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A Comparison of Resveratrol and Vitamin C Therapy on Expression of BDNF in Stressed Rat Brain Homogenate

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Abstract: *Prolonged exposure to stress results in neuronal loss, which is associated with depletion of Brain derived neurotrophic factor (BDNF) in rat brain. Resveratrol, an antioxidant is known to exert neuroprotective effect in both human and animal models. Vitamin C too has important anti-oxidant properties, and protects cells against oxidative stress. In the present study we have evaluated the effect of resveratrol and vitamin C on BDNF expression in stressed rat brain homogenate. Rats were treated with 10 or 20mg/kg body weight dose of resveratrol and 100 or 200mg/kg body weight dose of vitamin C for a period of 28 days, beginning one week prior to stress treatment (restraint stress for 6 hours daily for 21 days). Rats were sacrificed after stress treatment and the whole brain BDNF level was estimated using enzyme-linked immunosorbent assay (ELISA) kit. Our study confirms the neuroprotective effects of resveratrol by enhanced BDNF level in stressed rats. Though vitamin C has enhanced the BDNF expression in stressed rats interestingly it had an adverse effect when treated alone.*

Key words: *BDNF, Restraint stress, Resveratrol, Vitamin C*

I. INTRODUCTION

Neurotrophins are chemicals that help to stimulate and control neurogenesis [1,2]. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is known to be a strong survival-promoting factor against various neuronal insults[3]. It acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses [4, 5]. In the brain, it is active in the hippocampus, cerebral cortex,hypothalamus, brainstem and cerebellum [6, 7]. Neurogenesis occurs throughout life within two specialized neurogenic niches, the subventricular zone (SVZ) of the lateral ventricle [8] and subgranular zone of the dentate gyrus producing new cells throughout the life via proliferation [9]. This ongoing dentate neurogenesis is important for a broad range of hippocampal functions, which include learning, long-term spatial memory, and mood [10, 11].

Immobilization stress highly elevates the stress hormone corticosterone which has been shown to decrease the expression of BDNF in rats [12], and leads to an eventual atrophy of the hippocampus [13].Brain neurons are vulnerable to oxidative stress [14]. It is also clear that oxidative stress can interact with BDNF system to modulate synaptic plasticity and cognitive function [15].Numakawa et al in his work demonstrates that delicate balance between pro- and antioxidant reactions are critical for maintainingnormal neuronal function. Antioxidants including many phytochemicals and vitamins have been found to support the survival of neurons under oxidative stress.Stress being an integral part of life, the wear and tear in the neuronal circuit is inevitable. Antioxidant therapy in this regard, could be beneficial and enhance the quality of life [3].

Resveratrol is a polyphenolicphytoalexin produced by several plants, has also been produced by chemical synthesis [16]. Resveratrol was reported effective against neuronal cell dysfunction and cell death in diseases such as Huntington's disease [17] and Alzheimer's disease [18]. Treatment with single dose of resveratrol after trauma significantly ameliorated the trauma induced hippocampal neuronal loss in rats [19].Mokni et al report that resveratrol is able to cross the blood brain barrier and exerts potent antioxidant properties [20].Sahu et al study reported that gestational treatment of resveratrol prevented the prenatal stress induced memory impairment and the anxiogenic behavior, indicating its neuroprotective activity [21]. Resveratrol during pregnancy reversed the prenatal stress-induced memory impairment and oxidative damage in the offspring[22]. Hence in the present study we evaluated the role of resveratrol in BDNF expression in stressed and control rats. Vitamin C is a strongreducing agent and antioxidant, which is important in preventing the damaging effects of free radicals.It is important in the synthesis and stabilisation of neurotransmitters [23].

Further we also tested the role of vitamin C, which is less cost effective compared to resveratrol in BDNF expression in stressed and control rat.

II. MATERIALS AND METHODS

2.1 Animals

Four months old male Wistar rats (weighing 220g±20) bred in- house was used in the present study. Animals were maintained under controlled conditions of light (10h- light: 14h- dark), temperature (22±3°C), and humidity (approximately 50±10%). All rats were maintained on the standard rat food and water ad libitum. For housing the rats' plastic cages with paddy husk as bedding material was used. The institutional animal ethical committee has approved this research protocol.

2.1.1Stressing procedure

Four month old male and female *Wistar* rats were assigned to a daily restraint stress for 21 days in a wire mesh restrainer [24] for 6 hours. The wire mesh restrainer had a wooden base and stainless steel wire mesh restrainer hinged to the base. Pad lock and latch helped to secure the rat in the restrainer. The restrainer with dimensions of 11cm (L) x 8cm (B) x 8cm (H) was used to stress. This type of restrainer will restrict the movements of the animal without causing any pain, discomfort or suffocation.

2.1.2 Animal groups (n=6)

Group 1- Control and received sodium carboxy methylcellulose as vehicle
Group 2 - Received 10 mg/kg body weight dose of resveratrol (oral) for 28 days
Group 3 - Received 20 mg/kg body weight dose of resveratrol (oral) for 28 days
Group 4 - Received 21 days restraint stress (6h daily)
Group 5 - Received 21 days stress + resveratrol (10mg/kg body weight dose) for 28 days (Resveratrol was given a week prior to stress treatment)
Group 6 - Received 21 days stress + resveratrol (20mg/kg body weight dose) for 28 days (Resveratrol was given a week prior to stress treatment).

Another set of rats with Vitamin C therapy was also considered for BDNF estimation. The rat groups included
Group A: Received Vitamin C 100mg/kg body weight dose (oral) for 28 days.
Group B: Received Vitamin C 200mg/kg body weight dose (oral) for 28 days.
Group C: Received 21 days stress + Vitamin C 100mg/kg body weight dose (oral) for 28 days. (Vitamin C was given a week prior to stress treatment)
Group D: Received 21 days stress + Vitamin C 200mg/kg body weight dose (oral) for 28 days. (Vitamin C was given a week prior to stress treatment)

2.2 Chemicals:

Resveratrol (Cat. No. 70675) from Cayman Chemicals, USA and marketed in India by Pro Lab marketing, New Delhi was obtained. All other chemicals and reagents were of HPLC or analytical grade (Sigma, St. Louis, Mo.).

2.3 Estimation of Brain derived neurotrophic factor (BDNF) using an enzyme-linked immunosorbent assay (ELISA) kit. Hundred milligram of brain tissue was collected from the experimental groups soon after 28 days of treatment and were rinsed with 1X phosphate buffered saline (PBS), homogenized in 1 ml of 1X PBS and stored overnight at -20˚C. Thereafter two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 X g, 2-8˚C. The supernatants were used for ELISA analysis. BDNF protein was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (CUSABIO, catalogue number CSB-E04504r) according to manufacturer's protocol.

III. STATISTICAL ANALYSIS

The data was expressed as mean ± SE. The significance of differences among the groups were assessed using one way analysis of Variance (ANOVA) test followed by Bonferroni's multiple comparison test. P value < 0.05 was considered as significant.

IV. RESULTS

4.1 Comparison of BDNF expression in rats treated with various doses of resveratrol

Immobilization stress in rats resulted in significant ($p<0.01$) decline in whole brain homogenate BDNF level compared to that of control rats. Resveratrol at both the doses (10mg and 20mg/kg body weight) has not affected the BDNF level ($p>0.05$) in rats who have not received any stress when compared to that of control. Rats who

received resveratrol (at both doses) showed a significant ($p<0.001$) increase in the BDNF level when compared to rats who received only stress. Resveratrol at 10mg/kg dose has enhanced the BDNF level in stressed rats, but not at 20mg/kg dose (Fig.1).

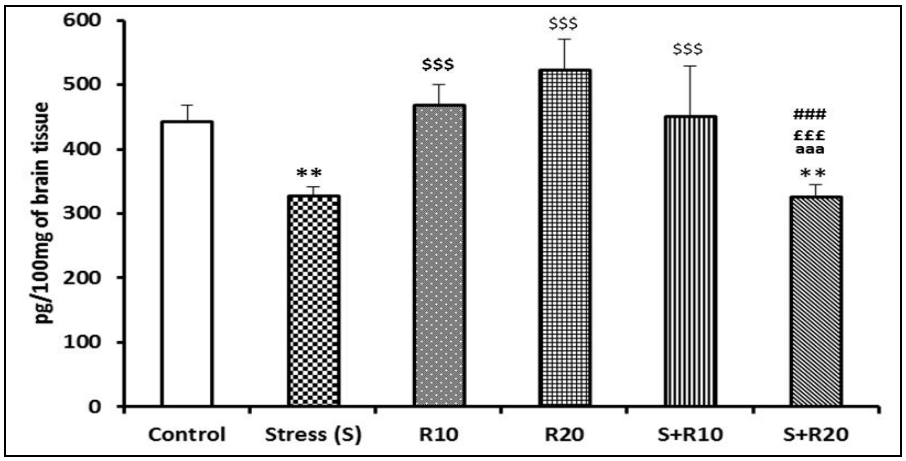

Fig 1 Comparison of BDNF expression in rats treated with various doses of resveratrol. Values expressed as Mean ±SE (n=6). controlvs others - ** $p<0.01$; stress (S) vs others - $$$ $p<0.001$; resveratrol 10mg (R10) vs others – aaa $p<0.001$; resveratrol 20mg (R20) vs others - £££ $p<0.001$; stress+R10 vs others - ### $p<0.001$

4.2 Comparison of BDNF expression in rats treated with various doses of vitamin C

Immobilization stress in rats resulted in significant ($p<0.001$) decline in BDNF level in whole brain homogenate compared to control rats. Vitamin C administration at both the doses to the rats which have not received the stress also showed a significant decline ($p<0.001$) in BDNF expression compared to control. Vitamin C at 200mg/kg dose had more severe effect (decline in BDNF level) when compared to 100mg/kg dose (Fig.2). Rats who received both stress and vitamin C (200mg/kg dose) has not shown any beneficiary effect, as its value did not differ significantly ($p>0.05$) from control group. Vitamin C at 200mg/kg dose in stressed rats has significantly enhanced ($p<0.01$) BDNF level when compared to rats who received only stress (Fig.2).

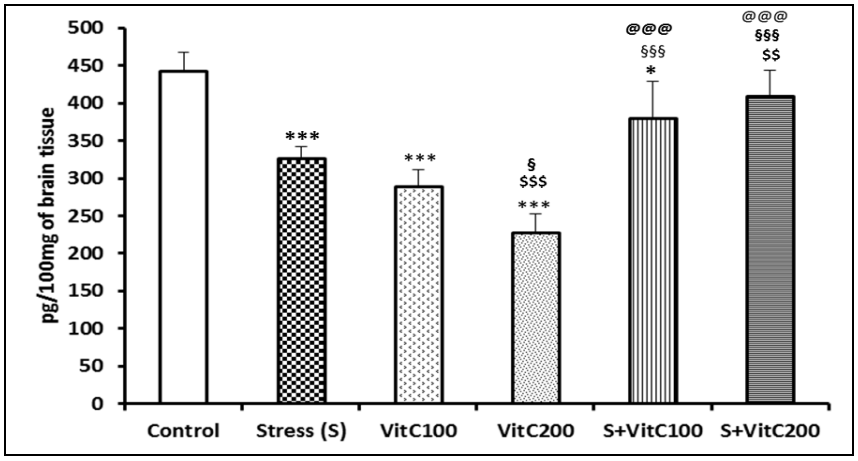

Fig 2 Comparison of BDNF expression in rats treated with various doses of vitamin C. Values expressed as Mean ±SE (n=6). controlvs others - *** $p<0.001$, * $p<0.05$; stress vs others -$$$ $p<0.001$, $$ $p<0.01$; VitC100 vs others –§§§ $p<0.001$,§ $p<0.05$; VitC200 vs others - @@@ $p<0.001$

4.3 Comparison of values of BDNF expression between Resveratrol and Vitamin C treatment

Comparison of effect of Resveratrol and Vitamin C in rats who have not received the stress showed that resveratrol (at both the dose) significantly ($p<0.001$) enhanced BDNF level. Resveratrol at 10mg/kg dose and Vitamin C at 200mg/kg dose showed a significant enhancement in BDNF levels in stressed rats. However when their effect was compared to each other there was no significant ($p>0.05$) difference (Fig.3).

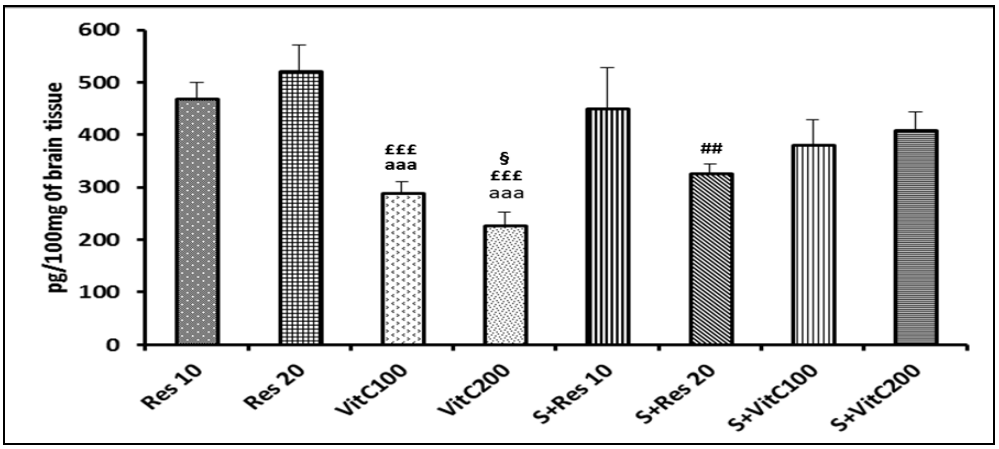

Fig 3 Comparison of BDNF expression between resveratrol and vitamin C treatment. Values expressed as Mean ±SE (n=6). R10 vs VitC100 & VitC200 – aaa $p<0.001$; R20 vs VitC100 & VitC200 - £££ $p<0.001$; VitC100 vs VitC200 - § $p< 0.05$; S+R10 vs S+R20 - ## $p<0.01$

V. DISSCUSSION

The present study clearly demonstrates that immobilization stress in rats resulted in a decline in BDNF level in whole brain homogenate. This result is consistent with few of the earlier studies [25-27], however a study by Tagliari et al, [28] claim that various chronic stress models used in rats has not affected BDNF expression in the hippocampus. Although the exact mechanism by which prolonged stressful experiences regulating BDNF is still unknown, the involvement of hormonal and neurotransmitter systems, has to be taken into consideration [29, 30].

To combat the stress-induced neurotoxic effects involving BDNF, we tested the neuroprotective effects of resveratrol and vitamin C. In the present study resveratrol treatment in stressed rats was able to enhance BDNF level. In an *in vivo* study model, resveratrol is proven to enhance hippocampal BDNF mRNA, demonstrating the neuroprotective effect of resveratrol [31]. In the present study we further proved that resveratrol can exert its neuroprotective effects in stress-induced oxidative damage affecting the brain. Resveratrol functions as an antioxidant, modulates mitochondrial activity and has shown beneficial effects in neurological disorders and is a sirtuin-activating compound (SIRT) [32]. SIRT1 is a major modulator of metabolism and also seems to be endowed with neuroprotective activities. A study by Madhyastha et al, [22] demonstrates the antioxidant potentials of resveratrol against prenatal stress-induced oxidative damage in rat neonates. In their study resveratrol crossed the placental barrier and exerted its antioxidant potentials in neonates. Liua et al in their work found that resveratrol can efficiently interrupt the neuronal apoptosis by regulating the expression of apoptosis proteins, thus enhancing recovery of the neurological function [33]. Resveratrol significantly promoted the recovery of rat dorsal neuronal function after spinal cord injury, and this effect is related to its characteristics of anti-oxidation, anti-inflammation and anti-apoptosis.

In the present study, rats who received both stress and vitamin C (200mg/kg dose) have not shown any beneficiary effect, as its value failed to enhance to the level of control group. Vitamin C at 200mg/kg dose in stressed rats has enhanced BDNF level when compared to rats who received only stress but not the level observed in the control groups. These results do not prove the neuroprotective effect of vitamin C in enhancing the BDNF expression. Taligiri et al, evaluated the neuroprotective effects of vitamin C and E against stress induced cognitive deficits [28]. The results of their study claim partial restoration of cognitive dysfunction after vitamin C as well as vitamin E therapy suggesting the role of oxidative damage. However this study does not focus on BDNF expression after vitamin C or E treatments. In the present study, vitamin C treatment in rats who have not received stress has an adverse effect on BDNF expression; however, in stressed rats they exerted a protective effect by enhancing BDNF expression. A study by El-Sokkary et al, showed administration of vitamin C attenuated the oxidative damage and morphological changes in rat brain [34]. There is also a study by Coskun et al, wherein vitamin C supplementation failed to protect the brain tissue against exercise-induced oxidative damage and behaving as pro-oxidant [35]. In the present study Vitamin C treatment resulted in reduced BDNF expression in rats who have not received stress suggests that vitamin C acted as pro-oxidant, linking the connectivity between the oxidative damage and BDNF expression. Wu et al showed BDNF and oxidative stress can interrelate to affect synaptic plasticity and cognitive function [15]. These studies suggest

that the expression of BDNF is also linked to the status of oxidative damage in brain tissue. Numakawa et al in their study state low levels of reactive oxygen species and reactive nitrogen species are important for maintenance of neuronal function, though elevated levels lead to neuronal cell death [3]. A complex series of events including excitotoxicity, Ca2+ overload, and mitochondrial dysfunction contributes to oxidative stress-mediated neurodegeneration. Oxidative stress-mediated toxicity may be closely related to the pathogenesis of neurodegenerative diseases [36]. Although the antioxidant nature in vivo of vitamin C has been questioned [37] it is nonetheless marketed as supplements in doses of 500 mg or more per day as an 'antioxidant'. In the present study vitamin C appear to act as pro-oxidant.

VI. CONCLUSIONS

The present study demonstrates that restraint stress and vitamin C individually suppressed BDNF expression in rats whereas resveratrol treatment enhanced the level of BDNF. Hence this study indicates the use of resveratrol as a therapeutic agent to combat the stress-induced neuronal dysfunctions.

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