



INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by Pharmascope Publications

Journal Home Page: www.pharmascope.org/ijrps

Antistress potential of *Convolvulus pluricaulis* choisy in chronic cold swimming stress rat model

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Article History:

Received on: 27.01.2018
 Revised on: 21.04.2018
 Accepted on: 24.04.2018

Keywords:

Anti stress,
C. pluricaulis,
 Cortisol,
 LPO,
 Depression

ABSTRACT

People are using this herb *Convolvulus pluricaulis* choisy for enhancing mind power- learning, memory and recall. Various toxicological evaluations of this herb have suggested that it is a relatively safe compound to treat many diseases. Anti-stress activity of aqueous extract of *C. pluricaulis* Choisy (Sankhapuspi) (AACP) was investigated against experimentally induced stress in rats using cold water forced swimming stress model in rats. Three doses 100 mg/kg body weight (bw), 150 mg/kg bw and 200 mg/kg bw of AACP was treated orally to the stress induced rats for the evaluation of anti-stress activity. The parameters like body weight measurement, lipid peroxidation and cortisol levels were estimated to determine the anti-stress activity. The stress induced animals post-treated with AACP-100, AACP - 150 and AACP-200 mg/kg bw significantly restored the altered body weight and serum cortisol and lipid peroxidation levels, when compared with stress control group. The results of this research showed that the AACP showed a significant dose-dependent anti-stress activity due to its neuroprotective and antioxidant activity.



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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v9i2.1479>

Production and Hosted by

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INTRODUCTION

The growing population with rapid globalisation has a direct effect on society thus making man easily prone to stress related conditions. The stress causes alterations in the physiological functioning of the body by generating free radicals which leads

to hypertension, neurosis, immune suppression and other physical and mental disorders (Seyle, 1973). Inadequate stress causes biochemical physiological and behavioural changes. Stress may be acute (single or short exposure to stress) or chronic (long-term exposure to stress).

Stress produces major changes at the behavioural, physiological and molecular levels (Benyo *et al.*, 2007). Drugs used to treat depression are the most successful drugs in patients with characteristics including psychomotor retardation, sleep pattern changes, anorexia and loss of weight. However different synthetic drugs have been found to have antidepressant activity and showed many side effects in human beings. Therefore, many researches are trying to develop from natural sources an anti stress drug to combat the altered physiological functions caused by stress. There is

great interest on screening of herbs for their antistress activity in recent times.

The Ayurvedic herbs used specially for brain tissue are called Medhya Rasayana. These Rasayanas helps in regeneration of neurons, thus relieving stress and enhancing memory, thereby retarding ageing of brain. *Convolvulus pluricaulis* Choisy (Sankhapuspi) comes under the category Medhya Rasayana which relieves various kinds of stress. *C. pluricaulis* (CP) Choisy belongs to the family Convolvulaceae. *Convolvulus pluricaulis* Choisy is commonly found in north India as a perennial wild herb growing in rocky or sandy ground in xerophytic conditions. (Singh *et al.*, 2008).

C. pluricaulis is a perennial herb that seems like morning glory. This herb improves digestion, voice and complexion. It is used to cure intestinal worms, cough, dyspnea, dysuria disorders of uterus, heart ailments, epilepsy (Kumar, 2006). The previous knowledge about *C. pluricaulis* help us to select this plant for antistress activity. Since there is no research has been conducted on this line, so this triggers the present experimental trail on stress related diseases. The aim of the research is to evaluate the protective effect of aqueous extract of *C. pluricaulis* in cold forced swimming stress induced rats.

MATERIALS & METHODS

Chemicals and reagents

The reagents used in the research were standardised and of analytical grade. Kits used for the estimation of LPO and cortisol content was purchased from Randox. The plant powder of *C. pluricaulis* were purchased from AGHP Enterprises, Chennai, India.

Aqueous extract preparation

Air-dried and powdered plant of *C. pluricaulis* (10.0 kg) were extracted thrice with water and the solvent was evaporated with a rotary evaporator to prepare an aqueous extract. This extract was freeze-dried and stored at -20°C . (Srishti Verma *et al.*, 2011)

Animals

The male wistar albino rats (150 - 200 g) were used for this research. They were housed in polypropylene cages and maintained under standard laboratory conditions at $22\pm 2^{\circ}\text{C}$ and alternating light-dark cycle. They were allowed access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee, (IAEC) reviewed the protocol and approved the use of animals for the studies, (Ethical clearance number: SU/BRULAC/RD/003/2014).

Cold water swimming stress

The rats were subjected to cold water swimming stress for 10 minutes/day for 30 days. Animals were forced to swim in a plastic container (Dimensions 45 cm height, 20cm in diameter) filled with 25cm depth of cold water ($10 \pm 2^{\circ}\text{C}$) under observation. (Bhatnagar and Nicola, 2009).

Experimental design

The male wistar rats (150-200g) were randomly divided into five groups of six animals each.

Group I: Normal control

Group II: Stress control

Group III: Stress induced + AECP (100 mg/kg, p.o.)

Group IV: Stress induced + AECP (150 mg/kg, p.o.)

Group V: Stress induced + AECP (200 mg/kg, p.o.)

After 30 days of stress 3 different doses of AECP was post treated as a single dose orally for 30 days for rats in groups III, IV and V. Animals were observed after giving dose once during the first 30 minutes and periodically during the first 24 hours. Rats were carefully observed during the first 4 hours, and daily thereafter, for a total of 30 days. Body weight was measured for the period of 1, 15, 30, 45 and 60 days. All the groups except group I were subjected to swimming. On the 60th day the animals of each group were anaesthetized with chloroform and blood was collected from jugular vein. Blood was collected in a EDTA containing tubes from the sacrificed animals and plasma was separated by centrifuging at 3500rpm for 10 mins at 4°C . The plasma cortisol and LPO was estimated using commercially available kits and the instructions were followed according to manufacturer's instruction.

Statistical Analysis

Results were statistically analysed and expressed as mean \pm SEM. The statistical significance between groups was compared using one-way ANOVA followed by Dunnett's t test.

RESULTS & DISCUSSION

The *C. pluricaulis* showed anti-anxiety and memory boosting effects and relieved nervousness, palpitation, lethargy and insomnia. Dietary feeding of this plant enhanced translation in the hippocampus, thus improving memory and learning in experimental animals (Nahata *et al.*, 2010).

It is observed that during stress, there is an initial decrease in the body weight to meet the extra metabolic demands of the tissues (Fig 1). Experimental models treated with AECP showed a dose-dependent increase in body weight for different days of treatment signifying its anti-stress potential. The stress induces the activity of

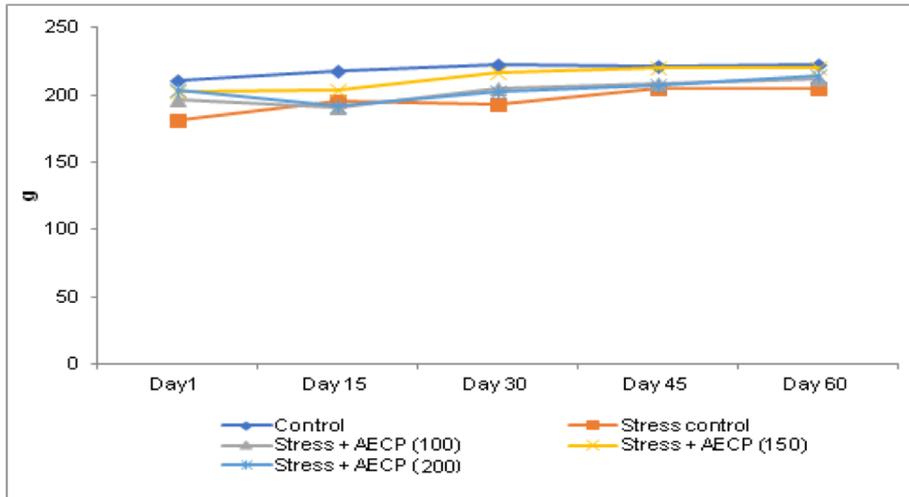


Figure 1: Body weight changes in AECP treated stress induced rats
Results are expressed as Mean (g body weight) (n=6)

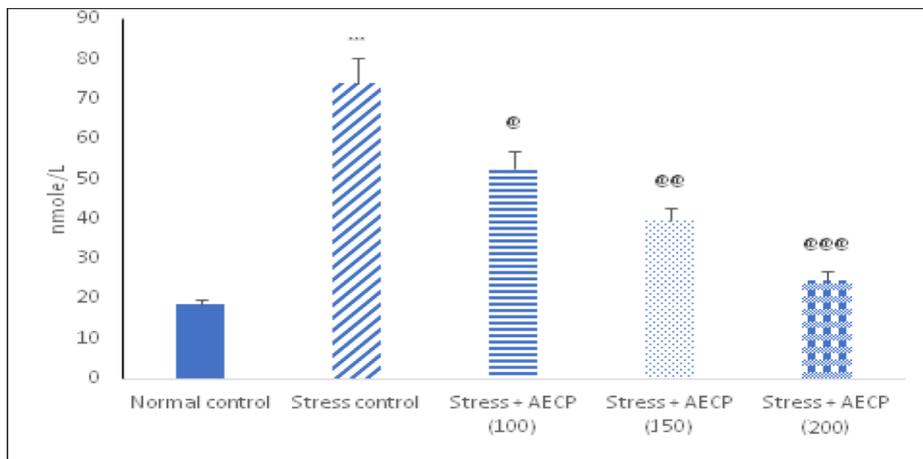


Figure 2: Levels of cortisol in AECP treated stress induced rats
Results are expressed as Mean ± SD (n =6); ***P < 0.001 as compared to normal control group. @P < 0.05; @@P < 0.01; @@@P < 0.001 as compared to stress control group

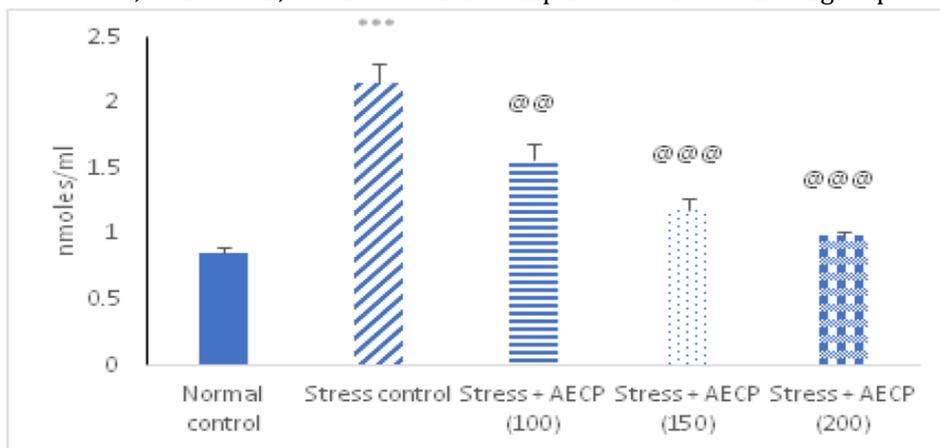


Figure 3: Levels of LPO in AECP treated stress induced rats
Results are expressed as Mean ± SD (n =6); ***P < 0.001 as compared to normal control group. @P < 0.05; @@P < 0.01; @@@P < 0.001 as compared to stress control group

the hypothalamus-pituitary-adrenal(HPA) axis and this results in hyper secretion of corticosteroids from the adrenal cortex. Cortisol and corticosterone are used as biomarkers for stress and depressive disorders. Cortisol is the

main glucocorticoid regulating stress responses in rodents (Shuai gong *et al*, 2015). The increased cortisol levels during stress increases adrenal hypertrophy and hyperplasia. The experiments conducted showed gradual decrease in cortisol

levels of AECF treated groups when compared with stress control group (Fig. 2).

High concentration of TBARS was detected in serum during brain injury in the stress induced group when compared with the control rats. This data support the hypothesis that stress induction may induce lipid peroxidative products as a pro-oxidant while an antioxidant effect in male rats brain was observed in AECF treated group (Fig. 3). In toxicology and tissue damage, the involvement of free radicals are usually found by measuring lipid peroxidation products (Gutteridge,1995). *C. pluricaulis* is a brain tonic, memory enhancer, and is also used as a tonic for those who want to achieve a healthy body (Debjit *et al.*, 2012). These actions of *C. pluricaulis* may be due to their antioxidant and neuroprotective properties.

CONCLUSION

The experimental results showed that the aqueous extract of *C. pluricaulis* plant possess significant dose-dependent anti-stress activity. The anti-stress activity of AECF might be due to decrease in cortisol and lipid peroxidation levels. The exact mechanism for the anti-stress activity AECF is still unclear. Further research may be needed to isolate the bioactive principles responsible for anti-stress activity and to determine the exact mechanism of action.

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