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Effect of *Convolvulus pluricaulis* aqueous extract on behavioural changes and antioxidants in stress induced rats

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ABSTRACT

Shankhapushpi (*Convolvulus pluricaulis*) helps to prevent memory loss and it is a wonderful neurotonic. Previous studies support the fact that chronic stress in brain causes alterations in cognitive functions. The present study evaluated the effects of aqueous extract of *Convolvulus pluricaulis* on forced swimming cold stress induced rats. Spatial and locomotor activity was evaluated by Morris Water Maze to observe behavioral changes of rats. Stress increased escape latencies (acquisition) in Morris Water Maze test after 60 days induction. Posttreatment with AECP (100, 150 and 200 mg/kg/day) orally for 30 days, reduced the induced cognitive defects in the Morris Water Maze test. The serum antioxidant enzymes Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Glutathione (GSH) concentration were significantly decreased in cold stress induced groups when compared with controls. The AECP treated rats showed neuroprotective effects, ameliorates the cognitive disability and associated biochemical changes in brain in experimental cold water stress model.

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INTRODUCTION

The stress encountered from the environment includes the pollution, very hot or very cold climates. It is inevitable to avoid the climate, as man has to migrate irrespective of the climate, he got adapted due to job opportunity and other personal reasons. The chronic stress induced by cold water swimming has been attributed to altered antioxidant enzyme activity and oxidative damage by free radicals to membrane lipids and DNA in brain. These alterations leads to deleterious effects of Reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical and hydrogen peroxide (Sikka 2001). Cold forced swimming stress has been documented to alter the oxidant and antioxidant balance in rat plasma.

Oxidative stress has been identified as the one of the major cause of several depression conditions (Chattopadhyay, 2008). Antidepressant drugs such as fluoxetine, paroxetine, fluvoxamine and sertraline have been found to have many side effects in human beings (Bhattacharjee *et al.,* 2018). So, the valid research was going globally for the development of an effective economical antistress drug from natural source without side effects in human body.

Convolvulus pluricaulis Choisy (Sankhapuspi) belongs to the family Convulvulaceae. It improves digestive power, complexion, strength, cures intestinal worms, cough, dysuria, dyspnea and uterine disorder (Kumar, 2006). Literature is available about the usage of this plant as a brain tonic in neurological disorder (Singh RH 2008; Na-hata A 2010). This medicinal plant enhances the feeling of peace, calmness, relieves stress and mental fatigue by alteration of neurochemistry of the brain. The herb is non-toxic and does not have any side effects (Sethiya *et al.,* 2018). This plant is used for CNS depression, it has anxiolytic, antidepressant, antistress, prevents neuro degeneration, antiamensic, analgesic, antioxidant, immunomodulatory, antibacterial and antidiabetic effects. Phytochemical analysis have shown the presence of glycosides, flavonids, beta-sitosterol glycoside, hydroxy cinnamic acids and alkaloids. Shankhapushpine, (the alkaloid) has been identified as active principle of this plant (Malik *et al.,* 2015).

However, to our knowledge, the effects of C. *pluricaulis* is after chronic stress on antioxidant activities has not been studied. Therefore, the aim of this study was to evaluate the effects of *C. pluricaulis* is after chronic stress on the behavioural changes and antioxidant enzymes, SOD, GPx activities and GSH levels in cold forced swimming stress induced rats.

MATERIAL AND METHODS

Reagents used

The chemicals and reagents used in this study were standardised and of analytical grade. The plant powder of *C. pluricaulis* were purchased from AGHP Enterprises, Chennai, India.

Aqueous extract preparation

Air-dried and powdered whole plant of *C. pluricaulis* (10.0 kg) were extracted thrice with water (3 × 20 L, 2 h each), then the solvent was evaporated using a rotary evaporator to prepare the aqueous extract. The extract which was obtained was freeze-dried and stored at −20°C. (Sristi Verma *et al.,* 2011)

Animals

The male Wistar albino rats (150 - 200 g) were used for this study were kept in clean polypropylene cages and maintained under standard laboratory condition at 22±2°C temperature with 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

Rats were forced to swim in the cold water maintained at 10°C. The rats will be subjected to cold water swimming stress for 10 minutes/day for a period of 30 days. Animals are forced to swim in a plastic container (Dimensions 45 cm height, 20cm in diameter) filled with 25cm depth of cold water (10°C) under observation. (Archana and Namasivayam, 1999).

Experimental design

The rats (150-200g) were randomly divided into five groups of six animals each. Cold water forced swimming stress was given for 30 days to the stress control group. After 30 days of stress 3 different doses (100mg, 150mg and 200mg/kg b.wt) of AECP was post treated as a single dose orally for 30 days to three different group of rats. On the 65th day the animals of each group were anaesthetized with chloroform and blood was collected from the retro orbital plexus under the guidance of veterinary surgeon. Serum was centrifuged at 3500rpm for 10 mins at 4°C and used for the assay of glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione (GSH) levels (Sinha 1972). SOD activity was measured as the degree of inhibition of autooxidation of pyrogallol at alkaline pH by the method of Marklund and Marklund (1974). GPx level was estimated by analysing the amount of reduced GSH consumed in the reaction mixture according to Rotruck *et al.,* method (1973).

Morris water maze study

The water maze study was an adaptation of the hidden escape paradigm described by Morris (1984). A small platform (10 cm dia 30 cm h) was placed in the centre of a quadrant in a circular tank (110 cm dia 50 cm h) containing warm water filled to a level just 1 cm above the platform. The rats was released in one of the other three quadrants and given a minute to find the hidden platform. The study consisted of 4 trials per day over 4 consecutive days. The rat was left on the platform for 10 seconds, if the rat does not locate the platform within a minute it was guided to the platform and left there for 10 seconds. The latency to locate the hidden platform was recorded for each trial as a measure of spatial learning performance.

Statistical Analysis

Analysis of data was conducted with Statistical Package for Social Sciences (SPSS) software (version 13.0; SPSS Inc, IL, USA) for Windows. The obtained results were expressed as the mean ±

standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Dunnett's t test was used to compare between group.

RESULTS AND DISCUSSION

Morris water maze, is a commonly used behavioural paradigm to evaluate learning and memory power in rodents when it is exposed to forced cold water swimming stress for a few minutes. Water temperature, perhaps through stress, influences the behavioural changes on water maze performance. The daily average escape latency was recorded as a measure of learning in the water maze. Moreover, AECP possesses a neuroprotective effect in a dose-dependent manner and showed significant improvements in behavioural parameters by gradual reduction in the latency period according to the low, moderate and high doses AECP (Fig 1).

Evidence suggesting that free radicals can attack and damage key biomolecule, including lipid, protein and DNA, causing them to lose their structure and function. Results obtained in this study showed that SOD activity decreases after 30 days of chronic stress due to increase in peroxide radical production. The decrease in SOD activity may be because of polypeptide chain oxidation during Reactive Oxygen Species (ROS) elevation (Goldstone *et al.,* 2006). In addition to this we found that chronic stress for 30 days caused a decrease in GPx activity that activates with low levels of Hydrogen peroxide (Forstorm, 1979). This explains the variations observed in the enzymatic activity of SOD and GPx. The results of

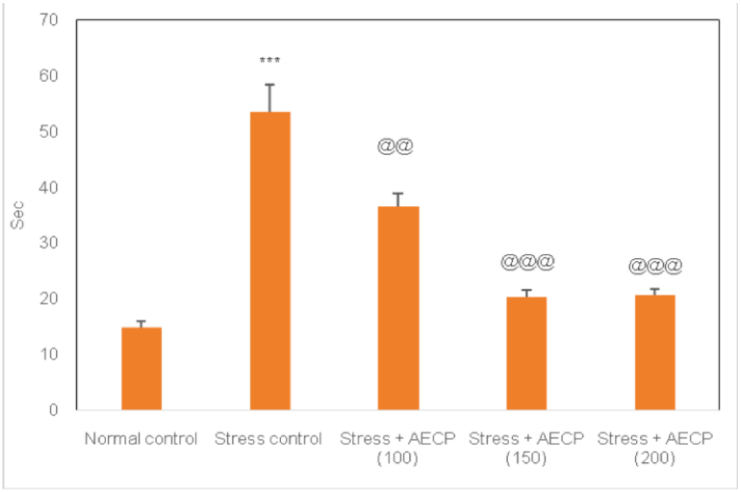

Figure 1: Behavioural changes in AECP treated cold stress induced rats

Results are expressed as Mean ± SD (n =6); ***P < 0.001 as compared to normal control group. @@P < 0.01; @@@P < 0.001 as compared to stress control group.

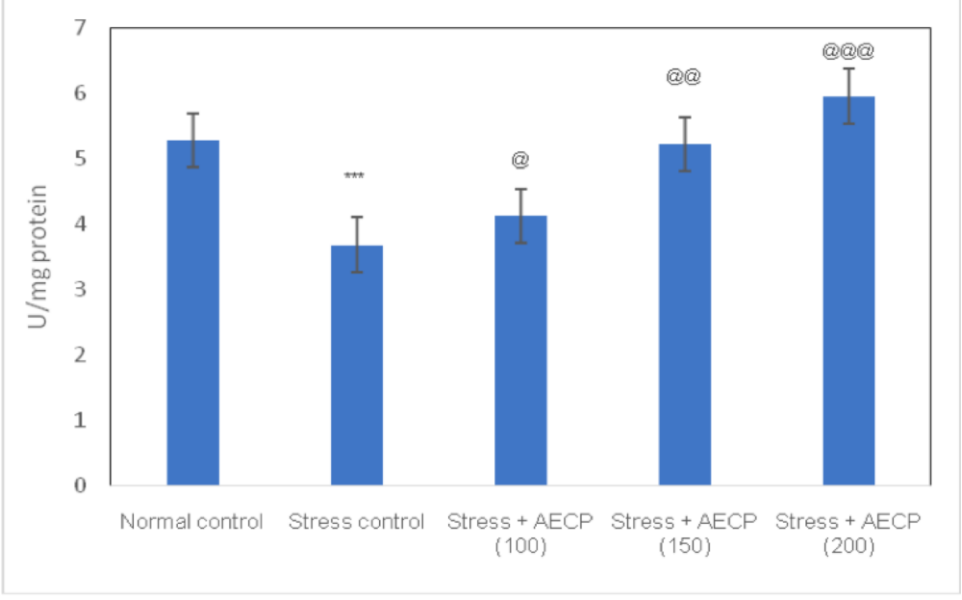

Figure 2: Activities of Superoxide dismutase enzyme in AECP treated cold stress induced rats

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

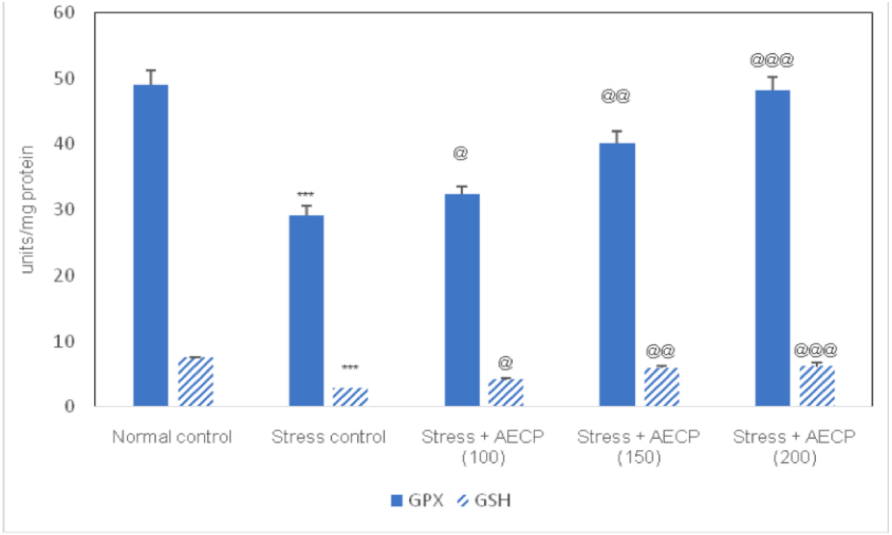

Figure 3: Activities of Glutathione peroxidase and glutathione levels in AECP treated cold stress induced rats

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.

SOD, GPx and GSH analysis are given in Fig 2 & 3. Rats exposed to cold water swimming stress showed a significant decrease in seum SOD, GPx and GSH levels. The level of these enzymes was significantly reduced in AECP treated rats exposed to cold water swimming stress.

The protective role AECP may be due to scavenging of the free radicals by the phytoconstituents and/or prevent the antioxidants from ROS and in addition the phytoconstituent compound can act by increasing endogenous antioxidant defences. The Direct reaction of AECP with ROS increased the level of these antioxidants in AECP administered rats exposed to cold water stress. *Convolvulus pluricaulis* is reported to be a strong antioxidant due to the presence of glycosides, flavonoids, beta-sitosterols and alkaloids.

CONCLUSION

The results obtained might be attributed to the neuroprotective effect of *Convolvulus pluricaulis* and its capacity to renew cognition and hippocampal function in brain regions. The present study proves that C. *pluricaulis* is significantly increases the scavenging of free radicals mechanisms in Cold water swimming stress exposed animals due to the presence of bioactive constituents which attributes to the antioxidant defensive mechanism.

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