ADAPTOGENIC POTENTIAL OF *ARTEMISIA VULGARIS* LINN. (1753) LEAF EXTRACT ON MALE ALBINO RATS


Department of Pharmacy, School of Health Care Professions, University of San Carlos, Robert Hoeppener Building, Nasipit Talamban, Cebu City - 6000, Philippines.

**ABSTRACT:** *Artemisia vulgaris* leaves are known to have anecdotal evidence of antioxidant activity which can be of use as an anti-stress remedy. However, this claim is yet to be proven and no available data on its adaptogenic potential hence, this study aimed to determine the adaptogenic potential of *Artemisia vulgaris* leaf extract on male albino rats using acute restraint stress. Six male albino rats were used as test animals and were subdivided into the following: control group, untreated normal group, negative group, and three doses of the test solution [500mg/200g rat (T1), 250mg/200g rat (T2), and 150 mg/200g rat (T3)]. T3 rats exhibited the highest adaptogenic activity of -3,111.11% based on cholesterol levels and -1916.67% based on glucose levels. Moreover, glucose levels of T2 and T1 rats were -345.41% and -6.60%, respectively while for their cholesterol levels, T2 exhibited -277.78% and T1 exhibited -90.05%. The median effective dose of the test suspension based on cholesterol levels and glucose levels is 169.70 mg/200g rat and 151.81 mg/200g rats, respectively. From this study, *Artemisia vulgaris* leaf extract has the potential protective effect against stress. Flavanoids, tannins and saponins in the leaf extract of *Artemisia vulgaris* were attributed for its adaptogenic activity.

**INTRODUCTION:** Stress has been defined as a biological response to events that threaten the balance or the normal conditions of the system 1. When under stress, levels of endogenous amines in the brain increase to prepare the organism to take action and to cope with the introduced stress. But beyond a certain point, stress starts to become a major drawback in a person’s life. The effect is exhibited when an individual identifies that the demands he is faced far exceeds its capability to mobilize 2.

Exposure to chronic stress, can lead to serious illness including cardiovascular abnormalities such as hypertension, stroke, and heart disease 3. Other stress related health problems include anxiety, depression, panic attacks, memory impairment, digestive disorders, autoimmune diseases and chronic fatigue syndrome 4. It has been reported that at least 60% of all illness can trace its roots back to the adverse effects of stress 5. All of these may cause further problems in an individual’s overall health, productivity and quality of life. Hence, it is important for an individual to cope with stress.

Some of the most extensively used drugs to combat stress are amphetamine, diazepam, caffeine and anabolic steroids.
But with reports of occurrence of side effects, dependence along with their high cost, the use of these drugs to control stress has been limited. Hence, many researchers focused to come up with safer and cheaper agents of plant origin.

In the past, the elders rely on plants to treat various disorders. The Philippines is rich in plants possessing potential therapeutic activities so most plants have well – known traditional uses.

*Artemisia vulgaris* is widely cultivated in the Philippines. It is traditionally used to alleviate various nervous system disorders such as depression, irritability, restlessness, insomnia and anxiety but there are still no scientific evidences to prove its adaptogenic activity. However, the anti-oxidant activity of *A. vulgaris* has been widely established. Most plants with known anti-oxidant activities contain flavanoids and saponins. Similarly, reports have also shown that these constituents are present in *A. vulgaris*. Anti-oxidants protect cells from the damage caused by unstable molecules known as free radicals. Free radicals are highly reactive particles, which react with other molecules in the body, causing damage to a number of cells. An imbalance between the production of free radicals and the ability of the body to counteract their harmful effects may cause oxidative stress. Oxidative stress leads to many pathophysiological conditions in the body.

There have been links made between oxidative stress and physiological stress. During a stressful situation, the energy requirement of the organism is increased resulting in enhanced generation of free radicals, which facilitates lipid peroxidation as well as oxidation of nucleic acids and proteins, thereby damaging cell membranes and compromising cell integrity and function. Reducing these free radicals by treatment with anti-oxidants may possibly reduce the damaging effects of stress brought about by these reactive molecules. To rationalize the plant’s mentioned traditional uses which are scientifically supported by the studies made about the plant’s anti-oxidant activity, adaptogenic potential of the plant was conducted. This study aimed to determine the percent adaptogenic activity based on blood glucose and cholesterol levels; differences in the grooming and behavioural patterns; and median effective dose of the leaf extract.

**MATERIALS AND METHODS:**

**Collection and Preparation of Plant Sample:** *Artemisia vulgaris* leaves were collected from a local area in San Francisco, Unidos Camotes, Cebu, Philippines and they were authenticated by the University of San Carlos - Department of Biology. The leaves were washed with water and wiped dry to remove adhering water.

**Extraction of Plant Sample:** The leaves were air dried at room temperature for seven days and ground using mortar and pestle. One hundred grams of the ground leaves were subjected to Soxhlet extraction at 92°C for four hours. The solvent for extraction was 500ml of 70% methanol. The extract obtained was concentrated in a rotary evaporator under a reduced pressure at 45°C. The extractive was further evaporated to dryness using water bath inside the fume hood. The resulting leaf extract was reduced to finer particles using the mortar and pestle. This was properly labelled as the leaf extract.

**Preparation of Stock Suspension:** The leaf extract has a weak to moderate solubility as 1g of the leaf extract still remained undissolved in 1000ml of distilled water. A stock suspension was prepared with a concentration of 0.5g/mL using a volumetric flask. Tween 80 (1%) was used as the suspending agent to maintain the stability of the formulation.

**Test Animals:** Six male albino rats weighing 100-170 grams were used in the experiment. Group N₀ was assigned as the Normal group which did not undergo stress. Group Uₜ was assigned as the untreated group which underwent stress, Group Nₙ was assigned as the negative control, which underwent stress and administered with distilled water, Group T₁ was administered with 500mg/200g dose, Group T₂ with 250mg/200g dose and Group T₃ with 150 mg/200g dose. The experiment was conducted in three trials. All rats were fed with 10-30g of commercial pellets per day and were provided access to water *ad libitum* before the induction of stress. This diet was given to the rats during the whole duration of the experiment.
They were acclimatized for one week inside five plastic cages and were kept in the University of San Carlos animal house. Rats were acclimatized to handling (gentling) to reduce stress. Their backs were gently stroked and they were handled frequently. The animals were re-used and wash-out period for two weeks were employed. The research protocol was conducted under the supervision of a certified and trained animal technician ensuring proper animal handling was observed throughout the duration of the study.

**Determination of Adaptogenic Potential by Acute Restraint Stress:**

**Baseline recording of levels of glucose and cholesterol:** Baseline blood glucose and cholesterol levels for non-stress were measured using a glucometer and cholesterol meter one hour after the last day of acclimatization to the environment. The blood was extracted from the saphenous vein of the rats. All groups of rats had undergone blood extraction.

**Acclimatization to the open field arena:** Thirty minutes after blood extraction, the rats were acclimatized for five minutes on the behavioural test equipment for three consecutive days before the induction of the stressor. It was necessary to acclimatize the animals to the open field arena to avoid potential effects induced by novelty of the apparatus that would reduce the variation in the experimental data.

**Induction of acute restraint stress before treatment:** Thirty minutes after the third day of acclimatization to the open field arena, the rats, except those belonging to No, were subjected to acute restraint stress. They were immobilized simultaneously for one hour in a cylindrical bamboo restraining tube with a diameter of 4.5 cm and a length of 12 cm which has its one end covered by wire mesh to allow ventilation. One session of acute restraint stress were conducted daily for three days.

**Assessment of behavioural and grooming patterns in the open field:** Directly after the last stress session, the test animal was placed in the open arena and was observed for ten minutes. The activities of the test animal were recorded on video. The latency to start grooming, total number of bouts/session, total time spent grooming, number of incorrect transitions and incomplete bouts, and time spent in the inner zone of the field were recorded.

**Measurement of glucose and cholesterol levels before treatment:** One hour after assessment in the open field, blood samples from each test animals, except No, were collected through the saphenous vein using digital glucometer and cholesterol home check (Easy Touch ET Glucose and Cholesterol Kit). The blood samples were used for the determination of blood glucose and cholesterol levels and these served as the biochemical parameters before treatment with the test drug.

**Administration of test drug:** Twenty-four hours after blood extraction, the test suspensions were administered to the test animals. Groups T₁, T₂, T₃ were treated with doses of 500mg/200g, 250mg/200g rat and 150mg/200g rat respectively through gastric lavage. It was done for five consecutive days.

**Induction of acute restraint stress after treatment:** The test animals were again subjected to stress one hour after the last day of the administration of the test solutions. The rats, except those belonging to No, were subjected to acute restraint stress. They were immobilized simultaneously for one hour in the same cylindrical tube. It was conducted for three days.

**Assessment of behavioural and grooming patterns in the open field:** The test animal was placed in the open arena and was observed for 10 minutes. The activities of the test animal were recorded on video. The latency to start grooming, total number of bouts/session, total time spent grooming, number of incorrect transitions and incomplete bouts, and time spent in the inner zone of the field were recorded.

**Measurement of glucose and cholesterol levels after treatment:** One hour after assessment in the open field, blood samples from each test animals were collected through the saphenous vein using digital glucometer and cholesterol home check (Easy Touch ET Glucose and Cholesterol Kit). The blood samples were used for the determination of blood glucose and cholesterol levels. These served as the blood levels of glucose and cholesterol after
treatment with the test drug of varying doses. Decrease in the blood glucose and cholesterol levels after treatment, increased time spent in the inner zone of the field, increased latency to start grooming, decreased number of bouts, decreased time spent grooming, low percentage of incorrect transitions and low percentage of incomplete bouts determined the adaptogenic potential of the plant to be studied.

Adaptogenic activity based on the blood glucose and cholesterol levels were assessed using the formula:

\[ \% \text{Adaptogenic Activity} \ (\%AA) = \left( \frac{B_2 - B_0}{B_1 - B_0} \right) \times 100 \]

Where:
- \( B_0 \) = baseline blood glucose/cholesterol levels
- \( B_1 \) = blood glucose/cholesterol levels before treatment
- \( B_2 \) = blood glucose/cholesterol levels after treatment

**Determination of the Median Effective Dose:**
The median effective dose (ED\(_{50}\)) were calculated based on the final blood levels of glucose and cholesterol and were obtained using the Pearson’s linear regression analysis given by the formula:

\[ y = mx + b \]

Where:
- \( y = 50 \) (half of its activity)
- \( m = \) Slope of the line (gradient)
- \( b = \) Intercept (The value of \( y \) when \( x = 0 \))
- \( x = \) dose of the test solution that will give 50\% effectiveness

**Statistical Analysis:** The difference between the test groups was evaluated using one-way Analysis of Variance (ANOVA). Confidence level was set at 99\% with 1\% chance of error. Statistical significance were accepted at \( p \)-level<0.01. Since a significant difference was obtained, post-hoc analysis (Tukey HSD) was done.

**RESULTS:**
**Percent Adaptogenic activity:** The percent adaptogenic activity (\%AA) was calculated based on the changes in the cholesterol and glucose levels. Based on the formula for computation of percent adaptogenic activity, the lower the percentage, the greater is the adaptogenic activity. **Fig. 1** shows the average \%AA.

**Behavioral Parameters:**
**Time spent in the inner circle of the open field arena:** The average time spent in the inner circle of the open field arena of the rats subjected to stress (\( U_T, N_c, T_1, T_2 \) and \( T_3 \)) decreased after induction of stress. On the other hand, rat \( N_0 \) only had a small change in the amount of time spent in the inner circle of the arena. All groups of rats had an increase in the time spent in the inner circle after the period of administration of the test suspensions to the treated groups. However, a greater increase in the time spent in the inner circle can be observed in rats \( T_1, T_2 \) and \( T_3 \) (**Fig.2**).

**Latency to start grooming:** The latency to start grooming decreased in rats induced with stress (\( U_T, N_c, T_1, T_2 \) and \( T_3 \)). After treatment, an increase in the latency to start grooming can be observed in all groups of rats. However, a greater increase in the latency is evident in rats \( T_1, T_2 \) and \( T_3 \) (**Fig. 3**).
**Number of bouts**: The average number of bouts increased in the rats induced with stress (UT, Nc, T1, T2 and T3). A decrease of these values occurred in all groups of rats after the treatment period but lower reduction can be observed from the rats treated with the test drug suspension (Fig. 4).

**Duration of grooming**: The average duration of grooming increased in rats induced with stress (UT, Nc, T1, T2 and T3). A decrease of these values occurred in all groups of rats after the treatment period but a lower reduction can be observed from the rats treated with the test drug suspension (Fig.5).

**Percentage of incorrect transitions**: The average percentage of incorrect transitions increased in rats induced with stress (UT, Nc, T1, T2 and T3). A decrease in these values occurred in all groups of rats, except Nc, after the treatment period but a lower reduction can be observed from the rats treated with the test drug suspension (Fig. 6).

**Percentage of incomplete bouts**: All rats have equal incomplete bouts all throughout the experiment. Only rats UT and T2 showed some degree of reduction in the percentage of incomplete bouts after the treatment period (Fig.7).

**Median Effective Dose**

**FIG. 8: LINEAR REGRESSION OF THE PERCENT ADAPTOGENIC ACTIVITY OF THE TEST SUSPENSION BASED ON CHOLESTEROL LEVELS**

\[
\% \text{ adaptogenic activity (cholesterol)} = -9.2686x + 162.9 \\
R^2 = 0.9665
\]
**Statistical Analysis:** The data from the percent adaptogenic activity based on the cholesterol and glucose levels were analysed using One-way Analysis of Variance (ANOVA). The p-level was set at 0.01 (Table 1 - 4). Since the p-level was <0.01, then there was a significant difference between the independent (test groups) and dependent (%AA) variables.

**TABLE 1: ONE-WAY ANALYSIS OF VARIANCE FOR CHOLESTEROL LEVELS**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5</td>
<td>40746031</td>
<td>8149206</td>
<td>253.65831</td>
<td>9.82x10^-12</td>
</tr>
<tr>
<td>Within Groups</td>
<td>12</td>
<td>385520.5</td>
<td>32126.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>41131551</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2: TUKEY HSD TEST FOR DIFFERENCE BETWEEN MEANS (CHOLESTEROL)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Difference</th>
<th>p-level</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>500mg v 250mg</td>
<td>-2741.15000</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v 150mg</td>
<td>-3069.42333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v negative</td>
<td>-3738.35333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v untreated</td>
<td>-3843.96000</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v normal</td>
<td>-4805.35333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>250 mg v150mg</td>
<td>-328.27333</td>
<td>0.287</td>
<td>Insignificant</td>
</tr>
<tr>
<td>250mg v negative</td>
<td>-997.20333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>250 mg v untreated</td>
<td>-1102.81000</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>250mg v normal</td>
<td>-2064.20333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>150mg v negative</td>
<td>-668.93000</td>
<td>0.007</td>
<td>Significant</td>
</tr>
<tr>
<td>150mg v untreated</td>
<td>-774.53667</td>
<td>0.002</td>
<td>Significant</td>
</tr>
<tr>
<td>150mg v normal</td>
<td>-1735.93000</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>negative v untreated</td>
<td>-105.60667</td>
<td>0.975</td>
<td>Insignificant</td>
</tr>
<tr>
<td>negative v normal</td>
<td>-1067.00000</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>untreated v normal</td>
<td>-961.39333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
</tbody>
</table>

**TABLE 3: ONE-WAY ANALYSIS OF VARIANCE FOR GLUCOSE LEVELS**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5</td>
<td>17597809</td>
<td>3519562</td>
<td>318.3119</td>
<td>2.55x10^-12</td>
</tr>
<tr>
<td>Within Groups</td>
<td>12</td>
<td>132683.5</td>
<td>11056.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>17730493</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4: TUKEY HSD TEST FOR DIFFERENCE BETWEEN MEANS (GLUCOSE)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Difference</th>
<th>p-level</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>500mg v 250mg</td>
<td>-1646.76333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v 150mg</td>
<td>-1833.92000</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v negative</td>
<td>-2230.65700</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v untreated</td>
<td>-2371.47620</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>500mg v normal</td>
<td>-3273.81333</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>250 mg v150mg</td>
<td>-187.15667</td>
<td>0.110</td>
<td>Insignificant</td>
</tr>
<tr>
<td>250mg v negative</td>
<td>-583.89367</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>250 mg v untreated</td>
<td>-724.71287</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>250mg v normal</td>
<td>-1627.05000</td>
<td>0.000</td>
<td>Significant</td>
</tr>
</tbody>
</table>
DISCUSSION: An increase in plasma glucose level will occur in response to stress. During stress, a series of events will occur and this includes the stimulation of the hypothalamus which proceeds to the secretion of corticotropin-releasing hormones (CRH), then the secretion of adrenocorticotropic hormones (ACTH) in to the bloodstream by the pituitary gland and finally the release of cortisol by the adrenal gland. The adrenal hormones secreted will induce insulin resistance and will then exhibit the said effect. The release of noradrenaline and corticosteroid will also enhance the synthesis of cholesterol. The transport of stored fat and carbohydrate reserves will also be affected by these changes causing an increase in the levels of blood cholesterol and glucose. The increased adrenal hormone levels and consequently, glucose and cholesterol levels are reversed by anti-stress agents.

In this study, the hypothesized anti-stress agent is the extract of the plant. The results show that groups T1, T2 and T3 had a significant reduction of glucose and cholesterol levels after administration of the test drug. Based on the formula for computation of percent adaptogenic activity, the smaller the percentage, the greater is the adaptogenic activity. These three groups yielded small percentages which can be translated as
having high adaptogenic activities. On the other hand, groups \( N_0 \), \( U_T \) and \( N_c \) yield large percentages of adaptogenic activity and therefore have low adaptogenic activities.

Stress affects psychomotor profiles and exploratory behaviour. One measure for stress using the open field area apparatus is the time the rat spends in the center of the arena \(^8\). In a study conducted by Faraji \(^19\), the behavioural patterns of the animals subjected to stress were characterized by less locomotion and less distance travelled. Studies which investigate the effects of anxiolytic drugs have also reported that the more stressed rats spend more time near the walls and less time near the center of the arena as compared to the less stressed rats \(^20\). Stressed rats will have a tendency to spend the majority of their time in close proximity to the walls, a phenomenon referred to as thigmotaxis. In this study, however, the results from a ANOVA show that there is no significant difference between the test groups and the time they spend in the inner circle of the arena. This means that the test doses have no direct effect on the time the subjects spent in the inner circle.

Studies \(^15, 21\) reported that shorter latencies to start grooming, and an increase in grooming frequency and duration have long been considered as behavioural markers of stress. Moreover, the percentage of incorrect transitions between grooming patterns and percentage of incomplete bouts may also be used as behavioural markers of stress in rats. The results of their study showed that the grooming analysis algorithm revealed a marked shift in grooming behavioural patterns, significantly increasing the percentage of incorrect transitions and incomplete bouts in more anxious rats. In this study, however, the results from ANOVA show that there is no significant difference between the test groups and all the other parameters of behaviour. This means that the test doses have no direct effect on the behaviour as well as grooming patterns of the test subjects. These results may be attributed to the claim of the researchers in the previously mentioned study that the grooming algorithm analysis may not be applicable to all strains of rats and when a study will be done using this analysis, specific rat strains should be carefully selected.

The median effective dose was calculated based on Pearson’s linear regression analysis. The values used in the computation were the average percent adaptogenic activity of the test suspensions based on cholesterol and glucose levels. Based on the cholesterol levels, the median effective dose is 169.702mg/200g rat. Based on glucose levels, the median effective dose is 151.8061mg/200g rat. It is only at the said doses that the test suspension of \( A. vulgaris \) leaf extract will be able to elicit 50% of the adaptogenic activity to 50% of the population. Previously done phytochemical tests show the presence of saponins, carbohydrates, proteins and amino acids, phenolic compounds, tannins, phytosterol and flavonoids in methanolic extracts of \( A. vulgaris \) leaves \(^22\). The main constituents responsible for the antioxidant properties and thus the adaptogenic activity of the plant are flavonoids, tannins and saponins. This activity is due to their ability to reduce free radical formation and to remove the said unhealthy substances \(^23\). Acute restraint stress caused an increase in blood glucose and cholesterol of the test animals. A decrease of these values was observed after administration of varying doses of the test suspension to the treated groups. Adjacent to these results is the study \(^24\) that had reported the ability of saponins to lower blood cholesterol levels.

Another study to support the results is the hypoglycemic effect of terpenes \(^25\). Flavonoids and Tannins would reduce plasma cholesterol levels by inhibiting LDL oxidation \(^26\). Saponins act in the gastrointestinal tract to inhibit the rate of gastric emptying at the intestinal brush border membranes as its mechanism of action \(^27\).

CONCLUSION: Leaf extract of \( Artemisia vulgaris \) Linn. Gilbas (1753) has the potential protective effect against stress.

ACKNOWLEDGEMENT: The researchers would like to express their gratitude to the following people who made contributions in the technical formation of this paper: Mrs. Yolanda C.
Deliman, Mrs. Nelly Nonette M. Quano, Mrs. Elizabeth Y. Tan, Mr. Jameross B. Tabiano, Mrs. Vanebth R. Camson, Mrs. Loujessa D. Arcelo, Mrs. Daisy C. Co. Also, we would like thank the University of San Carlos, Department of Pharmacy, School of Health Care Professions for the support in the conduct of this study.

CONFLICT OF INTEREST: The authors declare no known conflict of interest.

REFERENCES: