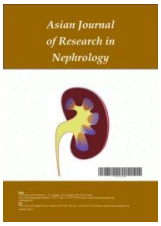

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Ascorbic Acid: A Prophylaxis Drug Therapy for Depression in Female Dam Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author HS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RS managed the analyses of the study. Author JKM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Ascorbic acid is extremely concentrated in the brain, being considered as a neuro-modulator. This vitamin possesses antioxidant properties due to its action either as an electron donor or as broad-spectrum radical scavenge. Maternal separation is used in a large number of studies on anti-depression. The present study demonstrates the long-term effect of ascorbic acid (10 mg/kg p.o) on maternal separation (MS) by isolation stress on the emotionality behaviour of female dam rats. The assessment of the 5-HT level in brain was analysed by HPLC, which revealed increased level of 5-HT when compared with control. Locomotor activity and FST showed that there were increased in % swimming & % climbing. Therefore, present finding relevance of ascorbic acid for the treatment of depression and as a co-adjuvant treatment with antidepressants.

Keywords: Ascorbic acid; MS; FST; serotonin (5-HT).

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1. INTRODUCTION

Depression is a condition of short mood and aversion to activity that can manipulate a person's thoughts, behaviour, feelings and physical well-being. It is a chronic, reoccurring and dilapidating illness that affect up to 20% of the population across the globe [1]. It is a leading cause of disability worldwide, bringing about considerable loss of life by suicide as well as being a risk factor for cardiovascular disease [2] and a multitude of neurological disorders, including dementia [3]. Despite intensive research on the neurobiology of depression and mechanisms of antidepressant action, current clinical management of the disease remains limited [4]. Depression is the generally frequent of the affective disorders (defined as disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. Furthermore, the disease produces a raise in health system utilization [5]. Everybody seems to recognize someone who is depressed whether a family member, a close friend or a co-worker. The World Health Organization estimates that by 2020 Unipolar major depression will become the second largest cause of global disease problems in the world, only behind ischemic heart disease [6]. Consequently, depression represents a chief medical and social problem. It is highly heritable, with roughly 40-50% of the risk for depression bring genetic, although the specific genes that underlie this risk have not yet been identified. The remaining 50-60% of the non-genetic risk also remains poorly defined, with suggestions that early childhood trauma, emotional stress, physical illness and even viral infection might be involve. Most experts concur that depression should be viewed as a syndrome, not a disease. Therefore, the highly variable completion of symptom that is used to define depression, [7] and the highly variable course of the illness and its response to various treatments, indicates that depression subsumes numerous disease states of distinct etiology, and perhaps distinct pathophysiology [8]. Depression often co-exists with other illness, such as anxiety disorders like post-traumatic stress disorder (PTSD), obsessive-compulsive disorder, pain disorder, social phobia and generalized anxiety disorder [9]. Studies have revealed that people who have depression in accumulation to another grave medical illness tend to have more severe symptoms of both depression and the medical illness. Treating the depression can help improve the outcome of treating the co-existing illness. Novel therapies for the treatment of this disorder will originate from preclinical research, which is based upon the use of animal models. Vitamin C (chemical names: ascorbic acid and ascorbate) is a six-carbon Lactone and a valuable food component because of its antioxidant and therapeutic properties. It helps the body in forming connective tissues, bones, teeth, blood vessels and plays a major role as an antioxidant that forms part of the body defense system against reactive oxygen species and free radicals, thereby preventing tissue damage. It is widely used in the treatment of certain diseases such as scurvy, common cold, anemia, hemorrhagic disorders, wound healing as well as infertility [10]. Vitamin C (ascorbic acid), which must be obtained from the diet, is an essential micronutrient required for normal metabolic functioning of the body. As a result, a lack of this vitamin marks in the symptoms of scurvy and death [11]. This potentially fatal disease can be prevented with as little as 10 mg of vitamin C per day, an amount easily obtained through consumption of fresh fruits and vegetables. Nevertheless, the recent recommended dietary allowance (RDA) for vitamin C is place at 60 mg per day to supply an sufficient margin of safety, as 60 mg/day would prevent the development of scurvy for about one month in a diet lacking vitamin C. Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine and neurotransmitters. A deficiency in vitamin C results in a weakening of collagenous structures, causing tooth loss, joint pains, bone and connective tissue disorder and poor wound healing, all of which are characteristic of scurvy. Vitamin C is an significant water-soluble antioxidant in biological fluids. It enthusiastically scavenges reactive oxygen and nitrogen species such as superoxide and hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitroxide radicals and hydrochlorous acid, in this manner effectively defensive other biomolecules from oxidative damage [12-14].

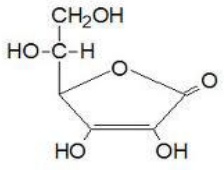

Fig. 1. Ascorbic acid

At physiological pH, ascorbic acid exists as a monovalent anion and its endogenous form is known as ascorbate. In humans, the brain accumulates ascorbate from the blood supply and maintains it at a relatively high concentration (milimolar) under widely varying conditions where it is proposed to exert neuroprotective effects as a result of its antioxidant action. Besides that, ascorbate is supposed to act as a neuromodulator in the brain, modulating both dopamine- and glutamate-mediated neurotransmission [13]. Although some clinical suggest antidepressant properties of ascorbic acid, there is no principle evidence this vitamin exert antidepressant effect. Vitamin C could improve conventional therapy because of it is inexpensive and having low toxicity. In the present study, at the first time, we provide evidence that maternal separation-induced depressive-like behaviors in rats can be reversed by the repeated administration of ascorbic acid showed significant effect comparable to that of the clinically active antidepressant, fluoxetine. A decrease in immobility time and increase in climbing and swimming time respectively, was observed on FST. Histopathology results also showed increase in neuronal cell density in dentate gyrus of hippocampus in rat brain. The 5-HT level in brain was determined by HPLC after treating female dam with ascorbic acid (10 mg/kg p.o) and the result was compared with standard antidepressant, fluoxetine (16 mg/kg p.o).

2. MATERIALS AND METHODS

2.1 Animals

Wistar, male and female rats purchased from IICB Kolkata and used in this project. The animals used were weighed between 230-260 g. They had free access to tap water and food. Housing conditions were controlled; temperature was maintained at 22±1^{o}C with 52±2% humidity. The animals were kept on a controlled 12:12 light/dark cycle with increasing lights from 0600h and fully lights on at 0700h. To minimize stress, the animals were allowed to remain in their transport cage for five days before they were housed individually. A period of at least one week for adaptation to the laboratory facilities was allowed. All handling were performed by the same person throughout the studies.

2.2 Drugs and Chemicals

Fluoxetine was received as a gift sample from Sigma limited, Ahmadabad. Ascorbic acid, CMC, Sucrose, Sodium chloride, Sodium hydrogen carbonate, Potassium chloride, Potassium hydrogen phosphate, Calcium chloride, magnesium chloride, sodium sulphate, D-glucose, Formaldehyde, Sodium hydroxide, Haematoxylin and eosin used were of analytical grade and were obtained from departmental laboratory.

2.3 Drug and Treatment

2.3.1 Maternal Separation Procedure (MS)

Twenty adult male and female wistar rats were separated for breeding. A female in estrous was placed overnight with a male in a breeding cage, and there after housed in her original group. At the end of pregnancy, females were housed singly and provided with nesting material. In our study 20 females and their offspring, were randomly divided into maternal separation treatment (n =6 in each group). Pups were separated daily from their dams for 3h during the first period of life (postnatal days 2-14 or 1-14). The dam was first removed from the home cage into a separate cage. Pups were there after remain in the cage, transferred to a room adjacent to the colony room. After this separation procedure, the dam was placed back into the maternity cage. Depression like behaviour was assessed by forced swim (FST) test [14].

Table 1. Animals were divided into six groups as follows (acute dosing): (n=6)

Sr. no.	Group	Drug treatment	Dose
1	Normal	Saline	0.5%
2	Depresssed	Saline	0.5%
3	Standard	Fluoxetine	16mg/kg
4	MS+A	Ascorbic acid	1mg/kg
5	MS+A	Ascorbic acid	5mg/kg
6	MS+A	Ascobic acid	10mg/kg

A = Ascorbic acid, MS = Maternal separation, Drug was administered 24, 5 and 1hrs before the behaviour test on 21 day

Table 2. Animals were divided into six groups as follows (Chronic dosing): (n=6)

Sr. no.	Drug treatment	Dose
1	Saline	0.5%
2	Saline	0.5%
3	Fluoxetine	16 mg/kg
4	Ascorbic acid	1 mg/kg
5	Ascorbic acid	5 mg/kg
6	Ascobic acid	10 mg/kg

A= Ascorbic acid, Drug was administered daily for 21 days and 24, 5 and1 hrs (p.o.) before the behaviour test

2.4 Behavioral Measures

2.4.1 Forced Swim Test (FST)

The rats were exposed to MS and received ascorbic acid, fluoxetine, 24, 5 and 1hrs for acute test and 21 days in chronic test and were individually forced to swim in an open aquarium (20×20×40 cm^3) containing 20 cm deep water (24±1°C) for 15 min pretest followed by the next day 5 min test session, both the swim test are conducted between 12.00-18.00 h. The rats in control received oral saline instead of drugs. The measurement of immobility time, climbing and swimming was carried out. The water was changed for each test session to avoid influence of alarm substance. Following the test animal were dried with towel and placed in a warm enclosure [15].

2.4.2 Locomotor activity

Assessment of locomotors activity was done by actophotometer, it is used to measure the spontaneous activity by means of infrared beams. Locomotor activity was counted initially before the start of stress application on and on the last day after behavior test [16].

% Change in locomotor activity = Final–Initial / Initial × 100

2.4.3 Histopathological study

The rats exposed to MS received ascorbic acid and fluoxetine for 21 days, on the last day after dosing two rats of each group were anaesthetised i.p with Xylazine and Ketamine in ratio 1:2 and then were perfused transcardially with artificial CSF (500 ml) followed by 0.05 M phosphate buffered saline (PBS), pH 7.4 (250 ml) and chilled 40% formaldehyde (500 ml). The whole brain was exised carefully and was further fixed in 40% formaldehyde for 1 week. The hippocampal region of each animal was sectioned into four section of 5µ from -1.80 cm from bregma region of dentate gyrus and stained with hematoxylin and eosin (HE). Sections were examined under light microscope [17].

2.4.4 Evaluation of 5-HT level in the brain of maternally separated dam

One rat from each group was anesthetised and sacrificed by decapitation immediately the brain was dissected out and cut into small pieces and kept in Hank's balanced salt solution in an eppendorfs tube and centrifuge at 3000 rpm for 10 min. After ten minute the supernatant were collected and 5-HT levels was determined using a high pressure liquid chromatographic system containing a UV detector [18].

2.4.5 5-HT level detection through HPLC

By using a LC-100, CyberlabTM, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector HPLC was performed. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of Mobile phase consist of 75 mM Na_2Hpo_4, 0.1 mM EDTA and 18% Methanol (pH was adjusted to 4.3). The flow rate was 0.5 mL/min, and a column temperature of 25°C. The injection volume was 20µl, and UV detection was effected at 260 nm.

3. RESULTS AND DISCUSSION

Animals were exposed to MS for 21 days and on last day FST was performed, dosing was done 24,5 and 1hrs before FST. The result revealed chronic dosing of ascorbic acid minimised MS induced depression. Animals treated with 1, 5 and 10 mg/kg p.o ascorbic acid. The ascorbic acid 10 mg/kg p.o showed significant result as compared fluoxetine 16 mg/kg. The increased in percentage swimming and % climbing 194.16%, 7.18 % and decrease in % immobility 28.53(↓)%. This shows that ascorbic acid show significant overcome MS induced damage on hippocampus neuron. The histopathology studied on

hippocampus of female rat dam brain showed increase in neuronal cell density in dentate gyrus (Figs. 2-5). HPLC determination of 5-HT level was tabulated in table. The increase in 5-HT level after treating with ascorbic acid on depressed rat provoked antidepressant activity of ascorbic acid.

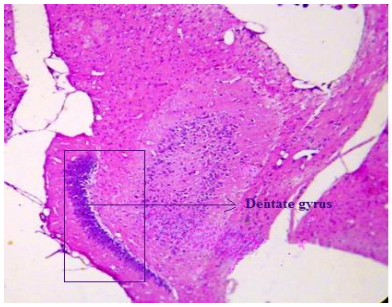

Fig. 2. Photomicrograph of hippocampus of normal rat brain showing neuronal cell density in dentate gyrus region

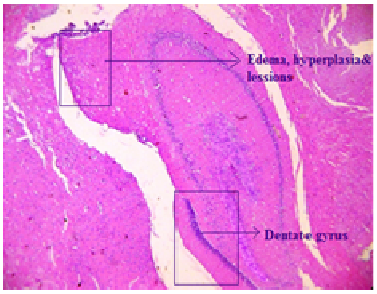

Fig. 3. Photomicrograph of hippocampus of depressed rat brain showing decreased neuronal cell density in dentate gyrus and edema, hyperplasia and lesions

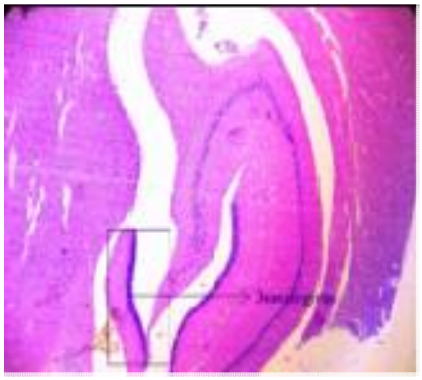

Fig. 4. Photomicrograph of hippocampus of standard (fluoxetine) drug treated rat brain showing increased neuronal cell density in dentate gyrus no edema, hyperplasia and lesions

Fig. 5. Photomicrograph of hippocampus of ascorbic acid (10 mg/kg) drug treated rat brain significant decrease in edema and pronounced increase in neurogenesis

Table 3. Change in % climbing in FST

Group	Treatment	% climbing	% change
Normal	Vehicle	22.10±0.06	------
Depressed	Vehicle	06.82±0.07^{ci}	067.61(↓)
Standard (Fluoxetine)	16mg/kg	13.23±0.08	097.08
Ascorbic acid	1 mg/kg	05.90±0.06	00.24(↓)
	5 mg/kg	12.52±0.08	098.54
	10 mg/kg	21.12±0.06	194.16

Table 4. Change in % swimming in FST

Group	Treatment	% swimming	% change
Normal	Vehicle	59.93±0.06	
Depressed	Vehicle	55.65±0.15^{b}	07.14(↓)
Standard (Fluoxetine)	16mg/kg	59.65±0.15^{af}	07.18
Ascorbic acid	1 mg/kg	62.35±0.15^{cfh}	12.03
	5 mg/kg	58.35±0.15^{be}	04.85
	10 mg/kg	59.35±0.15^{ae}	07.18

Table 5. Change in % immobility in FST

Group	Treatment	% immobility	% change
Normal	Vehicle	18.85±0.06	
Depressed	Vehicle	37.43±0.08	98.58
Standard (Fluoxetine)	16mg/kg	26.80±0.06	28.40(↓)
Ascorbic acid	1 mg/kg	30.80±0.0	17.71(↓)
	5 mg/kg	21.50±0.08	28.26(↓)
	10 mg/kg	26.85±0.07	28.53(↓)

Values are expressed as mean ± SEM (n=6). [a]p<0.05, [b]p<0.01,[c]p<0.001 as compared to normal. [d]p<0.05, [e]p<0.01,[f]p<0.001 as compared to depressed. [g]p<0.05, [h]p<0.01,[i]p<0.001 as compared to standard

Table 6. Effect of ascorbic acid on chronic dosing on locomotor activity in MS protocol

Group	Treatment	Initial count	Final count	change	Percent change
Normal	Vehicle	250.3	248.30	02.00	(↓) 00.79
Depressed	Vehicle	258.0	177.16	80.84[cf]	(↓)31.33
Standard (Fluoxetine)	16 mg/kg	130.0	125.17	04.83[f]	(↓)01.67
Ascorbic acid	1 mg/kg	226.6	204.30	22.30[f]	(↓)09.84
	5 mg/kg	144.0	127.50	16.50[f]	(↓)11.45
	10 mg/kg	125.0	117.50	07.50[af]	(↓)06.00

Values are expressed as mean ± SEM (n=6). [a]p<0.05, [b]p<0.01,[c]p<0.001 as compared to normal., [d]p<0.05, [e]p<0.01,[f]p<0.001 as compared to depressed, p<0.05, [h]p<0.01,[i]p<0.001 as compared to standard

Table 7. HPLC peak of standard drugs

S. no	Standard peak	Concentration	Retention time (min)
1	Serotonin	99.8%	8.10
2	Ascorbic acid	98.8%	4.06

Table 8. HPLC determination of 5-HT level in rat brain

Group	Treatment	5-HT level in brain	Retention time
Normal	Vehicle	71.01	7.85
Depressed	Vehicle	9.41	7.55
Standard (Fluoxetine)	16 mg/kg	61.89	8.10
Ascorbic acid	10 mg/kg	35.63	7.23

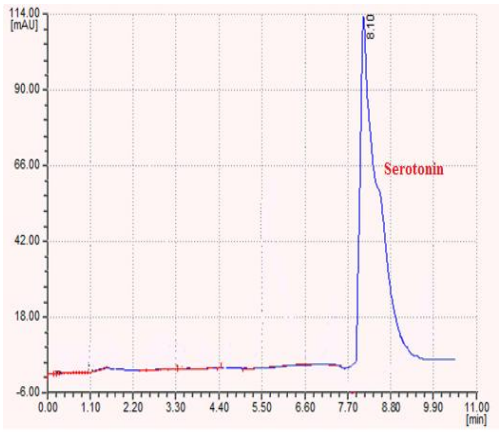

Fig. 6. HPLC chromatogram of standard serotonin

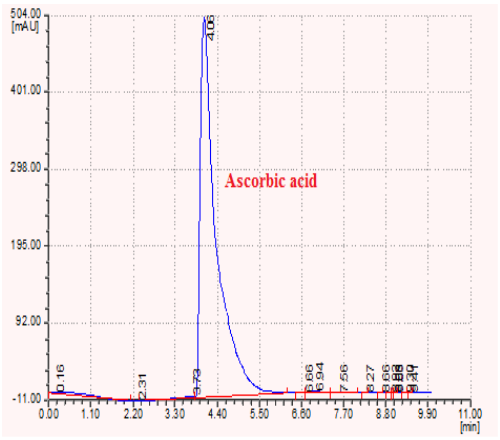

Fig. 7. HPLC chromatogram of ascorbic acid

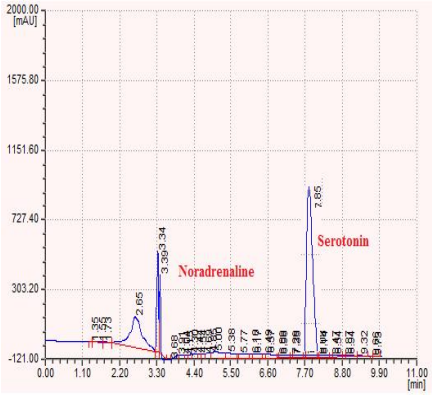

Fig. 8. HPLC chromatogram of 5-HT (Normal rat)

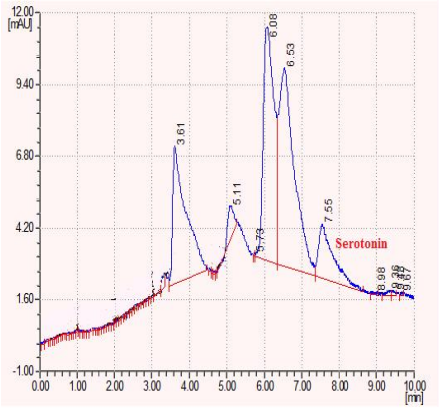

Fig. 9. HPLC chromatogram of 5-HT (Depressed rat)

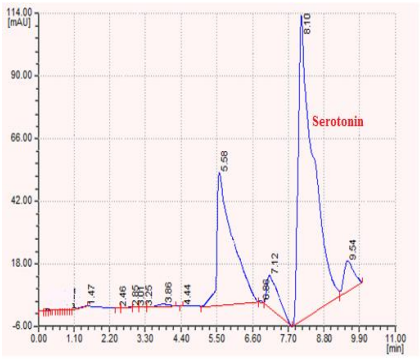

Fig. 10. HPLC chromatogram of 5-HT (Fluoxetine treated)

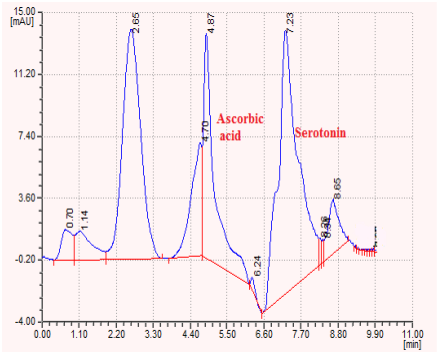

Fig. 11. HPLC chromatogram of 5-HT (Ascorbic acid treated)

4. CONCLUSION

Currently, because of a prevalent belief that "natural is better", depressed patients turn to complementary therapies owing to the lower side effects of medication, time and effort. For example, due to the concern about the effects of pharmacological treatment on breastfeeding, women with postpartum depression prefer to seek complementary and alternative medicine treatment [19-22]. The effects of Long term maternal separation (LMS) experience on rat mothers. FST performance may be the first rodent model of depression that is induced solely by disruption of a social relationship. FST is one of the most commonly animal models used to detect and characterize the efficacy of antidepressant drugs and are sensitive to these drugs after acute administration. In conclusion the results observed after study showed that oral administration of ascorbic acid (10 mg/kg p.o) was able to produce an antidepressant like effect in MS induced depression model.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocol was approved by Institutional Animal and Ethical Committee of SLT Institute of Pharmaceutical Sciences, Bilaspur (C.G.). (99/a/GO/06/CPSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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