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Beneficial effect of hydroethanolic extract of *Ocimum basilicum* L on enzymic and non enzymic antioxidant in depression induced rats

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Abstract
The antidepressant activity of *Ocimum basilicum* L was studied by administrating plant low dose (250 mg/Kg of bw) and high dose (500 mg/kg of bw) in depression induced rats. Imipramine (30 mg/Kg of bw) was selected as the standard drug which showed significant antidepressant activity. Hydroethanolic extract was orally administered to experimental rats for 14 days after the induction of depression. The status of depression was confirmed by using Forced Swimming Test (FST) in experimental rats. Enzymic and non- enzymic antioxidant level were analysed in control and experimental groups. The results showed that the extract decreased immobility time after the treatment. The level of enzymic and non - enzymic antioxidants were increased significantly levels in the brain tissues of experimental groups. Thus it may be concluded that *Ocimum basilicum* showed high propensity to improve the antioxidant status in rats. Hence, the antioxidant effect may be the cardinal mechanism of *Ocimum basilicum* in antidepressant activity. Further studies may elucidate the possibility of the commercial use of *Ocimum basilicum* for the benefit of patients suffering from depression.

Keywords: *Ocimum basilicum*, Antidepressant activity, forced swimming test (FST), Non-enzymic antioxidants, Enzymic antioxidants

1. Introduction
Depression is an important psychiatric disorder that affects individual's quality of life and social relations directly. Depression is characterised by emotional symptoms such as hopelessness, apathy, loss of self-confidence, sense of guilt, indecisiveness and a motivation as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances and loss of appetite. When the symptoms are very severe, major depression is considered [1].
Antidepressant drug such as tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI) are used to treat depression showing various side effects and thus, the search for a new antidepressant herb without side effect is important [2].
The incidence of anxiety and depression in the community is very high and is associated with lots of morbidity. Hence, it is very important to address these problems and final effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medication for these disorders [3].
The presently imposing drugs can impose a variety of side-effects including cardiac toxicity, sexual dysfunction, body weight gain and sleep disorder. During the last decade there is a growing interest in therapeutical effect of natural products on mental disorder [1].
Numerous antidepressant compounds are now available, presumably acting via different mechanism including serotonergic, noradnergic and/or dopamineregic systems [4].
Ocimum basilicum (belongs to Labiateae family), have been employed traditionally in Iranian and Indian medicine as folklore remedy for a wide spectrum of ailments, also incorporated in to a number of herbal medicinal preparations. It has been used as treatment of cold and persistent coughs. Some investigations shown its various protective effects including radiation protective efficacy, preventive potential against some chemicals, anti-inflammatory effect, stimulant agent in central nervous system, bactericidal activity, modulatory effect on glutathione and improvement in congenital task, antioxidant property, ulcer protective, antidiarrheal and blood-sugar (BS) lower efficacy [5].
Basil safety in human and animal models has been confirmed [6]. Recent study showed that basil could increase sperm health parameters and protect exposed animals by Electro Magnetic Field [7].

Materials and Methods

Plant collection and authentication

The whole plant of *Ocimum basilicum* was collected during the month of December, 2016 from the local areas of Coimbatore district, Tamil Nadu, India. The whole plant was submitted and authenticated (No. BSI/RC/5/23/2016/Tech-) at Botanical Survey of India, Southern Regional Centre, Coimbatore, India.

Preparation of hydroethanolic extract of plant

The hydroethanolic extract was prepared at large scale (100 g of seed powder in 500 ml water and 500 ml ethanol). It was filtered and the filtrate was then concentrated to dryness under controlled temperature in a microwave oven. The hydroethanolic extract yielded was stored air tight in desiccators for use in various assays.

Procurement of animals

Young male Albino rats of Wistar Strain (100±20 g) procured from KMCH, Coimbatore, India were used for the study. The ethical clearance for handling of experimental animals was obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India (351/2017/IAEC).

Experimental set up

The animals were divided in to 5 groups of six animals in each. The animals were acclimatized 3 days and maintained under standard laboratory conditions with controlled temperature (29±5˚c), humidity (55±5%) and 12 hours light/dark cycles throughout the experimental period. Group I rats were provided with only standard pallet and water for 21 days and were considered as normal control. Group II rats were depression induced by CUMS protocol for 7 days served as untreated depression control. Group III depressed rats were orally administered with the Standard drug, Imipramine (30 mg/Kg bw) for 14 days. Group IV depressed rats were orally administered with low dosage of hydroethanolic extract of *Ocimum basilicum* (250 mg/Kg of bw) for 14 days. Group V depressed rats were orally administered with high dosage of hydroethanolic extract of *Ocimum basilicum* (500 mg/Kg of bw) for 14 days.

Induction of depression

Depression was induced by CUMS protocol in albino rats. The status of depression was diagnosed by the forced swimming test (FST) [8] on the seventh day of induction, third and seventh day of treatment. Each stress regiment was carried out for 2 periods; the stressors are food deprivation for 24 hours, cage tilting (45 degree inclined) for 24 hours, crowded housing (10 animals per cage) and exposure to novel odour.

Confirmation of depression: Forced swimming test (FST)

The experimental group rats were placed in a cylindrical container of diameter 10 cm and height 25 cm with water level of 20 cm depth at 25˚c for a total of 6 minutes. The initial 2 minutes were not considered, the time period during which the animals try to escape out and hence the final 4 minutes were considered to calculate the immobility of animals inside the water container.

Preparation of tissue homogenate

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

Biochemical analysis

The amount of enzymic and non - enzymic antioxidants were analysed in experimental groups.

Estimation of superoxide dismutase (SOD)

SOD was assayed according to the method of Kakkar *et al* (1984) [9].Superoxide dismutase proportionately inhibits the formation of NADH, Phenazine, Methosulphate and Nitroblue Tetrazolium farmazan. The residual chromogen can be extracted into an organic solvent like butanol, the intensity of which is measured at 560 nm.

Estimation of catalase

Catalase was assayed according to the method of Sinha, (1972) [10]. Catalase cause rapid decomposition of hydrogen peroxide into water. This method is based on the fact that dichromate in acetic acid reduces to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchloric acid as unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 610 nm.

Estimation of glutathione peroxidase

This assay is based on the reaction between glutathione with the DNTB (Dithiobis nitrobenzoic acid) to form a colour complex which is measured spectrophotometrically at 412 nm.

Estimation of reduced glutathione

Reduced glutathione is measured by its reaction with DTNB (Dithiobis nitrobenzoic acid) to give a yellow coloured product that absorbs normally at 412 nm.

Estimation of ascorbic acid

Ascorbic acid is first dehydrogenised by bromination. The dehydro ascorbic acid is then reacted with 2,4 dynitrophenyl hydrazine to form osazone and dissolved in sulphuric acid to give an orange red colour solution which was measured as 540 nm[11].

Estimation of lipid peroxidation (LPO)

In these methods malonaldehyde and other thiobarbitric were measured by their reactivity with thiobarbitric acid in the acidic condition to generate a pink colour chromophore which was read at 535 nm [12].

Statistical analysis

All the data obtained was expressed as mean ± SD. Statistical analysis was performed by using the method of distribution statistics (standard descriptive analysis) and analysis of means (Student t test) using R - Statistical Computing and Graphical Tools (formerly AT & T, Lucent technology). A probability of $P<0.05$ was considered significant.

Results

Forced swimming test predicts the status of depression with the state of immobility. Depressed animals stay immobile for longer time during the final 4 minutes of analysis, whereas the

normal, control and depression treated animals showed better and more mobility than the depression control group. Depressed animals before treatment are shown to be more immobile due to their pessimistic behaviour whose attitude turns positive indicated by increase in mobility through active swimming which are indicated in minutes: seconds: microseconds. The final status at the end of experiment is shown in the figure 1.

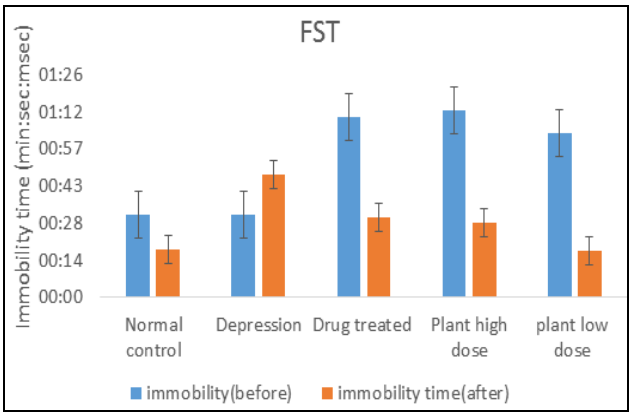

Fig 1: Immobility time period of experimental animals – Forced swim test (FST)

The current study the levels of enzymatic antioxidants SOD, GPx and catalase level are low in depression on comparison with the normal control group. On administration of standard drug (Imipramine), there was a significant increase in SOD, GPx and catalase due to the antidepressant activity of the drugs. Both the low and high plant doses are also found to possess antidepressant potential. Thus the study shows significance differences between each group that is statistically determined as shown in the table 1 and figure 2.

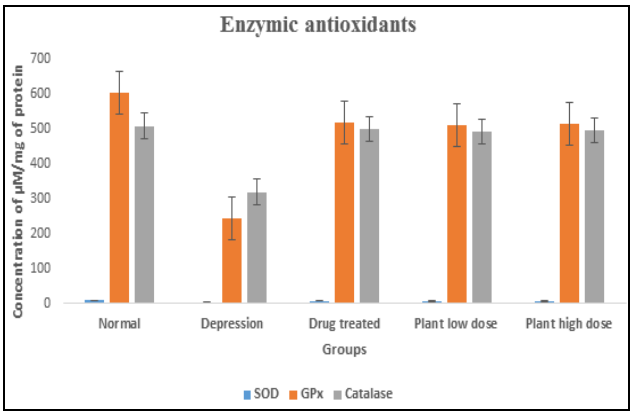

Fig 2: Activity of SOD, GPx and catalase in the brain tissues of experimental animals.

Table 2 and figure 3 give the activity of non – enzymic antioxidants, Glutathione reduced, ascorbic acid and lipid peroxidation. In the current study the levels of non - enzymatic antioxidants Glutathione reduced (GR) and ascorbic acid level are low in depression on comparison with the normal control group. On administration of standard drug (Imipramine), there is significant increases in GR and ascorbic acid due to the antidepressant activity of the drugs. Both the low and high plant doses are also found to possess antidepressant potential. In contrast LPO level is elevated in depression compared with treated and normal groups.

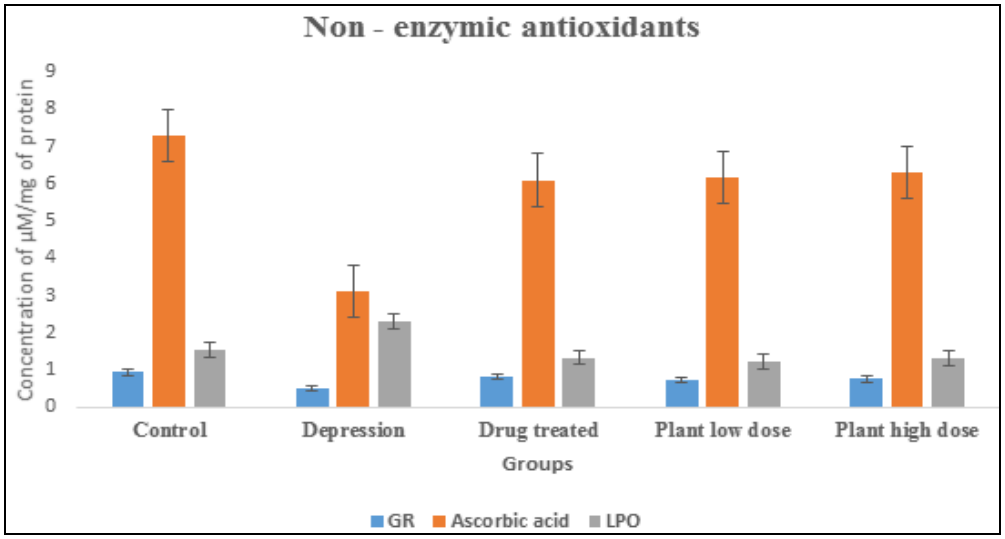

Fig 3: Activity of SOD, GPx and catalase in the brain tissues of experimental animals.

Discussion

In the current study, the hydroethanolic seed extract of *Ocimum basilicum* produced anti – depressant like activity in rats. The status of depression in the experimental rats was confirmed by FST (Forced swim test) method where in, the depressed rats show more immobility than the normal rats. The enzymatic and non- enzymatic antioxidants were analysed as the oxidative stress has major influence on depression. The enzymatic antioxidant study revealed the activity of SOD, Catalase, GPx and non- enzymatic antioxidants GR and ascorbic acid level to be decreased in depression but elevated levels in normal and treated groups. In contrast lipid peroxidation (LPO) level was decreased in normal and treated groups but elevated level in depression.

Some previous studies have investigated basil antioxidative property in vital organs [13]. The antioxidative effect is maintly due to phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes. The antioxidative activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and

neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [14].

Conclusion

The presently imposing drugs can impose a variety of side-effects including cardiac toxicity, sexual dysfunction, body weight gain and sleep disorder. *Ocimum basilicum* is one of the plant used in traditional medicine which has been proved to possess antidepressant like activity in our present study. Therefore, may be served as a potential source for natural psychotherapeutic agent against depression. However, further studies, were still required.

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