Evaluation of Adapogenic Activity of Extracts of *Apium graveolens* on Mice and Rats

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**Abstract:** Stress is defined as a nonspecific response of the body to any excessive demand imposed on it. Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis. Extreme stress leads to various complications like depression, anxiety, immunosuppressant and endocrine disorder including diabetes mellitus, ulcerative colitis, obesity etc. There is extensive search for plant products for use in stress condition which is devoid of adverse effects. This study was planned to investigate the adaptogenic activity (antistress) of the ethanolic and aqueous extracts of *Apium graveolens* at various dose using experimentally induced stress models in mice and rats like anoxia stress tolerance, swimming endurance and cold stress model were used for evaluation of adaptogenic activity. *Withania somnifera* (100 mg kg⁻¹, p.o.) was used as reference standard and it showed significant adaptogenic activity in all three models of stress. The aqueous (200 mg kg⁻¹) and ethanolic (400 mg kg⁻¹) extracts concomitant administration have shown increase in anoxia stress tolerance time and swimming endurance time as compared to control group and also there was marked decrease in blood glucose, cholesterol, triglycerides and BUN level as compare to stress control group in cold stress model. The liver and adrenal gland weight were markedly decreased but no weight change was observed in spleen and testes in this model. The presents study suggests that ethanolic extract of *Apium graveolens* possess more adaptogenic property than aqueous extracts.

**Key words:** Adaptogenic, stress, free radicals

**INTRODUCTION**

Stress is defined as a nonspecific response of the body to any demand imposed on it. Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis. Every individual is likely to face stressful situations in day to day life (Rai et al., 2003). When stress becomes extreme it is harmful for the body and hence need to be treated. Stress is involved in pathogenesis of variety of diseases and psychiatric disorder such as depression, anxiety, immunosuppressant and endocrine disorder including diabetes mellitus, male impotence, cognitive dysfunction, peptic ulcer, hypertension, ulcerative colitis (Singh et al., 2009) and influence on eating behavior in the humans (Gupta et al., 2009). Stress is a complex array of internal and external factors which influences on the appetite and consequently increases the amount and types of food intake by humans. When an acute stress is experienced, such as a threat to personal safety, there is an instant physiologic response “fight or flight” which results in the suppression of appetite. Exposure to chronic psychological stressors e.g., job pressure is reason for one of many mental health disorders that contribute to the global burden of stress associated disease. In the chronic stressful situations rather than avoiding food, individual seeks to consume energy dense foods. If stress causes some individuals to consume food in excess than requirement, this may culminate in weight gain and contribute to development of obesity. Internal factors have physiologic mechanisms that regulate appetite by hormone release such as neuropeptide-Y which stimulates food intake and hormone 1 Eptin reduce food intake (Torres and Nowson, 2007).

Chronic stress is one of the etiologies for obesity, the underlying physiological mechanisms explains potential link between stress and abdominal obesity. The release of glucocorticoids (e.g., cortisol) due to stress response may disrupt the food intake regulation in humans by stimulating the neuropeptide Y system (food intake stimulation) and blunting the effect of the 1 eptin system (food intake reduction) which leads to obesity (De Vriendt et al., 2009).

Obesity is a major cause of morbidity and mortality. The prevalence of obesity (BMI = 30) continues to be a health concern in individuals. Approximately 1.2 billion people in the world are overweight and at least 300 million of them are obese. According to the World Health Organization (WHO), obesity is one of the 10 most preventable health risks (Wolf and Colditz, 1998).

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Obesity is becoming an increasingly great threat to human health. Obesity affects nine organ systems and a major risk factor for gastroesophageal reflux disease, nonalcoholic fatty liver disease, cholelithiasis and colon cancer. The primary environmental determinants of obesity are high calorie intake and decrease in physical activity. It is a multi-factorial disorder, which is often associated with a considerable burden of ill health and other disorders such as diabetes; hypertension associated cardiovascular diseases, osteoarthritis and cancer (Liu et al., 2010).

As there is an increased scientific research in herbs, it is clear that the medicinal herbs are equally potent in today’s synthetic era. According to various studies it’s evident that only 20% of the plant flora has been studied and 60% of synthetic medicines owe their origin to plants. Hence, all over the world, there is increase in search of natural system of healing (Sarah et al., 2000).

The available synthetic antidepressants produce severe adverse effects like headaches, dry mouth, insomnia respiratory disturbance, constipation and trigger systemic arterial hypertension associated with cardiac arrhythmias. Herbs can be used on a long-term basis without a risk of serious side effects (Sarah et al., 2000).

Man approaches are put forth for treatment of stress, American ginseng is found to reduce stress, lower high blood sugar and adjust immunity. Various studies have shown that Ginsenoside, saponin, glycosides and flavonoid constituents of Panax quinquefolius L. (Nocerino et al., 2000) and Withania somnifera plant are responsible for adaptogenic activity.

The Apium graveolens extracts were traditionally used for diabetes, headache, hepatic disorder (Singh and Handa, 1995), hypercholesterolaemia (Tsi and Tan, 2000), stomach ache, CNS stimulant, antimicrobial (Momin and Nair, 2001), antioxidant (Momin and Nair, 2002), anti-inflammatory, inflammation and pain (associated with sprains, bruises, wounds, spasmodic colic and rheumatic arthritis (Lewis et al., 1985). This study was planned to screen adaptogenic of Apium graveolens having the similar chemical constituents as of Withania somnifera, Panax quinquefolius L., V. vinifera, Bacopamonnierii (Rai et al., 2003), Zinziber officinalis, Piper nigrum, Emblica officinalis and Terminalia bellirica (Guevara et al., 1999).

**MATERIALS AND METHODS**

**Extraction procedure:** The fresh leaves were thoroughly washed and dried in shade. The dried leaves were crushed to coarse powder using a hand mill and sieved (sieve No. 10). Coarse powder was successively extracted in soxhlet apparatus with 70% ethanol (60-80°C) for 24 h and cold maceration with distilled water and added few milliliter of chloroform to avoid fungal growth in aqueous extract. The extracts were concentrated by evaporating them at room temperature and air dried. The above obtained extracts of ethanolic and aqueous extracts were dissolved in distilled water (vehicle). All preparations (doses) were freshly prepared on the day of experiment and administered to the animals.

**Preliminary phytochemical analysis:** Preliminary phytochemical studies were carried out for all the two different extracts of Apium graveolens to find out the presence of different phyto-chemical constituents like carbohydrates, proteins, fats and oil, alkaloids, glycosides, terpenoids, flavonoids, tannins and polyphenols (Liu et al., 2008; Hu et al., 2008).

**Animal studies:** Swiss albino mice of either sex (25-35 g) or male wistar rats (200-250 g) were used. The animals were obtained from Drug Testing Laboratory (DTL) Bangalore, Karnataka. Animals were maintained in suitable nutritional and environmental condition throughout the experiment. The animals were acclimatized for 10 days under standard laboratory condition i.e., room temperature 25±2°C, relative humidity 65±10%, 12 h light/dark cycle. They were fed with standard pellet diet (Venkateshwara enterprises, Bangalore) and water ad libitum under hygienic condition.

**Acute toxicity studies (Klein et al., 2007):** Acute oral toxicity studies were carried out for ethanolic and aqueous extracts of Apium graveolens using “acute toxic class method” according to OECD guidelines No. 423. Healthy adult Swiss albino mice (female) weighing between 25 to 35 g was used for the study. Animals were divided in to four groups of three animals each, fasted overnight and administered different doses 5, 50, 300, 2000 mg kg⁻¹ b.wt. to each group of animals. The physiological changes in these animals like body temp, CNS activities, micturition and defecation etc were observed for 24 h.

**Swimming endurance test (Rangari, 2002):** Healthy adult Swiss albino mice of either sex weighing between 25 to 35 g were divided in to six groups of six animals each. Group I animals served as control were administered vehicle alone (1 mL/100 g, p.o.). Group II and III animals were administered aqueous extract of Apium graveolens at dose of 200 and 400 mg kg⁻¹, respectively and Group IV and V animals were administered ethanolic extracts of Apium graveolens at dose of 200 and 400 mg kg⁻¹. Group
VI animals were administered standard drug (Withania somnifera extract 100 mg kg⁻¹). All the extracts were administered orally once daily for 7 days and allowed for free access to food and water. On 7th day 1 h after extract treatment, all the mice were subjected to swimming endurance test. The mice were allowed to swim individually in a propylene tank of dimension 37×37×30 cm, filled with water to a height of 25 cm maintained at 26±1°C temperatures. The end point was death of animals due to drowning when the animals remained at the bottom of swimming tank. The mean swimming time for each group was calculated.

**Anoxia stress tolerance test (Rangari, 2002):** Healthy adult Swiss albino mice of either sex weighing between 25 to 35 g were divided in to six groups of six animals each. Group I animals served as control were administered vehicle alone (1 mL/100 g, p.o.). Group II and III animals were administered aqueous extract of Apium graveolens at dose of 200 and 400 mg kg⁻¹, respectively and Group IV and V animals were administered ethanolic extracts of Apium graveolens at close of 200 and 400 mg kg⁻¹. Group VI animals were administered standard drug (Withania somnifera extract 100 mg kg⁻¹). All the extracts were administered orally once daily for three weeks and were allowed for free access to food and water. At the end of 1st week (7th day), 2nd week (14th day) and 3rd week (21st day) week 1 h after the extract treatment, stress was induced in all mice by placing each animal individually in the hermetic vessel of 500 mL capacity to record anoxia tolerance time. The moment when the animal showed the first convulsions, it was immediately removed from the vessel and resuscitated if needed. The time duration of animal entry of the animal into the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia tolerance. Appearance of convulsion was very sharp end point, as delay by minute of removal of the animal from the vessel may lead to death of the animal.

**Cold restraint stress (Rangari, 2002):** Healthy adult Wistar rats were divided in to six groups of six animals each. Group I animals served as control were administered vehicle alone (1 mL/100 g, p.o.). Group II and III animals were administered aqueous extract of Apium graveolens at dose of 200 and 400 mg kg⁻¹, respectively and Group IV and V animals were administered ethanolic extracts of Apium graveolens at dose of 200 and 400 mg kg⁻¹. Group VI animals were administered standard drug (Withania somnifera extract 100 mg kg⁻¹). The extracts were administered orally once daily to respective groups for 10 days. All the animals were subjected to cold stress by exposing them to 4±1°C daily for 2 h. This procedure was repeated for 10 days between 11.00 am to 1.00 pm. On 10th day blood was collected from the hepatic portal vein under light ether anesthesia to estimate biochemical parameters like serum glucose, cholesterol, triglyceride and BUN (Blood Urea Nitrogen). Animals were sacrificed by cervical dislocation and the weight of organs such as liver, spleen, testes and adrenal gland were recorded per 100 g body weight of animal after washing them with alcohol (Hu et al., 2008).

**Statistical analysis:** Data obtained were expressed as Mean±Standard error of mean (SEM). The significance was determined by applying one-way ANNOVA using prism graph pad software. The statistical differences in the sample means were considered significant at p<0.05.

**RESULTS**

The preliminary phytochemical analysis of the ethanolic and aqueous extracts of Apium graveolens revealed the presence of the phyto-chemical constituents like alkaloids, carbohydrates (Reducing sugars Non-reducing Sugar), Glycosides (Coumarin Saponin Phenolic and Flavonoid), flavonoids, proteins and amino acids in both additionally ethanolic extract contains tannins, fats, terpenoids, tannins and polyphenols.

Acute oral toxicity studies of the ethanolic and aqueous extracts of Apium graveolens did not exhibit any sign of toxicity up to 2000 mg kg⁻¹ b.wt. Since, there was no mortality of the animals found at highest dose. In the present study dose was selected randomly i.e., 200 and 400 mg kg⁻¹.

It was found that ethanolic and aqueous extracts of Apium graveolens induced a striking increase in swimming endurance time in mice was found to be dose dependant. The extract shows significant increase in the percentage increase in swimming endurance time over vehicle treated animals by 26.82% in 200 mg kg⁻¹ and 38.05% in 400 mg kg⁻¹ of aqueous extracts of Apium graveolens and 48.65% in 200 mg kg⁻¹ and 52.70% in 400 mg kg⁻¹ of ethanolic extracts of Apium graveolens in the treated groups, respectively. Result are expressed as Mean±SEM (Table 1).

The effect of aqueous and ethanolic extracts of Apium graveolens on anoxia stress tolerance test in mice was found to be dose dependant increase (Table 2). Treatment with aqueous extract of 200 and 400 mg kg⁻¹ has produced stress tolerance time 33.9±1.38 and 36.65±1.31 in 1st h, 39.76±1.16 and 43.04±1.36 in 2nd h and 46.04±1.21 and 53.5±1.58 in 3rd h. The ethanolic extracts of 200 and 400 mg kg⁻¹ has produced stress tolerance time 34.03±0.27 and 37.38±0.3 in 1st h, 41.22±0.7 and 46.12±0.87
in 2nd h and 52.14±0.49 and 61.86±0.62 in 3rd h. Treatment with aqueous and ethanolic extracts as shown significant increase in anoxia stress tolerance time. The result express as mean (Table 2).

The chronic stress due to the cold resulted in significant rise in serum glucose, cholesterol, triglycerides and BUN. The aqueous extracts of dose 200 and 400 mg kg⁻¹ as shown reduction in the blood glucose 10.94±3.78 and 102.25±2.04, cholesterol 76.37±1.78 and 74.25±2.04, triglyceride74.61±2.31 and 81.17±4.5 and BUN 25.06±0.67 and 24.58±0.99. Ethanolic extract of dose 200 and 400 mg kg⁻¹ as shown reduction in blood glucose 91.95±1.27 and 84.66±1.57, cholesterol 68.75±2.51 and 55.22±1.61, triglyceride 79±2.01 and 77.73±1.40 and BUN 24.31±0.86 and 24.58±1.50. Concomitant treatment with ethanolic extracts has shown significant and greater extent than aqueous extracts alone in reduction of all the parameter (Table 3). The ethanolic and aqueous extracts were shown significant decrease in the cold stress induce elevated level of serum glucose, cholesterol and triglyceride with respect to Standard (Withania somnifera) activity expect BUN.

The chronic stress due to the cold resulted in significant rise of liver, adrenal gland weight and decrease in spleen and testes weight (Table 4). The aqueous extracts of dose 200 and 400 mg kg⁻¹ as shown decrease in the liver weight 7.48±0.18 and 6.30±0.16 and decrease in adrenal gland weight 0.02±0.00 and 0.027±0.00 and increase in spleen weight 1.16±1.16 and 1.50±0.22 and testes weight 1.60±0.00 1.76±0.00. Ethanolic extract of dose 200 and 400 mg kg⁻¹ as shown decrease in the liver weight 6.91±0.31 and 6.30±0.22 and decrease in adrenal gland weight 0.02±0.00 and 0.023±0.00 and increase in spleen weight 1.00±1.03 and 1.36±0.21 and testes weight 1.60±0.00 and 1.92±0.23. Concomitant treatment with ethanolic extracts has shown significant and greater extent than aqueous extracts alone in reduction of all biological parameters. The ethanolic and aqueous extracts were shown significant decrease in the cold stress induced increased liver, adrenal gland weight and increase in weight of spleen and testes respect to Standard activity.

Table 1: Effect of extract of Apium graveolens on swimming endurance test in mice. n = 6

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg kg⁻¹)</th>
<th>Swimming survival time in mice (min)</th>
<th>Percentage increase in swimming endurance time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (received vehicle)</td>
<td>1 mL/100 g</td>
<td>2.10</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>200</td>
<td>2.87±0.23*</td>
<td>26.82</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>400</td>
<td>3.39±0.17**</td>
<td>38.05</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>200</td>
<td>4.09±0.39**</td>
<td>48.56</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>400</td>
<td>4.44±0.28**</td>
<td>52.70</td>
</tr>
<tr>
<td>Standard (W. somnifera)</td>
<td>100</td>
<td>5.28±0.26**</td>
<td>60.22</td>
</tr>
</tbody>
</table>

**Significant at p<0.05, ***p<0.001 as compared to control group.

Table 2: Effect of ethanolic and aqueous extracts of Apium graveolens on anoxia stress tolerance test in mice. n = 6

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Duration of anoxia stress tolerance time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (received vehicle)</td>
<td>1 mL/100 g (received vehicle) 100 g</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>200</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>400</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>400</td>
</tr>
<tr>
<td>Standard (W. somnifera)</td>
<td>100</td>
</tr>
</tbody>
</table>

**Significant at **p<0.01, *p<0.05, ***p<0.001 as compared to control group.

Table 3: Effect of ethanolic and aqueous extracts of Apium graveolens on biochemical parameters of rats in cold restraint stress n = 6

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Biochemical parameters (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (received vehicle)</td>
<td>Dose-II (mg kg⁻¹)</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Glucose 89.9±1.65</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Cholesterol 76.37±1.78**</td>
</tr>
<tr>
<td>Eachanolic extract</td>
<td>Triglycerides 74.25±2.04*</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>BUN 68.75±2.51***</td>
</tr>
<tr>
<td>Standard (W. somnifera)</td>
<td>77.5±1.40***</td>
</tr>
</tbody>
</table>

| Treatments          | Significance at *p<0.05, **p<0.01, ***p<0.001 as compared to positive control group (stress rats).

Table 4: Effect of ethanolic and aqueous extracts of Apium graveolens on organ weights of rats in cold restraint stress n = 6

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Biological parameters (g/b wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (received vehicle)</td>
<td>Dose-II (mg kg⁻¹)</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Liver 86.6±0.42</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Spleen 0.03±0.001</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Adrenal gland 0.96±0.01</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>BUN 1.08±0.02</td>
</tr>
<tr>
<td>Standard (W. somnifera)</td>
<td>1.50±0.22***</td>
</tr>
</tbody>
</table>

| Treatments          | Significance at *p<0.05, **p<0.01, ***p<0.001 as compared to positive control group.
DISCUSSION

The swimming endurance test was conducted in mice has shown significant increase in the swimming endurance time with both doses of ethanolic and aqueous extracts. The ethanolic extract adaptogenic activity was potential than aqueous extracts. The activity produced may be possibly due to normalization of plasma levels of catecholamine and MAC, which are decreased during stress situation or by decreasing muscle glycogen and preventing accumulation of fat (Friedewald et al., 1972). The other possible mechanism for this adaptogenic activity may also be mediated by slowing glycogen depletion, decreases in concentrations of muscle lactic acid and ammonia. The anti-oxidant property of the Apium graveolens extracts has already reported and which may also contributes for the adaptogenic activity.

The anoxia tolerance test was conducted in mice has produced increase in the anoxia tolerance time with both doses of ethanolic and aqueous extracts. The anoxia tolerance test of adaptogenic property of the extracts may due to increasing succinate dehydrogenase (SDH) in the brain. This enzyme is responsible for utilization and conservation of energy in the cellular system of the organism which helps adaptive processes during stress (Bhatwadikar et al., 1999). The effect is probably related to an increase in cerebral resistance to anoxia and reducing the cerebral consumption of oxygen in anoxic stress. The present study has given emphasis on estimation of stress induced biochemical and biological parameters by in vitro method.

Stressful conditions lead to formation of excessive free radicals which are the major internal treat to cellular homeostasis of aerobic organisms. Free radicals are formed in human body both in physiological and pathological conditions in cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes (Patil et al., 2006). These free radicals are extremely reactive and unstable chemical species which reacts with proteins, lipids, carbohydrates and nucleic acids in the body. Exposure of lipids in cell membrane to free radicals stimulates the process of lipid peroxidation. The free radical activity and the extent of tissue damage are related quantitatively to the amount of lipid peroxide level in the blood. There are reports suggesting that there is a stress induced accelerated lipid peroxidation (Han et al., 2005) (increased MDA) and alterations in lipid profile affecting serum total cholesterol, LDL, VLDL and triglycerides. The aqueous and ethanolic extracts were shown significant reduction in stress induced elevated levels of serum glucose, cholesterol and triglyceride levels (Kaur and Kulkarni, 2001) except BUN in a dose of 200 and 400 mg kg\(^{-1}\) with respect to standard (Withania somnifera). Concomitant administration of aqueous extracts and ethanolic extracts with a dose of 200 and 400 mg kg\(^{-1}\) were shown significant decrease in liver and adrenal gland weight and increase in weight of spleen and testes with respect to standard (Withania somnifera).

Both aqueous and ethanolic extracts has produced significant change in the reduction of stress induced biochemical and biological changes in rats. The reduction in the stress induced all the above parameters which may likely to prevent formation of excessive free radicals resulting in preventing internal treat to cellular haemostasis, thus depressing the processes of lipid peroxidation and normalizing the elevated serum glucose, cholesterol and triglycerides.

CONCLUSION

The ethanolic extract of the plant has produced significant effect in swimming endurance time, anoxia tolerance time and biochemical and biological parameters compared to aqueous extracts. Hence, extracts of Apium graveolens considered to be having potent antistress effect. These extracts might help in preventing stress induced complications, these results obtained in the study are encouraging to pursuing further studies on the isolated active constituent present in the extracts responsible for the activity.

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