

## Effect of *Ocimum Sanctum* Linn (Tulsi) on body weight and some biochemical parameters in restraint stressed albino rats

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**Background:** With the increased trend of urbanization of our society, the incidence of various stress related diseases are increasing day by day. People under chronic stress often tends to seek relief through drugs, which may have side effects. But some natural supplements called adaptogen, can be more beneficial in reducing symptoms of stress. **Objective:** The present animal model experimental study was designed to observe the effects of restraint stress on body weight, serum ALT, AST, Glucose, Cholesterol and their modifications by *Ocimum sanctum* Linn (*tulsi*) pretreatment which is an Ayurvedic adaptogen and has a long history of therapeutic use. **Study design:** For this purpose, 30 albino rats aged 90 to 120 days were included in this study. Twenty rats of experimental group were further subdivided into two groups. One group consisted of 10 rats exposed to one hour restraint stress daily for 7 days and 10 rats of the second group were pretreated with *tulsi* for 7 days before exposure to stress in the same way. 10 non stressed, non pretreated rats were taken into control group. This study was undertaken in the laboratory of the Department of Physiology, BSMMU during the period from July 2003 to June 2004. **Methods:** The body weight of all control rats were recorded daily in the morning. Body weight of the experimental rats were recorded just before exposure to stress. After completing the experiment, the animals were sacrificed and blood was collected. Serum glucose, cholesterol and ALT, AST were determined by standard laboratory technique. Data were compared among the groups and the results were statistically analyzed using unpaired student t' test. **Results:** The body weight in untreated stressed group was significantly lower ( $p < 0.001$ ) than those of the control group and *tulsi* pretreated group. Serum levels of glucose, cholesterol, aminotransferases (ALT and AST) were significantly higher ( $p < 0.001$ ) in stressed group than those of control. Again in *Tulsi* treated group all these biochemical parameters were significantly lower ( $p < 0.001$ ) than those of stressed group. This restraint stress-induced changes in body weight and biochemical parameters may be due to hypophagia, altered secretion of various metabolic hormones and neurotransmitters, changes in membrane permeability & hypovolaemia resulting from stress induced secretion of corticosterone and epinephrine through hypothalamo-pituitary adrenal axis activation. **Conclusion:** Prevention of stress induced changes in biochemical parameter by *tulsi* pre treatment indicates its anti stressor effect.

**Key words:** Restraint stress; adaptogen; *Ocimum sanctum* Linn (*tulsi*); glucose; cholesterol; ALT; AST.

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### Introduction:

**S**tress is a condition of highly individualized response of an organism to external or internal challenges which one can control with difficulties or cannot control. Most of the scientists define, stress as the situation when

activation of hypothalamo-pituitary adrenocortical (HPA) axis occurs with consequent elevated adrenocorticotrophic hormone level<sup>1</sup>. Other investigators suggest the involvement of activation of different

systems, such as autonomic nervous system with or without elevation of ACTH<sup>2,3</sup>.

Stressor may be external or internal. External stressors include adverse physical environment such as exposure to excessive light, sound, immobilization etc. and stressful psychological environment like poor working condition or abusing relationship. Similarly internal stressors can also be physical like infection, inflammation or psychological such as anxiety, fear and frustration.

It has been accepted that central nervous system plays an important role on elicitation and modulation of compensatory stress response pattern. A variety of neurotransmitters and neuromodulators are released in various brain regions during exposure to stress, which then act on specific neuronal circuits to optimize an effective, rapid and efficient responses to restore disturbed homeostasis and ensure minimal damage to other organs<sup>4</sup>. Acute stress responses are usually of short lasting and less marked. In chronic stressful situations, responses are prolonged, and hyper active due to repeated stimulation. Elevated hormone levels for prolonged period can become dangerous for an organism manifested by significant changes in behavior, functional ability, body weight, biochemical and immunological systems. So, overstimulated stress response can cause damage to the various organs<sup>5</sup>.

Seelye et al. was the first researcher who used immobilization stress (restraint stress) in rats to explore the manifestation of stress syndrome i.e., adrenal hypertrophy, gastric ulceration and thymicolymphatic involution<sup>6</sup>. In stress management, relaxation and biofeedback can help to treat chronic stress hazards especially in combination with medicine<sup>7</sup>. But all these may lead to side effects to the patient. Now a days, a range of natural supplement such as herbal medicine, known as adaptogens which are devoid of side effects, can be more beneficial in reducing stress-induced symptoms<sup>7</sup>. Adaptogen is an agent that allows the organism to encounter adverse physical, chemical or biological stressors by raising nonspecific resistance towards such stress though

the mode of action is not fully understood, but considerable amount of evidence suggest that its effects are mediated by the pituitary and hypothalamo- pituitary adrenal (HPA) axis<sup>8</sup>. In addition, Adaptogens are also having antioxidant, anti-cancer, immunomodulator, hypocholesterolaemic and hypoglycemic effects<sup>9</sup>. One of the ayurvedic adaptogen is *Ocimum sanctum* Linn known as holy basil in English, as *tulsi* in Hindi. It has a long history of medicinal use in pain, fever, vomiting, bronchitis, earache and disease of heart and blood<sup>10</sup>. To combat restraint stress induced changes and their prevention by treatment with adaptogen especially by *O. sanctum* Linn (*tulsi*) are not well documented. It has been speculated that restraint stress induced changes are noticeable in body physiology which is reflected by changes in body weight and some biochemical parameters. Again, such changes can be reversed or prevented with an adaptogen such as *tulsi*. So, this study was designed to observe the effects of *tulsi* on some biochemical parameters like serum glucose, cholesterol, alanine aminotranfease (ALT) and aspartate aminotransferase (AST) as well as on body weight of a group of rats exposed to restraint stress.

#### Methods:

This is a prospective experimental study carried out in the department of Physiology, BSMMU Dhaka during the period from July 2003 to June 2004. For the purpose of study, 30 male long Evans strain Albino rats of 90 to 120 days old, weighing 150 to 200 g were included and collected from animal house of pharmacology department, BSMMU. The rats were housed properly in numbered iron cages in a clean animal house with an optimal room temperature (25 to 30° C) and were kept on basal laboratory diet and water ad libitum for one week before commencing the experiment for acclimatization.

Rats were divided into three groups according to the state of exposure to stress. Each group consisted of 10 rats. Non stressed control group (Group A) were not put on restrain stress. Stress group (Group B) were exposed to restrain stress and *tulsi* pretreated group (Group C) was treated

with *tulsi* extract before exposure to restrain stress. Body weight of all control rats were recorded daily at the morning for 7 days. For the experimental rats, body weights were recorded just before exposure to stress. After 7 day's experiment body weight of all the rats were recorded and then they were sacrificed. The percent of change in body weight was calculated by the formula (Final weight-Initial weight/ Initial weight x 100) Stress procedure was done by immobilizing each rat of stressed group separately for 1 hour daily in the morning for 7 days, in an aerated conical transparent plastic flask (12.7 x 6.8 x 4.8 cm). For preparing the plant extract, powder of sun dried leaves of *O. sanctum* Linn (*tulsi*) was collected from Hamdard pharmaceuticals, Dhaka. This powder was extracted by percolation with 70% ethyl alcohol and the extract was concentrated in vacuo below 50<sup>0</sup> C till a residue was obtained, then this powder was dissolved in propylene glycol (10 g/ 100ml) for experimental use<sup>11</sup>. The freshly prepared *O. sanctum* Linn (*tulsi*) extract was administered at a dose of 100mg / kg body weight daily for 7 days into stomach of each rat of group C through a plastic feeding tube.

After the experiment, the rats were anaesthetized with chloroform and 5ml blood was collected from abdominal aorta after opening the abdominal cavity of each rat and sera were separated by centrifugation. From their sera, the levels of serum glucose, serum cholesterol were determined by GOD-PAP, CHOD-PAP method respectively.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by the single vial method using kit.

The results were compared among the different groups. The data were statistically analyzed using computer based SPSS by unpaired t test.

### Results:

Result of the percent change of body weight are presented in Table-I. The mean body weight was markedly decreased in stressed group at the end of experiment compared to that of their initial mean body weight. On the other hand, the mean body weight of the control and *tulsi* pretreated group were noticeably increased at the time of their sacrifice compared to those of their initial body weight. But the statistical analysis was not done between their initial body weight and final body weight. The percent of change in body weight was significantly lower ( $p < 0.001$ ) in group B when compared with those of group A and group C. Again, the percent of change in body weight was though lower in group C than that of group A but this differences was not statistically significant. ( $P > 0.10$ ) (table-I).

Results of serum glucose, cholesterol, ALT, AST are shown in Table-II and III.

Mean serum glucose, cholesterol, ALT, AST levels were significantly ( $p < 0.001$ ) higher in stressed group (B) compared to those of control group (A) and *tulsi* pretreated group (C). But all these values were almost similar in control group A and *tulsi* pretreated group (C). No statistically significant differences were observed.

**Table – I**

*Mean (±SE) initial and final body weight and their percent change in different study groups. (n=30)*

Group	n	Body weight (Kg)		% change in final weight in respect to initial weight
		Initial (1 <sup>st</sup> day)	Final (7 <sup>th</sup> day)	
A	10	179.7 ± 2.3	198.9 ± 1.8	+ 10.9
B	10	192.7 ± 4.8	183.8 ± 4.4	- 4.4
C	10	167.2 ± 5.9	181.3 ± 5.0	+ 8.8

### Statistical Analysis

	t value	P value
A vs B	6.172	< 0.001
B vs C	- 5.511	< 0.001
C vs A	0.957	> 0.10

Group A : Control ( non stressed, non treated).

Group B : Experimental ( exposed to restraint stress)

Group C : Experimental ( tulsi pretreated and exposed to restraint stress)

**Table –II***Mean ( $\pm$ SE) of Serum Glucose and Cholesterol levels in different study groups(n=30)*

Group	n	Glucose (mmol/L)	Cholesterol(mg/dl)
A	10	5.9 $\pm$ 0.3	72.3 $\pm$ 6.8
B	10	7.8 $\pm$ 0.2	104.3 $\pm$ 2.6
C	10	5.6 $\pm$ 0.2	61 $\pm$ 5.3

  

Statistical analysis			
Groups		P value	P value
Avs B		<.001	<.001
BvsC		<.001	<.001
CvsA		0.50	0.50

Group A : Control ( non stressed, non treated).

Group B : Experimental ( exposed to restraint stress)

Group C : Experimental ( tulsi pretreated and exposed to restraint stress)

**Table –III***Mean ( $\pm$ SE) of Serum ALT and AST levels in different study groups(n=30)*

Group	n	ALT(IU/l)	AST (IU/l)
A	10	49.6 $\pm$ 2.8	141.3 $\pm$ 6.1
B	10	77 $\pm$ 3.4	164.2 $\pm$ 7.3
C	10	46.1 $\pm$ 1.4	138.41 $\pm$ 4.8

  

Statistical analysis			
Groups		P value	P value
Avs B		<.001	<.001
BvsC		<.001	<.001
CvsA		0.10	0.10

Group A : Control ( non stressed, non treated).

Group B : Experimental ( exposed to restraint stress)

Group C : Experimental ( tulsi pretreated and exposed to restraint stress)

**Discussion :**

This study was undertaken to observe the effects of restraint stress on some biochemical parameters, and their modifications by *tulsi* pretreatment in albino rats. The results of body weight changes are similar to those of other researchers<sup>12</sup>, but it was lowered than that of Harris *et al*<sup>13</sup>. It is well established that corticotrophin – releasing hormone influences

feeding behavior and mediate in behavioral and physiological response to stress<sup>14</sup>. Several investigators have mentioned, CRH induced anorexia during stress. This may be due to either activation of serotonin pathways or inhibition of neuropeptide Y release.<sup>15 - 16</sup>. Neuropeptide Y is a potent stimulator of food intake<sup>17</sup>. It has been suggested that restraint stress induces suppression of weight gain occur due to depression and anorexia<sup>16</sup>.

Serum glucose level in this study was found higher in stress group than those of *Tulsi* pretreated group which is similar to those of control group. Several studies shown that *Tulsi* has a hypoglycemic action<sup>18-20</sup>.

Cholesterol level was significantly higher in stress group which is similar to those reported by others<sup>21</sup>. It has been suggested that various forms of stress raised the cholesterol level by disturbing rate of synthesis and excretion<sup>22</sup>. Various authors have suggested that this change is due to the effect of epinephrine on lipoprotein lipase, hormone sensitive lipase and hepatic lipase<sup>23,24</sup>. Patterson et al. suggested that psychological stress caused decreased volume, producing hemaconcentration which might be a secondary cause of increased cholesterol level<sup>25</sup>.

Stress induced cholesterol level was also lowered in *Tulsi* pretreated group which is similar to the results reported by some other groups of investigators<sup>26, 27, 18</sup>.

ALT and AST were significantly higher in stress group. Similar observations were also made by some investigators<sup>28-30</sup>. *Tulsi* has significantly controlled the stress induced alteration of serum ALT and AST level. Stress causes alteration in the membrane permeability of the cells or injury to the cells due to release of intracellular enzymes into the systemic circulation.<sup>18, 23</sup>. Autonomic nervous system plays a great role in this change by modulation of cell structure as well as cell permeability resulting in excessive Ca influx which in turn damages the cytoskeleton and cause leakage of intracellular enzyme to the outside<sup>31</sup>. Although there is no such available data to compare the effect of *tulsi* on restrained stress induced changes in relation to serum levels of ALT and AST but it was reported that elevation of corticosterone was prevented by *tulsi*<sup>32</sup>. They also suggested that such prevention may be due to the blockade in release of ACTH through HPA axis. So *tulsi* extract may have a central action.

### Conclusion:

From the result of this study it can be concluded that stress may cause reduction in blood glucose, cholesterol ALT and AST status and body weight as well which can be prevented by pretreatment with *tulsi* extract.

Although this study may confirm the anti stress effect of *tulsi* but the mechanism of this adaptogen was not studied. But before recommending the extract of *tulsi* as a therapeutically active agent against stress, further study should be done in this field.

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