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# ADAPTOGENIC ACTIVITY OF *TABERNAEMONTANA ALTERNIFOLIA* LEAVES ON RODENTS

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**KEYWORDS:**

Adaptogen, Anoxia stress tolerance, *Tabernaemontana alternifolia*, Immobilisation stress.

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## ABSTRACT

**Objective:** To screen the adaptogenic activity of ethanolic and aqueous extracts of *Tabernaemontana alternifoli*a leaves using anoxia stress tolerance model and immobilization induced stress model. **Method:** In both the stress models a well known adaptogen *Withania somnifera* was selected as reference standard. The adaptogenic effect was assessed by recording the parameters like anoxia stress tolerance time in anoxia stress model and by estimation of biochemical parameters like cholesterol, glucose, triglycerides, blood urea nitrogen in immobilisation stress model. **Results:** Ethanolic extract exhibited significant adaptogenic activity by increasing the anoxia stress tolerance time and by reducing the elevated level of biochemical parameters due to the stress compared to control groups. **Conclusion:** The results obtained in this study indicate that EETA possess significant adaptogenic activity may be due to the presence of various phytochemicals in test extracts.

## 1. INTRODUCTION:

Stress is a state of threatened homeostasis or disharmony and it is the major cause for anxiety, depression, hypertension, peptic ulcer, metabolic disorders, endocrine disorders etc[1]. Several attempts have been made to counter the effects of stress which includes meditation, yoga and there are certain antistress drugs which induce a state of non-specific resistance against stressful condition[2]. Drugs such as benzodiazepines, CNS stimulants and some anabolic steroids are commonly used by people to combat stress. However, the incidence of toxicity, dependence and other adverse effects has limited the therapeutic usefulness of these drugs[3]. Hence it is imperative to find out drugs with minimum side effect and for its effective adaptogenic activity. Adaptogens are plant based formulation claimed to enhance physical endurance, mental function and non specific resistance of the body[4]. The potential utilities of cheaper, safer and effective herbal medicines as antistress agents have been reported as they can withstand stress without altering the normal physiological functions of the body[5].

*Tabernaemontana alternifolia* (family: Apocynaceae) is one traditionally used plant which has reported to have medicinal properties and was used to cure many disorders. Folk usage of the plant suggested its use in snake bite, gonorrhea, venereal diseases, nervous disorders, diabetes, chronic bronchitis, rheumatism, respiratory problems, cardio tonic ailments, antioxidant and antiproliferative[6]. However the plant has not been evaluated scientifically for adaptogenic activity. Hence, the present study was conducted with the aim to investigate the adaptogenic activity of ethanolic and aqueous extracts of *Tabernaemontana alternifolia* leaves using anoxia stress and immobilization induced stress models in rodents.

## 2. MATERIALS AND METHOD

### 2.1. Collection of plant material

The leaves of *Tabernaemontana alternifolia* was collected from the Neralamane Village, Thirthahalli taluk, Shivamogga district**,** Karnataka, India in the month of November 2019 and authenticated by Ms. Soukya N., Dept of Botany, S.R.N.M College, Shivamogga. The leaves were shade dried and pulverized to obtain coarse powder.

### 2.2. Preparation of extract[7]

**Aqueous extract**

The aqueous extract was prepared by maceration process. In a 2000ml conical flask 100gm of powdered drug was taken with 500ml of distilled water, 10ml of chloroform is added as preservative and kept for extraction up to 7 days with occasional stirring. After 7 days the extract was filtered through muslin cloth and the marc was discarded. The filtrate obtained was concentrated to semisolid

mass and the dried extract was stored in airtight container in refrigerator below 10°C. The percentage yield of extract was found to be 20%.

**Ethanolic extract**

The powdered material of *Tabernaemontana alternifolia* leaves was defatted with petroleum ether (60-80°C) in a soxhlet extractor. The marc obtained was subjected to extraction with 95% ethanol for 48 hrs in 2 batches of 250g each. The extract was concentrated and stored in airtight container in refrigerator below 10°C. The percentage yield of extract was found to be 19.6%

**2.3. Preliminary Phytochemical screening**

Preliminary phytochemical screening was performed on EETA and AETA for the detection of phytochemicals as per the standard methods described in practical pharmacognosy by K.R. Kandelwal and Dr. C. K. Kokate[8,9].

**2.4. Experimental animals**

Healthy albino mice (20-25g) and wistar rats (150-200g) of either sex were used for the acute toxicity and pharmacological studies. The animals were procured from central animal house, National College of Pharmacy, Shivamogga, Karnataka. After randomization into various groups, animals were acclimatized for period of 7 days under standard husbandry conditions; room temperature 27°±2°C, relative humidity 65% ± 10% and 12 hours-light/dark cycle. All the animals were fed with rodent pellet diet (Krishvet feeds, Bangaluru) and water was allowed *ad libitum* under strict hygienic condition. Ethical clearance (Clearence number: NCP/IAEC/CL/03/2018-2019) for performing experiments on animals was obtained from Institutional Animal Ethical Committee (IAEC).

**2.5. Acute toxicity study[10]**

Healthy albino mice (20–25 gm) were used for acute toxicity study. The animals were fasted over night prior to the experiment. Acute oral toxicity study for the test extracts was carried out as per OECD guideline-425.

**2.6. Evaluation of adaptogenic activity[11- 13]**

**2.6.1. Anoxia stress tolerance in mice.**

Albino mice (20-25g) of either sex were randomly assigned to six experimental groups with 6 animals each as **Group-I** Control (treated with vehicle 1ml/kg b.w *p.o*), **Group-II** Standard (treated with *Withania somnifera* 100mg/kg b.w *p.o*), **Group-III** Animals treated with EETA (200mg/kg b.w *p.o*), **Group-IV** Animals treated with EETA (400mg/kg b.w *p.o*),**Group-V:** Animals treated with AETA (200mg/kg b.w *p.o*), **Group-VI** Animals treated with AETA (400mg/kg b.w *p.o*). The animals under particular group were treated as stated above for three weeks. On 7$^{th}$, 14$^{th}$, 21$^{st}$ day after one hour of treatment, anoxia stress tolerance time was recorded by placing each animal individually in the

hermetic vessel of 1 lit. air capacity. The time duration from the entry of the mice to hermetic vessel till the appearance of first convulsion was considered as anoxia stress tolerance time. Appearance of the first convulsion was very sharp end point and at that point animal should be immediately removed from vessel, delay of even a minute in removal of animal may lead to their death.

**2.6.2. Immobilisation induced stress in rats.**

Albino rats (150-200g) of either sex were randomly assigned to seven experimental groups with 6 animals per group as **Group-I** Negative control (unstressed, untreated), **Group-II** Positive control (stressed, treated with vehicle), **Group-III** Standard group (treated with *Withania somnifera* 100mg/kg b.o *p.o.*), **Group-IV** Animals treated with EETA (200mg/kg b.o *p.o.*), **Group-V** Animals treated with EETA (400mg/kg b.o *p.o.*), **Group-VI** Animals treated with AETA (200mg/kg b.o *p.o.*), **Group-VII** Animals treated with AETA (400mg/kg b.o *p.o.*). The animals under particular group were treated as stated above for 10 days. After one hour of the treatment, stress was induced by immobilizing rats in supine position by fixing the hindlimbs and forelimbs to wooden board inclined at 60°, 2 hours daily for a period of 10 days. The animals were sacrificed after 10 days and blood was collected by cardiac puncture under mild ether anesthesia. Biochemical parameters such as blood glucose, triglycerides, cholesterol and blood urea nitrogen were estimated for the collected blood samples.

**2.7. Statistical analysis**

All the values were expressed as mean ± S.E.M. Statistical analysis was carried out by performing one-way ANOVA followed by Graphpad 8.0 pair wise comparisons of Tukey's test. The experimental groups were compared with control in anoxia stress and positive control in immobilisation model. A probability level of $P<0.05$ was considered moderately significant, $P<0.01$ is considered as significant and $P<0.001$ is considered as highly significant.

**3. RESULTS**

**3.1. Phytochemical analysis**

Preliminary phytochemical analysis reveals that saponins, terpenoids, flavonoids, alkaloids, steroids, anthraquinones, carbohydrates, mucilage and tannins were present in EETA and AETA. Cardiac glycosides were present in EETA but absent in AETA.

**3.2. Acute toxicity study**

The acute toxicity study was carried out for EETA and AETA at the dose of 2000mg/kg b.w. Both the extracts were found to be safe and no mortality of the animals observed. hence, we have selected $1/10^{th}$ of $LD_{50}$ (200mg/kg) and $1/5^{th}$ of $LD_{50}$ (400mg/kg) for screening adaptogenic activity.

### 3.3. Adaptogenic activity

#### 3.3.1. Anoxia stress model

In case of anoxia stress tolerance test the ethanolic extract of *Tabernaemontana alternifolia* at a dose of 400mg/kg b.w exhibited highly significant antistress activity as indicated by increase in duration of anoxia stress tolerance time in 7$^{th}$, 14$^{th}$ and 21$^{st}$ day. The test drug also produced moderate increase in anoxia stress tolerance time at the dose of 200mg/kg b.w. The aqueous extract of *Tabernaemontana alternifolia* at the dose of 200mg/kg b.w produced mild increase in anoxia stress tolerance time which was however, not significant statistically but the aqueous extract at the of 400mg/kg b.w exhibited moderate increase in anoxia stress tolerance time in 14$^{th}$, 21$^{st}$ day. The results are shown in Table 1.

Table 1: Effect of EETA and AETA on anoxia stress tolerance in mice.

| Sl.No. | Groups | Dose (mg/kg) | Duration of anoxia stress tolerance(min) | | |
|---|---|---|---|---|---|
| | | | 7$^{th}$ Day | 14$^{th}$ Day | 21$^{st}$ Day |
| 1 | Control | - | 41.17±1.973 | 42.13±1.12 | 42.48±1.7 |
| 2 | Standard | 100 | 65.5±1.976*** | 68.76±1.01*** | 68.966±0.89*** |
| 3 | EETA | 200 | 46.5±0.763$^{ns}$ | 46.67±0.494* | 48.5±0.67* |
| 4 | EETA | 400 | 48.17±0.79* | 49.83±0.477*** | 52.6±0.405*** |
| 5 | AETA | 200 | 40.17±0.609$^{ns}$ | 41.33±2.124$^{ns}$ | 44.7±1.66$^{ns}$ |
| 6 | AETA | 400 | 43.5±2.125$^{ns}$ | 46.3±0.494* | 48.17±0.401* |

Values are expressed as mean ± S.E.M. n=6, ***P < 0.001 is considered as highly significant.

#### 3.3.2. Immobilisation stress

In case of immobilisation stress model, immobilisation stress caused marked increased in blood glucose, cholesterol, triglycerides and BUN in rats. This stress induced elevated levels of biochemical parameters were reversed significantly by ethanolic extract at dose of 400mg/kg and moderately reversed at the dose of 200mg/kg. The aqueous extracts however failed to reverse the stress induced change in biochemical parameters. The results are shown in Table 2.

Table 2: Effect of EETA and AETA on immobilisation stress induced biochemical parameters in rats.

| Sl.No. | Groups | Dose (mg/kg | Biochemical estimations (mg/dl) | | | |
|---|---|---|---|---|---|---|
| | | | Glucose | Cholesterol | Triglycerides | BUN |
| 1. | Positive control | - | 143±0.2740 | 86.7±0.5891 | 111.1±0.3567 | 39.2±0.2418 |
| 2. | Negative control | - | 92.2±0.2*** | 55.7±0.200*** | 67.99±0.84*** | 26±0.6874*** |
| 3. | Standard | 100 | 98.1±0.26*** | 64.8±0.815*** | 74.92±0.83*** | 27.9±0.2168*** |
| 4. | EETA | 200 | 139±0.642* | 82.8±0.4330** | 104.8±0.456** | 36.6±0.4702* |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5. | EETA | 400 | 120±0.545** | 77±0.966*** | 84.93±0.12*** | 29.9±0.5019*** |
| 5. | AETA | 200 | 142±1.573ns | 85.5±0.307ns | 107.9±1.73ns | 38.7±0.6776ns |
| 7. | AETA | 400 | 140±0.459ns | 83.6±0.5820* | 106.4±1.234* | 37.2±0.3647* |

Values are expressed as mean ± S.E.M. n=6, ***P < 0.001 is considered as highly significant.

## 4. DISCUSSION:

In the present investigation ethanol and aqueous extracts of *Tabernaemontana alternifolia* were evaluated for the antistress activity against different types of stress viz. anoxia and immobilisation stress models. The well known adaptogen *Withania somnifera* was taken as a standard in this study. The result of the present study reveals that the test extracts possess adaptogenic activity which may be due to the presence of various phytochemicals.

The Phytochemical studies of ethanonic and aqueous extracts revealed the presence of steroids which are thought to be involved in the inactivation of the stress system and in protecting the organism from over reaction in response to stresssors. In addition, there are reports that polyphenolic compounds like flavonoids and tannins are useful as antioxidants. These antioxidants will inhibit the accumulation of the free radicals which in-turn put the body in a state of oxidative stress and will bring injury to the body by attacking large molecules and cell organs. Thus, the presence of polyphenolic compounds and other phytochemicals in the test extracts gave strong evidence to adaptogenic activity[14].

The enhanced activity of hypothalamo-hypophyseal axis (HAP) during the stress results in liberation of catecholamines and corticosteroids which in-turn lead to increase in blood cholesterol level since epinephrine is known to mobilise lipids from adipose tissues. The increased release of catacholamines also leads to elevation in blood glucose and BUN level. The effect of stress on serum triglycerides has been shown to be variable[15].

The human body has a complex antioxidant defence system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT). These block the initiation of free radical chain reactions. The nonenzymatic antioxidant components consist of molecules such as alphatocopherol, glutathione, ascorbic acid and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions. However, when free radicals are generated in excess or when the cellular antioxidant defence system is defective, they can stimulate chain reactions by interacting with proteins, lipids and nucleic acids causing cellular dysfunction and even death[16].

Impairment in antioxidant defence mechanism or increase in generation of oxidative free radicals have been implicated in chronic stress induced diabetes mellitus, hypertension, immunosuppression,

reproductive dysfunctions, peptic ulceration, inflammation and behavior dysfunction like anxiety due to involvement of the central nervous system, endocrine system and metabolic system[15].

The leaves of *Tabernaemontana alternifolia* have exerted significant antioxidant activity induced by augmented activity of OFR scavenging enzymes like catalase (CA), glutathione peroxidise (GPx) and superoxide dismutase (SOD).

Experimental studies have confirmed the adaptogenic properties of *Tabernaemontana alternifolia* and the effects are apparently due to presence of various phytochemicals in the leaves like flavonoids, steroids, tannins, saponins, glycosides and tannins.

## 5. CONCLUSION:

The present study concludes that the ethanolic extract of the *Tabernaemontana alternifolia leaves* possess adaptogenic activity and the phytoconstituents present in the test extract may be responsible for adaptogenic activity.

However, further studies are necessary to find the exact mechanism of adaptogenic effect and to isolate the active compounds responsible for this pharmacological activity.

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