An Experimental Evaluation of Adaptogenic Potential of Standardized Epipremnum Aureum Leaf Extract

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**Background:** Stress is a normal part of everyday life but chronic stress can lead to a variety of stress-related illnesses including hypertension, anxiety, and depression. In the present investigation, standardized leaf extract of *Epipremnum aureum* was evaluated for its anti-stress potential. **Materials and Methods:** For the evaluation of anti-stress activity, groups of mice (*n* = 6) were subjected to forced swim stress and anoxic stress tolerance test in mice 1 h after daily treatment of *E. aureum* extract. Diazepam (5 mg/kg) was taken as a reference standard. Urinary vanillylmandelic acid (VMA) and ascorbic acid were selected as noninvasive biomarkers to assess the anti-stress activity and plasma cortisol, blood ascorbic acid, and weight of adrenal were measured. The 24 h urinary excretion of VMA and ascorbic acid were determined by spectrophotometric methods in all groups under normal and stressed conditions. The hematological parameters (neutrophils, lymphocytes, and eosinophils) were also determined. **Results:** Administration of *E. aureum* doses of 400 and 600 mg/kg was found to be effective in inhibiting the stress-induced urinary biochemical changes in a dose-dependent manner. Treatment with *E. aureum* extract prevents the rise in blood ascorbic acid and plasma cortisol. Moreover, the extract prevented the increase in weight of adrenal gland also significantly increased the anoxia stress tolerance time. Dose-dependent significant reduction in white blood cell count was observed in anoxic stress tolerance test as compared to stressed group. **Conclusion:** Hence, the present study provides scientific support for the positive adaptogenic effect of *E. aureum* extract.

**Keywords:** Anorexia tolerance test, anti-stress, cortisol, *Epipremnum aureum*, forced swimming test

**INTRODUCTION**

Modern life is full of competition, hassles, deadlines, frustration, and demands which gradually results in stress if not fulfilled. Stress can be defined as the sum total of all the reactions of the body, which disturb the normal physiological condition and results in a state of disrupted homeostasis. Stress is one of the common factors which cause a number of chronic diseases such as atherosclerosis, coronary heart disease, aging, and liver disease. According to the report of WHO, approximately 450 million people suffer from mental or behavioral disorders like stress. This amounts to 12.3% of the global burden of disease and is predicted to rise up to 15% by 2020. Though many synthetic medications are available for treating stress, they are associated with long-term side effects. In this regard, adaptogens can play an important role. Adaptogens are the agents that improve adaptation capacity of the organism during stress and “Anti-stress” agent is a pharmacological word for the same, meaning an agent, which nullifies or prevents ill effects of stress and improves adaptation. These are natural herb product that is proposed to increase the body’s resistance to stress, trauma, anxiety and fatigue, commonly found in Traditional Chinese Medicine. They work at the cellular level to help normalize the body’s various functions and stimulate the recovery processes needed to adapt to

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all types of stress. The beneficial effects of adaptogens are mainly associated with the hypothalamic–pituitary–adrenal axis, a part of the stress system that is believed to play a primary role in the reactions of the body to repeated stress and adaptation by balancing the releases of adrenaline.

*Epipremnum aureum* is known by many names but most common is “Money Plant.” It is a large root-climber belongs to the botanical family of *Araceae* and a common house plant with several cultivars and capable of removing indoor air pollutants such as xylene, formaldehyde, and benzene. The plant generally stands at a height of between 5 m and 9 m and has a total spread of 1.5–2.5 m. *E. aureum* produces small green flowers in summer. This plant is widely known in Malaysia and Singapore and has a reputation as a traditional anticancer preparation as well as a remedy for skin diseases. A decoction of the fresh leaves with meat or eggs or as tea was reported to be a common practice among the locals. Aerial roots and leaves of *E. aureum* show great potential for antioxidant activity. Antioxidants play an important role in resisting stress. In the present study, an attempt has been made to investigate the adaptogenic activity using ethanolic extract of *E. aureum* in view of reported antioxidant activity of this plant.

**Materials and Methods**

**Collection and authentication of plant**

The fresh whole plant of *E. aureum* was collected from Kepong district, Malaysia. The plant was identified by Miss Tan Ai Lee, Research Officer, Natural Products, Forest Research Institute Malaysia. The voucher specimen (No. SBID: 001/15) was prepared and deposited in the Faculty of Pharmacy, Lincoln University College, Malaysia for imminent reference.

**Plant material**

The authenticated leaves were washed with fresh water and dried under shade of sunlight for 5 days. The dried plant leaves were coarsely powdered with the help of mechanical grinder. The powder was stored in an airtight container for further use. The ethanolic extract was provided by the method of hot percolation using soxhlet apparatus and 90% ethanol. After completion of extraction, the resulting extract was concentrated using rotary evaporator and stored in desiccator.

**Standardization of ethanol extract**

**Determination of total flavonoids**

The total flavonoid contents of crude extract were estimated by aluminium chloride colorimetric method.[3] Sodium nitrate (2.5 g) was taken in a volumetric flash (50 mL) and added water up to the mark that was 5% sodium nitrate. Sodium hydroxide (2.5 g) was taken in another volumetric flash (50 mL) and added water up to the mark that was 4% sodium hydroxide. Then, 10% aluminum chloride solution was prepared the same procedure. The different crude extracts (0.25 mg) were taken in a test tube and added water (1.25 mL) and sodium nitrate (0.75 µL) then mixed. All the test tubes were kept in the dark place for 6 min. Then, 10% aluminum chloride (0.150 µL) was added to the test tube and wait for 5 min in the dark for complete reaction. Finally, 5% sodium hydroxide (0.5 mL) and water (0.275 mL) were added to the test tube. The absorbance was measured of all samples at a fixed wavelength 510 nm using ultraviolet spectrophotometer. Quercetin standard was used for the calibration curve. The estimation of total flavonoids contents in the crude extracts was carried out in triplicate, and the results were averaged. The total flavonoid was calculated by the following formula:

\[ X = \frac{(A - m)}{A_o} \]

Where “X” is the flavonoid content, mg/g plant extract, “A” is the absorption of plant crude extract solution, “Ao” is the absorption of standard quercetin solution, “m” is the weight of crude drug extract in mg and “mo” is the weight of quercetin in the solution in mg.

**Experimental animals**

Swiss albino mice (18–25 g) were selected for the study. They were kept in the departmental animal house at 26 ± 2°C and relative humidity 44–56%, light and dark cycles of 12 h respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet, and the food was withdrawn 24 h before the experiment though water was allowed *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Ethics Committee, Lincoln University College, Malaysia.

**Determination of acute toxicity lethal dose 50**

The determination of lethal dose 50 was performed according to OECD guideline 423. The extract at a dose of 2000 mg/kg did not produce any observable toxic effects during entire duration of the study and all animals survived 14 days of observation.[4]

**Anti-stress activity**

**Forced swimming test**

In this method, mice were forced to swim until exhausted (3–4 min) in a cylindrical vessel of (25 cm height × 18 cm in diameter) which was filled with water to a 15-cm depth at a temperature maintained at 25°C. The mice were again placed individually in the water-filled glass cylinder and the duration of immobility was recorded during the last 4 min of a 6 min test. The duration of stress induced immobility was recorded. Decrease in
the duration of immobility during the forced swimming test (FST) was taken as a measure of anti-stress activity. The 24 h urinary levels of vanillylmandelic acid (VMA) and ascorbic acid were determined for both normal and stress-induced animals.

The experimental protocol

Mice of either sex weighing between 18 and 25 g were divided into five groups (I, II, III, IV, and V) each containing six animals. The 24 h urine sample from each group was collected into two different beakers, one containing 5 mL of 10% oxalic acid for the determination of ascobic acid at 550 nm and the other containing 0.5 mL of 6 N hydrochloric acid for the determination of VMA spectrophotometrically at 360 nm.

In the first phase of the experiment, 24 h urine samples were collected in all the four groups and analyzed for both VMA and ascorbic acid. The normal values were recorded for 7, consecutive days.

In the second phase, the animals in each group were subjected to fresh water swimming stress individually. The 24 h urinary levels of VMA and ascorbic acid under stressed conditions were determined for 7 consecutive days and the swimming induced stress immobility was recorded.

In the third phase of the experiment, Group III and IV were administered orally with E. aureum (suspended in 1% carboxymethyl cellulose [CMC]) at daily doses of 400 and 600 mg/kg body weight respectively for 7 consecutive days after having recovered completely. Group I served as control and Group II received diazepam 5 mg/kg as a standard drug. The 24 h urine samples were collected, and the levels of both VMA and ascorbic acid were determined. The swimming induced stress immobility was recorded after treatment.

The final phase of the experiment consisted of administration of E. aureum extract to the same groups of animals after a recovery period of 1 week. One hour before the daily induction of stress, Groups III and IV were administered orally with E. aureum at doses of 400 and 600 mg/kg body weight, respectively, for 7 consecutive days. The 24 h urine samples were collected and analyzed for VMA and ascorbic acid for 7 consecutive days to study the effect of extract on the stress-induced biochemical changes.

Treatment protocol

- Group I: Vehicle control (received 1% CMC in normal saline (1 mL) p.o saline water once daily for 7 days) without stress
- Group II: Positive control (received 1% CMC in normal saline (1 mL) p.o saline water once daily for 7 days) with stress
- Group III: Standard (received diazepam 5 mg/kg (Standard drug) once daily for 7 days) with stress
- Group IV: Received 400 mg/kg extract (test sample) once daily for 7 days with stress
- Group V: Received 600 mg/kg extract (test sample) once daily for 7 days with stress.

Anoxic stress tolerance test

Swiss albino mice (18–25 g) were used for anoxic stress tolerance test. The animals were divided into four groups having six animals in each group, and all were treated as per the following drug treatments.

- Group I: Received 1% CMC in normal saline (1 mL) p.o saline water once daily for 7 days
- Group II: Positive control (received 1% CMC in normal saline (1 mL) p.o saline water once daily for 7 days) with stress
- Group III: Received diazepam5 mg/kg (standard drug) once daily for 7 days with stress
- Group IV: Received 400 mg/kg extract (test sample) once daily for 7 days with stress
- Group V: Received 600 mg/kg extract (test sample) once daily for 7 days with stress.

At the end of drug treatment, the animals were exposed to the anoxia stress and anoxia tolerance time was noted. Conical flasks of 250 mL capacity were used for the study. These flasks were made airtight using rubber cork before beginning the experiment. Each animal was kept in the airtight vessel and time was noted using a stopwatch. The moment an animal showed first convulsion, it was removed immediately from the vessel and resuscitated if needed. The time duration from the entry of the animal in the hermetic (conical flask) vessel to the appearance of the first convulsion was taken as the time of “anoxic stress tolerance.” The mean time to convulsion was recorded and animal was removed at onset of convulsion. All the animals were anesthetized using ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) and blood samples were collected via cardiac puncture. The collected blood samples were assayed for biochemical and hematological parameters, such as plasma cortisol, blood ascorbic acid, adrenal weight, blood glucose, neutrophils, lymphocytes, and eosinophils, respectively.

Data and statistical analysis

All values were expressed as mean ± standard error of the mean. The result was analyzed statistically using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. P ≤ 0.05 was considered statistically significant.
Results
The total flavonoid content in the extract was found to be 275.5 mg equivalent to quercetin for ethanol as the extracting solvent.

In FST model, the swimming survival time was increased in test Group V (200.67 ± 1.61) as compared to positive control group (122.67 ± 1.28) after 7 days treatment with E. aureum extract. Consequently, higher dose of E. aureum is more efficacious with respect to lower dose of extract as shown in Table 1.

The level of levels of vanillylmandelic acid stress-induced rats were found to be decreased as compared to control group and stress induced group after administration of Epipremnum aureum extract as shown in Table 2. Post administration of Epipremnum aureum extract have shown to increased 24 h urinary levels of ascorbic acid in stress induced rats as compared to control group as shown in Table 3.

In anorexia stress tolerance model, both the test group increases the duration of anoxic tolerance stress time in 8th day. Group V at a dose of 600 mg/kg has shown a highly significant increase in duration of anoxic tolerance stress time (39.83 ± 0.90) as compared to positive control Group II (20.83 ± 0.94) while Group IV has moderately increased the stress time to (26.66 ± 0.84) as shown in Table 4.

On 8th day of the study, the elevated biochemical parameters were found to be reduced in both the test groups in a dose dependant manner. The level of plasma cortisol in Group V was found to be reduced to 17.34 ± 0.69 as compared to positive control (26.42 ± 0.76). Blood ascorbic acid level was found to be 668.42 ± 3.42 in Group V with respect to 873.79 ± 3.41. The increase in adrenal weight (16.02 ± 0.46) in Group II is reduced to 11.84 ± 0.54 in Group V. The elevated level of glucose (168.89 ± 0.46) in Group II was reduced (120.31 ± 0.40) in Group V as shown in Table 5.

Table 1: Effect of Epipremnum aureum extract on swimming survival time (s) for forced swimming endurance test in Swiss albino mice for 7-day study

| Group                      | Treatment                                      | Swimming survival time (s) |
|----------------------------|------------------------------------------------|-----------------------------|
| Group I control (vehicle)  | 1% CMC in normal saline 1 mL p.o               | 142.67±1.28                |
| Group II positive control + stress | 1% CMC in normal saline 1 mL p.o               | 122.67±1.28                |
| Group III standard         | Diazepam 5 mg/kg p.o                           | 238.67±1.17                |
| Group IV extract           | E. aureum extract 400 mg/kg p.o                | 129.17±1.74*               |
| Group V extract            | E. aureum extract 600 mg/kg p.o                | 200.67±1.61**              |

Values are mean±SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. *P<0.05 not significant, **P<0.01 considered significant. SEM: Standard error of mean, CMC: Carboxymethyl cellulose, E. aureum: Epipremnum aureum

Table 2: Effect of Epipremnum aureum extract on the 24 h urinary levels of vanillylmandelic acid in normal and stress-induced rats

| Group                      | Treatment                                      | Level of urinary VMA µg/kg |
|----------------------------|------------------------------------------------|-----------------------------|
| Group I control (vehicle)  | 1% CMC in normal saline 1 mL p.o               | 191.33±1.46                |
| Group II positive control + stress | 1% CMC in normal saline 1 mL p.o               | 364.67±0.84                |
| Group III standard         | Diazepam 5 mg/kg p.o                           | 204.17±1.35                |
| Group IV extract           | E. aureum extract 400 mg/kg p.o                | 341.11±1.47***             |
| Group V extract            | E. aureum extract 600 mg/kg p.o                | 252.51±1.02***             |

Values are mean±SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control, ***P<0.05 considered statistically significant. SEM: Standard error of mean, CMC: Carboxymethyl cellulose, E. aureum: Epipremnum aureum, VMA: Vanillylmandelic acid

Table 3: Effect of Epipremnum aureum extract on the 24 h urinary levels of ascorbic acid in normal and stress-induced rats

| Group                      | Treatment                                      | Level of urinary ascorbic acid µg/kg |
|----------------------------|------------------------------------------------|--------------------------------------|
| Group I control (vehicle)  | 1% CMC in normal saline 1 mL p.o               | 121.50±0.67                          |
| Group II positive control + stress | 1% CMC in normal saline 1 mL p.o               | 51.66±0.55                           |
| Group III standard         | Diazepam 5 mg/kg p.o                           | 112.50±1.12                          |
| Group IV extract           | E. aureum extract 400 mg/kg p.o                | 74.00±1.06                           |
| Group V extract            | E. aureum extract 600 mg/kg p.o                | 104.11±1.07***                       |

Values are mean±SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. ***P<0.05 considered statistically significant. SEM: Standard error of mean, CMC: Carboxymethyl cellulose, E. aureum: Epipremnum aureum
As shown in Table 6 the lymphocyte count of Groups IV (64.79 ± 0.98) and V (1.94 ± 0.12) have reduced due to pretreatment of extracts with respect to Group I (42.01 ± 0.28). The eosinophil count of animals of Group I, II, III, IV, and V was found as 1.23 ± 0.15, 3.87 ± 0.23, 1.70 ± 0.19, 3.04 ± 0.10, and 1.94 ± 0.12, respectively.

DISCUSSION

Adaptogens are agents which act as smooth anti-stressor by reducing the reactivity of host defense system resulting in decreasing the damaging effect of various stressors due to increase of basal level mediators involved in the stress response.[7] The FST is the most preferred screening model for evaluating the anti-stress activity of a compound. This model is based on the observation that when animals are forced to swim in water, eventually they assumed characteristics of immobile posture and devoid of any activity. The appearance of immobility, therefore, reflects a state of tiredness, fatigue and reduced stamina with the end point being the moment when the mice could not swim further. In the present study, the increased swimming survival time has been observed in mice pretreated with *E. aureum* as compared to stressed mice. The physical performance was enhanced significantly longer than untreated (control).

### Table 4: Effect of *Epipremnum aureum* on duration of anorexic stress tolerance for anorexic stress tolerance test in Swiss albino mice for 7-day study

| Group                     | Treatment                          | Duration of anoxic stress tolerance (min) |
|---------------------------|------------------------------------|------------------------------------------|
| Group I negative control  | 1% CMC in normal saline 1 mL p.o   | 18.12±0.21                               |
| Group II positive control | 1% CMC in normal saline 1 mL p.o + stress | 20.83±0.94                               |
| Group III standard        | Diazepam 5 mg/kg p.o               | 50.16±0.87                               |
| Group IV extract          | *E. aureum* extract 400 mg/kg p.o  | 26.66±0.84**                             |
| Group V extract           | *E. aureum* extract 600 mg/kg p.o  | 39.83±0.90***                            |

Values are mean±SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. **P<0.05 considered statistically significant. SEM: Standard error of mean, CMC: Carboxymethyl cellulose, *E. aureum*: *Epipremnum aureum*

### Table 5: Effect of *Epipremnum aureum* extract on biochemical parameters for anorexia stress tolerance test in Swiss albino mice for 7-day study

| Group                     | Treatment                          | Plasma cortisol µg/100 mL | Blood ascorbic acid µg % | Adrenal weight mg/100 g of b.w | Glucose (mg/dl) |
|---------------------------|------------------------------------|---------------------------|--------------------------|---------------------------------|-----------------|
| Group I negative control  | 1% CMC in normal saline 1 mL p.o   | 13.74±0.24                | 571.29±2.78              | 9.01±0.28                       | 99.17±0.79      |
| Group II positive control | 1% CMC in normal saline 1 mL p.o + stress | 26.42±0.76               | 873.79±3.41              | 16.02±0.46                      | 168.89±0.46     |
| Group III standard        | Diazepam 5 mg/kg p.o               | 14.46±0.31***             | 619.12±3.12***           | 10.28±0.17***                   | 100.97±1.16***  |
| Group IV extract          | *E. aureum* extract 400 mg/kg p.o  | 22.20±0.60*               | 810.46±3.54*             | 14.25±0.21*                     | 148.04±0.68*    |
| Group V extract           | *E. aureum* extract 600 mg/kg p.o  | 17.34±0.69***             | 668.42±3.42***           | 11.84±0.54***                   | 120.31±0.40***  |

Values are mean±SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. *P<0.01 considered non significant. **P<0.05 considered statistically significant. SEM: Standard error of mean, CMC: Carboxymethyl cellulose, *E. aureum*: *Epipremnum aureum*.

### Table 6: Effect of *Epipremnum aureum* extract on hematological parameters for anorexia stress tolerance test in Swiss albino mice for 7-day study

| Group                     | Treatment                          | Neutrophils | Lymphocytes | Eosinophils |
|---------------------------|------------------------------------|-------------|-------------|-------------|
| Group I control           | 1% CMC in normal saline 1 mL p.o   | 14.19±0.12  | 42.01±0.28  | 1.23±0.15   |
| Group II positive control | 1% CMC in normal saline 1 mL p.o + stress | 20.29±0.32  | 68.30±0.54  | 3.87±0.23   |
| Group III standard        | Diazepam 5 mg/kg p.o               | 15.20±0.25  | 51.72±0.50**| 1.70±0.19** |
| Group IV extract          | *E. aureum* extract 400 mg/kg p.o  | 17.88±0.28* | 64.79±0.98* | 3.04±0.10*  |
| Group V extract           | *E. aureum* extract 600 mg/kg p.o  | 16.17±0.32**| 61.61±0.42**| 1.94±0.12** |

Values are mean±SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. *P<0.01 considered non significant. **P<0.05 considered statistically significant. SEM: Standard error of mean, CMC: Carboxymethyl cellulose, *E. aureum*: *Epipremnum aureum*.
group and thus confirmed the adaptogenic potential of *E. aureum*.\(^9\)

In case of stressful conditions, noradrenaline is released and metabolized to VMA peripherally and 3-methoxy-4-hydroxyphenyl glycol centrally.\(^9\) In the present study, VMA, the major metabolite of sympathetic amines, was taken as an indirect biochemical marker to represent the increase in peripheral sympathetic activity during stress. The increase in the urinary VMA excretion during stress was used as a noninvasive biochemical marker to study the anti-stress activity of *E. aureum*.

The urinary level of ascorbic acid decreases with the application of stress. Ascorbic acid is a free radical scavenger which is more likely to be utilized in scavenging the free radicals involved in stress. Administration of *E. aureum* extract in stress-induced mice reversed the stress-induced biochemical changes i.e., increase in urinary VMA levels and decrease in urinary ascorbic acid levels, in a dose-dependent manner.

The anoxia stress model was used to extensively evaluate the anti-stress activity. Anorexia is a very severe stressor. All the body functions including cellular respiration depend on oxygen supply to them. Any lack of this vital element plays an important role on all body mechanisms. *E. aureum* extract significantly prolonged the meantime to convulsion as compared to positive control group. In response to stress, the weight of adrenal glands was increased which was due to hypertrophy of adrenal glands.\(^\text{10}\) ACTH is released, which acts on the adrenal cortex to stimulate the synthesis and release of cortisol and ascorbic acid.\(^\text{11}\) Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves which in turn increased blood glucose level.\(^\text{12}\) *E. aureum* significantly prevents the hypertrophy of adrenal glands and decreases the stress induced elevated levels of cortisol, ascorbic acid and blood glucose. In stress-induced animals, spleen contracts and releases more amounts of lymphocytes, neutrophils and eosinophils into circulations.\(^\text{13}\) Pretreatment with *E. aureum* extract have shown to significantly reduce the number of white blood cells parameters. The adaptogenic activity may be attributed due to the presence of flavonoids in the *E. aureum* extract.

**Conclusion**

The present study provides adequate data to support the adaptogenic potential of *E. aureum* extract as confirmed by results obtained. Further investigations are required to characterize the active constituent(s) responsible for observed activities of the plant extract.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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