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Antidepressant Effect of *Plectranthus amboinicus* on Chronic Unpredictable Mild Stress Induced Rats

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[1]Victor Arokia Doss. D, [ǂ]Nithyanand. P and [*ǂ]Sowndarya. R

[1]Associate Professor, [1ǂ]Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India.

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ABSTRACT

Plectranthus amboinicus contains flavonoids, alkaloid and terpenoids as its major component. The purpose of the study is to explore the antidepressant activity of hydroethanolic extract of *Plectranthus amboinicus* leaf in rats. Chronic unpredictable mild stress (CUMS) was employed to induce stress in rats. *Plectranthus amboinicus* (200mg/kg, body weight) and Fluoxetine (20mg/kg, body weight) were administered during the 28 day stress exposure period. Antidepressant activity of ethanolic extract of leaf of *Plectranthus amboinicus* was investigated by using forced swim test (FST). Enzymatic antioxidants such as SOD, GPx, GSH and non enzymatic antioxidants such as vitamin C and lipid peroxidation show the significant increase and improved scavenging activity in treated rats when compared to depressed rats. So, it was concluded that *Plectranthus amboinicus* possesses potential antidepressant which may be due to its antioxidant effect.

INTRODUCTION

Depression is a chronic illness that affects mental stability, personal and social relations. Herbal drugs possess least side effects compared to synthetic medicines. It interferes with daily life and normal functions. According to WHO estimated, 121 million people suffer from clinical depression. The high relevance of suicide in depressed patients (up to 15%) and the other complications arising from stress and its effects on the cardiovascular system has suggested that it will be the second leading cause of death by the year 2020. Prevalence rate for all mental disorders in India was observed to be 65.4/1000 population. Out of which prevalence rate for affective disorders is estimated to be 31.2/1000 population [1].

Symptoms of depression include biological and emotional components. In biological symptoms include retardation of action and thoughts, sleep disturbance etc., emotional symptoms include pessimism, ugliness, loss of motivation and loss of self system. It occurs usually in the early adult life of patients with depression have more symptoms include decrease in monoamine neurotransmitters particularly and dopamine [2]. Numerous antidepressant compounds are now available presumably acting via different mechanism including serotonergic, noradrenergic and dopaminergic system. Medical plants therapies may be effective alternatives in the treatment of depression and has progressed significantly in the past decade [3].

Herbal drugs possess least side effects compared to synthetic medicines. Medicinal plants became an important source for novel antidepressant drugs. The analyses of *Plectranthus amboinicus* leaves revealed the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids. Alkaloids have pronounced physiological effect particularly on the nervous system.

MATERIALS AND METHODS

Collection of Plant Material

The young leaves of *Plectranthus amboinicus* were collected from Coimbatore and authenticated by Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SRC/5/23/2015 /Tech). The specimen was later shade dried, powdered and stored in an airtight container for further use.

The powdered material was used for pharmacological investigation while for phytochemical screening, the powder was extracted with different solvents in their increasing order of polarities such as petroleum ether, chloroform, 50% ethanol, water and ethanol. The crude extracted were collected and stored in bottles.

Preparation of Extract Solution

The hydroethanolic extract of *Plectranthus amboinicus* was prepared at large scale (100g leaf extract powder in 500ml water and 500ml ethanol). It was filtered and the filtrate was then evaporated to dryness using rotatory evaporator and crystals were obtained. The crystals obtained were weighed and dissolved in sufficient quantity of water. This solution was administrated orally to the experimental animal for the treatment of depression.

Experimental Setup

The experimental rats were divided into four groups of six animals in each group.

TABLE 1: Experimental setup of animals

GROUPS	EXPERIMENTAL SETUP
GROUP I	Normal rats
GROUP II	Depression Induced rats
GROUP III	Depression Induced + 20mg/kg of Fluoxetine
GROUP IV	Depression induced + 200mg/kg of Plant leaf extract

Induction of Depression

Depression was induced by Chronic Unpredictable Mild Stress (CUMS) [4] in rats. The status of depression was confirmed by the Forced Swim Test (FST) [5].

Organ Collection

After treatment, the animals were sacrificed by chloroform anaesthesia, the brain was excised immediately and thoroughly washed in saline and brain tissue homogenate was prepared.

Preparation of Brain Tissue Homogenate

A 10% homogenate of the washed animal tissue were prepared using 0.1M cold Tris-HCl buffer (pH 7.4) in potter homogenizer fitted with Teflon plunger running at 600 rpm for 3 minutes. Thus, prepared homogenate was used for various biochemical assays.

Estimation of Enzymic and Non-Enzymic Antioxidants

Enzymic antioxidant such as superoxide dismutase (SOD) was measured according to the method of Kakkar *et al*., 1984[6], Glutathione peroxidase was measured according to the method of Rotruck *et al.,* 1973 [7]. Reduced Glutathione was measured according to Beutler *et al.,* 1963 [8]. Ascorbic acid (Vitamin C) was estimated according to the method of Roe and Kuether, 1943 [9] and the levels of tissue thiobarbituric acid reactive substances (TBARS) was measured according to the method of Ohkawa *et al.,* 1979 [10].

Statistical analysis

Data obtained were expressed as mean ± SD. The level of significance was determined by performing Students "t" test using R – Statistical Computing and Graphical Tools. A probability of $p<0.05$ was considered to be significant.

RESULTS AND DISSCUSION

Immobility time of rats in FST method was calculated and it was expressed as Mean ± Standard Deviation (S.D) for six animals in each group in Figure 1. In the present study, the depression treated rats showed more immobility than the normal control groups.

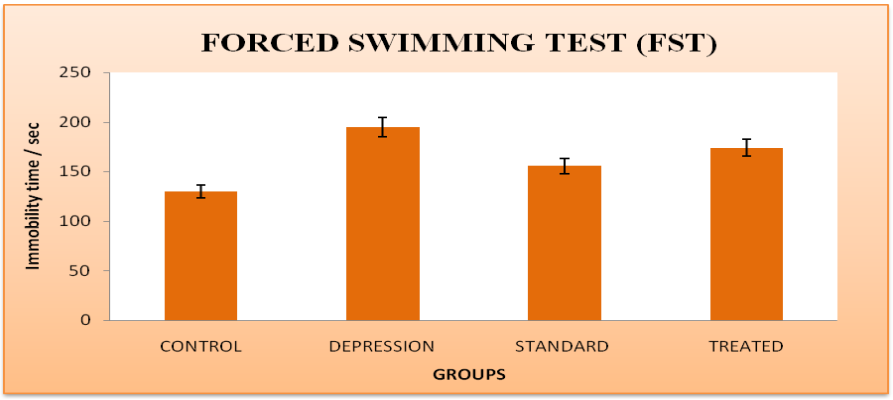

Figure-1: FORCED SWIM TEST

Estimation of Enzymic Antioxidants

Table 1 and figure 2 gives the activity of SOD, GPx**,** GSH, Vit C and LPO in the brain of control and experimental rats. In the present study, depression group animals show low levels of SOD, GPx, GSH and Vitamin C when compared to that of normal control group. This shows that during depression, the activity of enzymic and non-enzymic antioxidants was found to least against free radicals, thereby prone to higher rates of oxidative stress.

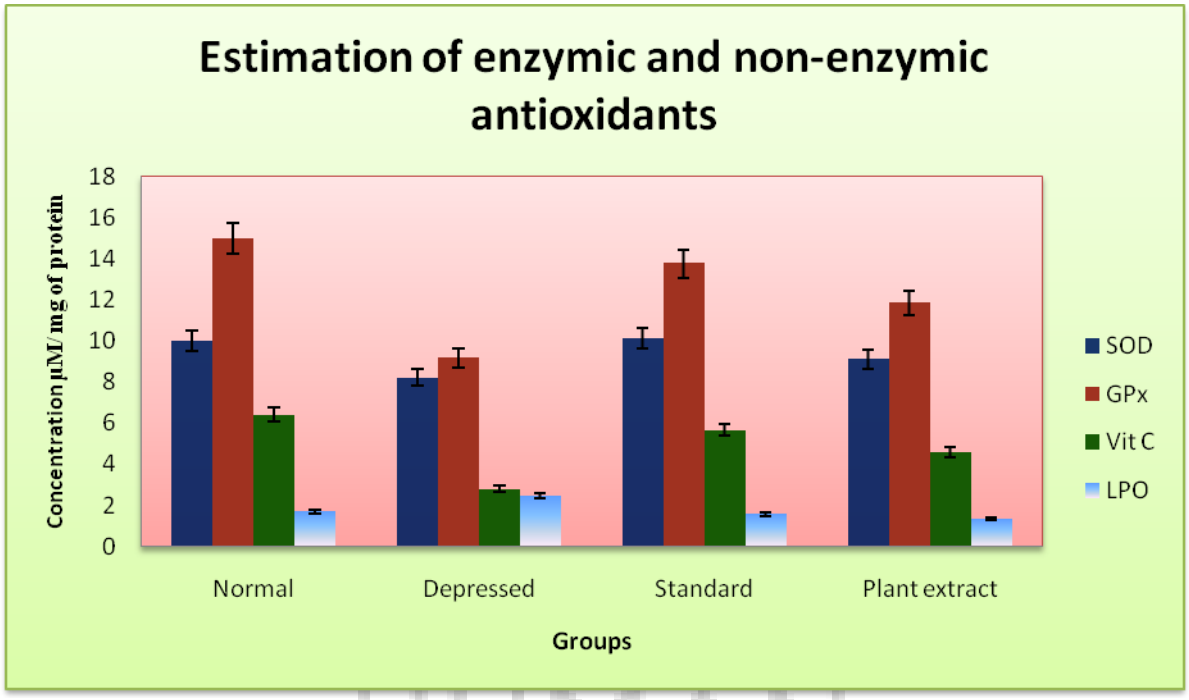

Figure 2: Activity of Enzymic and Non-Enzymic antioxidants in the brain tissue of experimental animals

On further treatment with Fluoxetine and plant extract, it was found that the levels of SOD, GPx, GSH and Vitamin C had increased significantly when compared to that of the depressed rats.

GROUPS	SOD mg/dl	Gpx	GSH	Vit C	LPO
CONTROL	10.02 ± 0.62	14.98 ± 0.59	0.98 ± 0.05	6.42 ± 0.33	1.69 ± 0.09
DEPRESSION	8.22 ± 0.34*	9.16 ± 0.57*	0.50 ± 0.08*	2.79 ± 0.18*	2.47 ±0.42 *
STANDARD	10.0 ± 0.26*	13.75 ± 0.33*	0.88 ± 0.04*	5.67 ± 0.28*	1.57 ± 0.07*
TREATED	9.10 ± 0.30*	11.84 ± 0.41*	0.80 ± 0.03*	4.58 ± 0.23*	1.32 ± 0.14*

The MDA level was found to be increased in group II (depressed) when compared to that of normal rats. After the administration of hydroethanolic extract of *Cardiospermum halicacabum* and standard drug Fluoxetine, the level was found to be decreased.

CONCLUSION

Antidepressant drugs used for the treatment of depression may cause side effects. So in order to overcome this, herbal medicines are used for the treatment of depression. *Cardiospermum halicacabum* is one of the plants used as a traditional medicine for various diseases, possess antidepressant like activity in rats in our present study. The results are found to similar to standard drug. Our study clearly indicated a significant antidepressant activity as well as antioxidant activity of *Plectranthus amboinicus* on depression induced rats. However, further studies are needed to understand the mechanism of action and the active component responsible for antidepressant activity.

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