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# Evaluation of Adaptogenic Activity of *Anacyclus pyrethrum* L. in Animal Models

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**Original Research Article**

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**Abstract:** *Anacyclus pyrethrum* L. root is one among the ingredients of polyherbal formulation frequently employed for the treatment of stress by Ayurvedic practioners. So, the objective of this study was to scientifically validate its use. Animals were administered graded doses of ethanolic extract of *Anacyclus pyrethrum* root and subjected for anoxia stress tolerance test, swimming endurance test and acetic acid induced writhing test models and its adaptogenic potential was assessed by estimating various parameters. Stressed animals demonstrated altered values of these parameters. Ethanolic extract of *Anacyclus pyrethrum* root exhibited significant anti-stress activity by restoration of all the altered values. Present findings validate its use in Aurvedic system of medicine.

**Keywords:** Antistress**,** *Anacyclus pyrethrum*, anoxia stress tolerance test.

## INTRODUCTION

In the present era, stress commonly affects all age group persons as it is inevitable factor in the modern day society. Persistent stress leads to many ailments such as diseases of CNS, immune system etc [1]. To combat stress and stress related disorders, different approaches are practiced like exercise and relaxation. But these techniques offer mixed results and often not satisfactory results [2].

Indian systems of Ayurvedic medicine practioners employ several medicinal plants as adaptogens for the treatment of stress [3]. Traditionally *Anacyclus pyrethrum* L. root is known to possess many medicinal uses [4].

This plant was used for its properties such as analgesic, antibacterial, antiviral, diuretic, etc [5]. Literature report reveals the extensive researches done on *A. pyrethrum* are antidepressant, antibacterial, immunostimulating and antioxidant [6-9]. The purpose of this study is to demonstrate antistress effect of *Anacyclus pyrethrum* root in experimental animals.

## MATERIALS AND METHODS

### Evaluation of antistress activity of EEAPR (Ethanolic extract of *Anacyclus pyrethrum* root)

### Anoxic tolerance test

Albino mice of either sex weighing 20 -30 g were selected and divided into five groups of six each.
Group I : Stress control, received vehicle only
Group II: Standard (*Withania somnifera*, 100 mg/kg, p.o.(per oral))
Group III: EEAPR 125 mg/kg p.o. ($1/20^{th}$ of $LD_{50}$ cut off value)
Group IV: EEAPR 250 mg/kg p.o. ($1/10^{th}$ of $LD_{50}$ cut off value)
Group V: EEAPR 500 mg/kg p.o. ($1/5^{th}$ of $LD_{50}$ cut off value)

As shown above all animals were treated for three weeks. Stress was induced on $7^{th}$, $14^{th}$ and $21^{st}$ day 1 hr. after the treatment, in all the groups of animals by placing each mouse individually in the hermetic vessel of 300 ml capacity to record post anoxic tolerance time. The animal was removed immediately from the vessel and resuscitated if needed, when it exhibits the first convulsions. The time duration of animal entry into the hermetic vessel and the appearance of the first convulsion was recorded as anoxia tolerance time [10].

### Swimming endurance test

Albino mice of either sex weighing 20 -30 g divided into six groups of six animals each
Group I : Normal control, untreated
Group II: Control, received vehicle only
Group III: Standard (*Withania somnifera*, 100 mg/kg, p.o.)
Group IV: EEAPR 125 mg/kg p.o.
Group V: EEAPR 250 mg/kg p.o.
Group VI: EEAPR 500 mg/kg p.o.

Above treatment was given to mice for 7 days. On $7^{th}$ day 1 hr. after treatment, all the mice (except normal control) were subjected to swimming endurance

test. The mice were allowed to swim individually in swimming tank (30 cm height with 20 cm diameter) containing water of 25 cm height maintained at 26 ± $1^0$C temperature. The mice were allowed to swim till exhausted and moment when animal drowned is considered as the endpoint. The mean swimming time for each group was calculated.

**Post swimming antifatigue and motor coordination test**

The animal was immediately taken out, dried with tissue paper and subsequently all the animals placed on digital rotarod (15 rpm) to monitor antifatigue and motor coordination effects.

**Biochemical estimations**

The blood was collected (orbital sinus) from all the animals subjected to post swimming antifatigue effect for estimation of serum cortisol level. Then all the animals were sacrificed for the removal of adrenal glands to record their weight and also used for the estimation of ascorbic acid and cortisol levels [11].

**Acetic acid induced writhing test**

The mice of either sex weighing between 20-30g were randomly divided into five groups of six animals each.

Group I : Normal control, untreated
Group II : Stress control, received vehicle only
Group III : Standard (Diazepam 2 mg / kg, p.o.)
Group IV : EEAPR 125 mg/kg p.o.
Group V : EEAPR 250 mg/kg p.o.
Group VI : EEAPR 500 mg/kg p.o.

Control group animals were received vehicle only. Animals of group II treated with standard drug, diazepam (2 mg / kg, p.o.). The groups III, IV and V were treated with different doses of the test extract p.o. for 15 days. On 15$^{th}$ day 1 hour after drug treatment all the animals received 0.1 ml of 6% (v/v) glacial acetic acid intra-peritoneally and the number of writhing responses observed in all the groups for 20 min [12,13].

## STATISTICAL ANALYSIS

The data obtained from the above findings was subjected to statistical analysis following one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results using Graph Pad Prism-5 software p-values less than 0.05 were considered as statistically significant.

## RESULTS

**Anoxia stress tolerance test**

Mice pretreated with graded doses of *Anacyclus pyrethrum* roots extract and standard drug demonstrated significant increase in anoxia stress tolerance time. The activity was found to be dose and duration dependent manner, except the lower dose i.e. 125 mg/kg p.o which exhibited statistically non-significant results. Results are given in table.1.

**Swimming endurance test**

**Effect on swimming performance time**

It is evident from the results given in the table-2, ethanolic extract of *Anacyclus pyrethrum* roots has shown dose dependent significant increase in swimming time in mice at medium and at higher dose levels i.e. 250 mg/kg and 500 mg/kg p.o. The standard drug has significantly increased the swimming time when compared with control animals. The extract at the lower dose i.e.125 mg/kg p.o also demonstrated considerable increase in swimming performance time (5.79%) over the stress control group, but the result found to be statistically not significant.

**Antifatigue effect**

This extract at higher doses (250 mg/kg and 500 mg/kg p.o) certainly increased the post swimming antifatigue effect. The results were comparable with swim stress control group. The antifatigue effect of lower dose (125 mg/kg p.o) of the extract was found to be statistically not significant. Standard drug has also demonstrated significant antifatigue effect.

**Effect on biochemical parameters**

Further, extract at 500 mg/kg p.o has significantly reversed the altered adrenal gland weight and other biochemical parameters in this model. The results are given table-3.

**Acetic acid induced writhing test**

Animals pretreated with extract at 250 mg/kg and 500 mg/kg p.o. significantly reduced the number of writhing responses, however 125 mg/kg p.o was statistically non-significant as compared with stress control group. The results are evident from the table-4. Standard drug also exhibited significant reduction of writhing responses.

**Table-1: Effect of *Anacyclus pyrethrum* root extract on anoxia stress tolerance time in mice**

| Groups | Treatment | Dose (mg/kg) | Duration of anoxia stress tolerance time (min) | | |
|---|---|---|---|---|---|
| | | | 7$^{th}$ Day | 14$^{th}$ Day | 21$^{st}$ Day |
| I | Stress control | -- | 22.32±0.52 | 29.63±1.54 | 33.55±1.42 |
| II | Std. (*W. somnifera*) | 100 | 45.59±3.88*** | 53.94±3.67*** | 58.19±2.35*** |
| III | EEAPR | 125 | 23.18±2.38$^{ns}$ | 26.82±2.09$^{ns}$ | 36.40±2.22$^{ns}$ |
| IV | EEAPR | 250 | 34.51±2.53* | 40.59±2.91* | 45.39±2.42** |
| V | EEAPR | 500 | 40.23±3.67** | 47.08±2.38*** | 49.21±2.66*** |

The values are expressed as Mean ± SE, (n=6).$^{ns}$ Non-significant, * p < 0.05, ** p < 0.01, *** p < 0.001 as compared to stress control.

**Table-2: Effect of *Anacyclus pyrethrum* extract on swimming endurance time and post swimming antifatigue effect in mice**

| Groups | Treatment | Dose (mg/kg) | Swimming endurance time (min) | % increase in swimming time | Fall of animal from rotarod (sec) | % antifatigue effect |
|---|---|---|---|---|---|---|
| I | Normal control | -- | -- | -- | 109.72±4.78 | -- |
| II | Stress control | -- | 223.76±8.65 | -- | 24.29±2.30$^{@}$ | -- |
| III | Std. (*W. somnifera*) | 100 | 372.09±9.28*** | 39.86 | 63.19±2.88*** | 61.56 |
| IV | EEAPR | 125 | 237.52±4.76$^{ns}$ | 5.79 | 27.17±1.56$^{ns}$ | 10.59 |
| V | EEAPR | 250 | 258.63±4.20* | 15.58 | 38.89±2.51** | 37.54 |
| VI | EEAPR | 500 | 320.49±5.83*** | 30.18 | 43.07±2.39*** | 48.29 |

Values are Mean ± SEM, (n=6), $^{@}$ $p < 0.001$ compared with normal control,
$^{ns}$ Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

**Table-3: Effect of *Anacyclus pyrethrum* extract on swimming stress induced biochemical parameters in mice**

| Groups | Treatment | Dose (mg/kg) | Adrenal gland weight (mg/100 g b.w) | Serum cortisol (µg/dl) | Ascorbic acid (mg/100 g of adrenal wt.) | Cortisol (mg/100 g of adrenal wt.) |
|---|---|---|---|---|---|---|
| I | Normal control | -- | 6.75±0.36 | 22.52±0.76 | 262.24±3.07 | 3.73±0.40 |
| II | Stress control | -- | 18.30±0.51$^{@}$ | 37.62±1.76$^{@}$ | 138.52 ± 3.21$^{@}$ | 1.14±0.21$^{@}$ |
| III | Std. (*W. somnifera*) | 100 | 10.39±0.62*** | 26.44±1.05*** | 238.57±3.59*** | 3.42±0.32*** |
| IV | EEAPR | 125 | 17.28±0.76$^{ns}$ | 35.97±1.22$^{ns}$ | 138.02±2.45$^{ns}$ | 1.33±0.23$^{ns}$ |
| V | EEAPR | 250 | 14.42±0.93** | 31.49±1.37* | 157.78±3.26** | 2.68±0.22* |
| VI | EEAPR | 500 | 11.53±0.74*** | 28.03±1.92*** | 218.37±2.93*** | 3.20±0.31*** |

Values are Mean ± SEM, (n=6), $^{@}$ $p < 0.001$ compared with normal control,
$^{ns}$ Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

**Table-4: Effect of *Anacyclus pyrethrum* extract in acetic acid induced writhing test**

| Groups | Treatment | Dose (mg/kg) | No. of writhes per 20 min | % Inhibition |
|---|---|---|---|---|
| I | Stress control | --- | 69.59 ± 3.14 | ---- |
| II | Std. drug (Diazepam) | 2 | 22.36 ± 2.53*** | 67.86 |
| III | EEAPR | 125 | 64.72 ± 3.08$^{ns}$ | 6.99 |
| IV | EEAPR | 250 | 53.32 ± 3.47** | 23.37 |
| V | EEAPR | 500 | 32.88 ± 2.36*** | 52.75 |

Values are Mean ± SEM, (n=6), $^{ns}$ Non-significant, ** $P < 0.01$,
*** $P < 0.001$ as compared to control.

## DISCUSSION

Medicinal plant research is constantly progressing field in the search of new drugs for treatment of many neurological diseases. Literature report reveals that many plant species have demonstrated therapeutic potential in various *in-vivo* and *in-vitro* models. Stress is an aversive stimulus, which will cause alteration in physiological homeostasis. This shows an effect on variety of biological systems. Various changes occurs during excess stress phenomenon includes behavioral, endocrinological and visceral changes [14]. There are reports which postulate that, stress is involved in many disease conditions like anxiety, depression, endocrine disorders, immunosuppression etc [15,16]. In the regulation of stress physiology, adrenal glands and hypothalamic-pituitary-adrenal (HPA) axis plays an important role [14]. It has also been postulated more recently that involvement of central nervous system (CNS) in the stress phenomenon.

Adaptogens are substances, which prepare the organisms to resist stressors and helps in adaptation phenomenon to challenges. They protect organisms

against a variety of emotional and environmental stress factors by normalizing the body functions, strengthening the systems and functions which are compromised during stress phenomenon [17].

The acute restraint stress like forced swimming is a widely used behavioral model to investigate the antistress property of novel compound [18, 19]. In this model, mice are forced to swim in a restricted space from that they cannot escape, this result in immobility of animal. It has been postulated that drugs with anti-stress activity increase swimming endurance and latency of post-anoxic convulsions. Adrenal glands contain high amounts of cortisol and ascorbic acid. Swimming stress leads to alteration of these levels and also hypertrophy of adrenal gland. Typical response of stress is characterized by the stimulation of hypothalamus-pituitary-adrenal axis, results in an increase in blood cortisol level. The results of our study also reveal that treatment of *Anacyclus pyrethrum* extract has significantly reversed the stress-induced altered cortisol and ascorbic acid levels and adrenal gland weight. The therapeutic potential of most of the herbal extracts is attributed to various phytoconstituents [20]. Results of preliminary phytochemical screening (data given in the second progress report) showed the presence of phenolic compounds, flavonoids, tannins, and saponins in the extract. It is therefore probable that the components that are present in abundance in the extract might contribute in part to the observed antistress effect.

Lack of oxygen supply will play havoc on all body mechanisms since it is needed for all the functions, including cellular respiration. Adaptogenic agents exhibit their beneficial effects in these kinds of stress conditions by increasing the tolerance. This is believed to act by increasing non-specific resistance. In our study, depletion of oxygen in hermetic vessel leads to convulsions in mice and pretreatment with EEAPR had demonstrated significant increase in anoxia stress tolerance time suggests its anti-stress property. During anoxia stress the enzyme, succinate dehydrogenase (SDH) plays an important role in utilization and conservation of energy in the cellular system of the organism and which helps in adaptive processes [21]. In the present study also adaptogenic potential of EEAPR may be due to its ability to elevate succinate dehydrogenase concentration in the brain.

To study the antinociceptive agents, the widely used model is acetic acid induced writhing test [22]. When acetic acid is injected in to abdomen produces peritoneal inflammation triggering a response of writhing [23]. This writhing response is mediated by the release of various endogenous mediators of pain such as prostaglandins, bradykinine and cytokines that activate the nociceptive neurons [24]. Previous researchers reported that the plants having anti-stress property significantly reduces the number of acetic acid induced writhes [11]. In our study also, pretreatment of EEAPR has demonstrated significant reduction in number of writhings thereby indicates its antistress effect this could be due to presence of several phytoconstituents which may be responsible for inhibition of pain pathway.

## CONCLUSION

The results of the present study showed an increase in swimming endurance and duration of anoxia stress tolerance time in animals pre-treated with ethanolic extract of *Anacyclus pyrethrum* root. Extract also restored the altered biochemical parameters and organ weight in swimming endurance test. So, the results of our study suggest that *Anacyclus pyrethrum* root extract possess significant antistress potential. Hence, *Anacyclus pyrethrum* can be considered as adaptogenic plant.

### Conflict of interest statement

The authors declared that, there is no conflict of interest.

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