exposed to different types of stress confirms the various reports published already (12, 13). Increased weight of the heart in the initial period of exposure to stress could be because of the stress induced increase in the workload on the heart. Stress is known to induce more secretion of epinephrine and norepinephrine from adrenal gland (14). These hormones acting on the heart exert positive influence on the force of contraction (inotropism) which gradually might have caused the cardiac hypertrophy. When the stress period was prolonged to 30 days, there was no further significant increase in the weight of the heart. This type of response for the prolonged stress explains the partial adaptation of the animals to the stressful stimuli. During stress there is uniform arousal of both the fight-flight sympatho-adrenal and the pituitary adrenal cortical systems. These two systems acting together

TABLE III : Serum Transaminase level (IU/L) in Isolation, Immobilization and Overcrowding Stress.

Parameter	Control (n=10)	Isolation stress			Immobilization stress			Overcrowding stress		
		7 Days (n=10)	15 Days (n=10)	30 Days (n=10)	7 Days (n=10)	15 Days (n=10)	30 Days (n=10)	7 Days (n=7)	15 Days (n=7)	30 Days (n=7)
SGOT	0.585 ± 0.029	4.337 ± 0.452**	7.678 ± 0.382**▲▲	10.100 ± 0.398**	3.772 ± 0.477**	4.574 ± 0.452**	5.310 ± 0.112**▲▲	7.310 ± 0.427**	7.813 ± 0.405**▲▲	2.971 ± 0.200**◆◆
SGPT	0.648 ± 0.021	9.590 ± 0.043****	9.681 ± 0.543**	3.693 ± 0.139**◆◆	2.815 ± 0.141**	6.747 ± 0.189*φφ	3.521 ± 0.136*φφ	5.142 ± 0.215**	4.112 ± 0.263*††	2.745 ± 0.148**

Values are expressed as Mean ± SEM

Significance :

- ** P<0.00001 compared to control
- ▲▲ P<0.00001 compared to 7 days
- ◆◆ P<0.00001 compared to 7 & 15 days
- †† P<0.00001 compared to 30 days
- φφ P<0.00001 compared to 7 & 30 days

participate in the stress response of an organism. The observed increase in the adrenal weight could be attributed to the well documented hypertrophy and hyperplasia of the adrenal cortical cells under the influence of stress (15, 16).

The heart rate increase was seen when the animals were exposed to different types of stress only in the initial period of exposure to stress, upto 7 days. But when the stress period was prolonged to 15 to 30 days, the tachycardiac response was reduced gradually. Increased rate and force of cardiac contraction is considered as the immediate response of the organism to stress (17). Tachycardia was reported by several workers as a response to both acute and chronic stress (18, 19). In the case of P-R interval, not much significant change was observed after exposing the animals to stress. Eventhough a significant change was seen in the heart rate, no significant difference was seen in the P-R interval in the different types of stress. Different types of stresses employed in this study could not have affected the conduction of cardiac impulse and hence the normal P-R interval.

The observed increase in the serum SGOT and SGPT was in accordance with the various other reports already published (20, 21). SGOT and SGPT are better markers for hepatic functional status as they indicate parenchymal liver damage. The observed increase in these transaminases in stressful situations might be as a result of cortisol induced gluconeogenesis in the liver (22, 23). Of the two transaminases, SGOT level is particularly interesting to correlate with

TABLE IV: Serum transaminase level (IU/L) in fresh water swimming and cold water swimming stress.

Parameter	Control (n=10)	Fresh water swimming stress			Cold water swimming stress		
		1 Day (n=9)	7 Days (n=10)	15 Days (n=10)	1 Day (n=10)	7 Days (n=10)	15 Days (n=10)
SGOT	0.585 ± 0.029	6.488 ± 0.049***	4.459 ± 0.230**	5.155 ± 0.292**	3.520 ± 0.056**	4.181 ± 0.109**	4.194 ± 0.095**
SGPT	0.648 ± 0.021	10.461 ± 0.094***	4.207 ± 0.120**	5.278 ± 0.178**	2.450 ± 0.026**	3.997 ± 0.208**	4.866 ± 0.196**

Values are expressed as Mean ± SEM

Significance:

**P<0.00001 compared to control

♦♦P<0.00001 compared to 7 & 15 days

myocardial damage also because, the duration and extent of increase in this transaminase can be related to the size of infarct if any. It was reported that, in about 3-8 hours after the onset of myocardial infarction, SGOT level increased significantly, but the SGPT level was known to increase only to a small extent (24, 25). Interestingly our ECG analysis revealed no possible myocardial infarction after exposure to stress. In our study, there was persistent increase in the SGOT and SGPT level in different types of stress even when the stress duration was prolonged. This increase may also be due to the change in the cell membrane characteristics induced by stress. There could be increase in the cardiac cell membrane permeability in the tissues which might have released these intracellular enzymes into circulation. Probably circulating level of creatine phosphokinase (CPK) might be a better index for cardiac cell membrane permeability changes.

In the present study, there was a significant rise in the heart rate, heart weight, adrenal weight and also serum transaminase level after different types of

stress. Significant increase in the organ weight reflect the intensity of the stressor in rats. The heart rate change provides indirect information on the activity of the autonomic nervous system which play a major role in response to stress. Psychological stressors like immobilization and the physical stressor like forced swimming had greater effect on the cardiovascular response. It was reported that during stress, baroreceptor reflex control of heart rate was lessened and this reduced baroreceptor sensitivity might enhance the cardiovascular response to stress (26). The observation in this study indicated that after an initial increase in the heart rate, the tachycardiac response was reduced when the stress period was prolonged beyond 7 days. Weight of the heart also did not increase further after 15 and 30 days of stress exposure. These changes indicate that there could be partial adaptation to stressful stimulations. This may be because of the adaptation of the hypothalamo-pituitary-adrenal axis when same kind of stress is repeatedly applied for a long period of time (27).

In conclusion, stressors like forced swimming, isolation, immobilization and overcrowding produced a more significant increase in the heart rate, heart weight and serum transaminase levels. Heart rate

increased only in the initial period of exposure to the stressor. Physical stressor like forced swimming produced a significant cardiovascular response than the

psychological stressors like isolation and overcrowding stress. Partial adaptation was seen when the animals were exposed to chronic stress.

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