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Dose Dependent Activity of *Benincasa hispida* on Colchicine Induced Experimental Rat Model of Alzheimer's Disease

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Abstract: The present study focused the dose dependent protective effects of water extract of *Benincasa hispida* (BH) pulp on colchicine induced experimental rat model of Alzheimer's disease (AD). The effect of chronic oral treatment of aqueous pulp extract of BH (400 mg kg^{-1} b.wt.) was studied in Holtzman strain adult albino rats of both sexes. The behaviour study, antioxidant level Superoxide dismutase (SOD), Catalase (CAT), Reduced glutathione level and Lipid peroxidation level were studied in different brain areas such as cerebral cortex (CC), cerebellum (CB), midbrain (MB), caudate nucleus (CN) and pons and medulla (PM) in colchicine induced experimental Alzheimer rat model before and after treatment with BH. Results indicate that chronic treatment with BH at different doses (100, 200, 300, 350, 400 and 450 mg kg^{-1} body weight), BH increased the CAT, SOD, GSH level and the number of correct choices out of 10 daily trials along with decreased latency time (in seconds) and LPO level dose dependently. These changes were statistically significant in some doses not in all doses. The effect of BH was most effective at 400 mg kg^{-1} body weight, compared to other doses on all parameters of different brain parts of colchicine induced Alzheimer's rat model. Antioxidant plays a crucial role in the management of neurodegenarative diseases including Alzheimer's disease. A number of Indian medicinal plants have been used in the traditional system of medicine (Ayurveda) for the management of neurodegenarative diseases including Alzheimer's disease. Some of these plants have already been reported to possess strong antioxidant activity. BH, a fruit of common use, is rich in vit-E, beta-carotene, flavonoids and flavonols. Colchicine produces Reactive Oxygen Species (ROS) by binding with tubulin, which is the structural and functional protein of microtubule and ultimately helps in neurodegeneration leading to experimental AD. The results convey the message that at a dose of 400 mg kg^{-1} body weight BH has protective effect on colchicine induced Alzheimer's disease.

Key words: *Benincasa hispida*, Alzheimer's disease, colchicine, antioxidant, reactive oxygen species

INTRODUCTION

The *Benincasa hispida* (BH) tree are like annual vines, have thick furrowed stems with coarse hairs, their leaves are triangular and irregularly lobed. The fruit BH is an important ingredient of Kusmanda lehyam (Ayurvedic medicine), which is widely used, in nervous disorders. The fruits and seeds of BH possess a number of pharmacological properties and uses: laxative, tonic, diuretic, aphrodisiac, antiperiodic, inhaemoptysis, other internal hemorrhages, in insanity, epilepsy and other nervous disorders (Chopra, 1956). Some of the important isolated compounds of BH reported are triterpenes, sterols and glycosides (Kumar and Vimalavathini, 2004) and volatile oils (Kumar and Vimalavathini, 2004). It has been observed that BH has significant antiulcer activity and antidepressant activity (Kumar and Vimalavathini, 2004). Fruit juice (dose -50 mg mL^{-1}) was supplied internally once a day for 3 days with sugar as folklore medicine in Phulbani district for the management of jaundice.

Alzheimer's disease is a complete neurodegenerative disorders characterized by the loss of learning, memory and other cognitive functions. It is pathophysiologically characterized by the presence of extracellular deposition of senile plaques and intracellular deposition of neurofibrillary tangles. Senile plaques and neurofibrillary tangles are salient features in AD brains at autopsy and the histopathological hallmarks of clinical dementia. The etiology of AD is multifactorial (Hardy, 1997). In the framework of such a concept, several authors have proposed a pivotal role for oxidation in the pathogenesis of AD in recent years (Smith *et al*., 1991; Dyrks *et al*., 1992; Markesbery, 1997; Multhaup *et al*., 1997).

Oxidative stress due to increase in free radical generation of impaired endogenous antioxidant

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mechanism is an important factor that has been implicated in neuronal damage and in AD and cognitive defects seen in elderly (Pratico and Delanty, 2000; Cantuti *et al*., 2000). Vitamin C has been described to be a major hydrophilic antioxidant in human plasma (Frei *et al*., 1989), CSF (Spector and Lorenzo, 1973; Lonnrot *et al*., 1996) and the central nervous system (Rice, 2000). Both vitamin E and beta-carotene were found to protect rat neurons against oxidative stress related disorders. BH are rich in Vitamin E and beta-carotene.

Thus the present study was undertaken to determine the dose dependent activity of BH pulp extract on colchicine induced Alzheimer's rat model.

MATERIALS AND METHODS

Subjects: Eighty-four male Holtzman strain adult albino rats approximately 120 days old and weighing 250-300 g were used in the following studies. The animals were individually housed and maintained under standard laboratory conditions with natural dark and light cycle (approximately 12-h light/10-h dark cycle) and room temperature (27±1°C) and constant humidity (60%) in accordance with Institutional Ethical Committee rules and regulations. This study was conducted everyday between 12:00 to 14:00 h for 14 days and thereafter behavior study was done. Food and water were freely available except during testing. Drinking water was supplied ad libitum. Five days prior to behavioral training, animals were reduced to 85% of their free feeding weight by limiting their daily ration of food. Food deprivation was maintained throughout testing except for 3 days immediately prior to and following surgery. Body weights of the rats were recorded everyday and maintained in the laboratory throughout the experimental period. The behavioral procedure was carried out between 12:00 and 14:00 h.

Collection and preparation of water extract from the pulp of BH: The fruit of BH were purchased from the local market and the identity of the plant was authenticated by the Botanical Survey of India, Howrah and kept in S.N. Pradhan Centre for Neurosciences, University of Calcutta.

The pulp of BH fruit was used throughout the experimental study. The fruit of BH were cut into pieces, Sun-dried and ground with the help of an electrical grinder to get a free flowing powder. This powder was subjected to extraction with water (1:3) at room temperature for 48 h. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40-50°C to get a dry powder, which was dissolved in double distilled water for final use (Roy *et al*., 2007).

Animal treatment

Schedule 1: Forty-two Holtzman strain adult albino rats (250-300 g) of either sex were divided into control (group-1) and experimental (group-2, 3, 4, 5, 6 and 7). Group-1 rats were treated with saline (5 mL kg^{-1}) for a period of 14 days. Group-2, 3, 4, 5, 6 and 7 rats received the BH pulp extract 200-300 μL (100, 200, 300, 350, 400 and 450 mg kg^{-1} body weight) once daily for 14 consecutive days between 9:00 and 11:00 am. From 14th day, animals were given 7 days habituation trials through Radial-Y-arm maze study. After 7 days habituation trials, all the animals were sacrificed by cervical dislocation (between 11:00 and 12:00 am) and the biochemical estimation of Superoxide dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH) and Lipid peroxidation (LPO) in different brain areas was performed.

Schedule 2: Forty-two Holtzman strain adult albino rats (250-300 g) of either sex were divided into colchicine induced Alzheimer's animal (group-8) and BH pulp extract pretreated experimental Alzheimer's animals (group-9, 10, 11, 12, 13 and 14). Rats of group 9, 10, 11, 12, 13 and 14 received BH extract (100, 200, 300, 350, 400 and 450 mg kg^{-1}, orally) once daily for a period of 14 days. On the 15th day Alzheimer's animals were prepared by intracerebroventricular injection of colchicine. The toxicity study of BH was done in our laboratory.

Behavior study by radial Y-arm maze training: Radial Y-arm maze study was used to assess cognitive function. The apparatus is a four Y-arm connected together in which the animals were trained to perform a standard radial arm maze (RAM) task. Rats were given 7 days habituation trials in which food pellets (chocolate chips) were scattered throughout the maze and the rats were allowed to freely explore inside it for 5 min. Following habituation sessions, the animals were trained for 10 daily trials on RAM task (10 trials/day). In this task, an animal was placed in the centre of the maze and allowed to visit each of the 4 arms, which were baited with single food pellet. Entry into an arm previously visited within any daily trial was scored as an error. Animals not reaching this criterion were discarded from the study.

Preparation of experimental Alzheimer's rat model by colchicine: Prior to surgery, all the animals were subjected to overnight fasting though drinking water was not withdrawn. During procedures, the animals were anaesthetized with sodium pentobarbital (50 mg kg^{-1} b.wt.) and re-strained in a stereotaxic apparatus (INCO, India Ltd.) equipped with a custom-made ear bar, which prevents the damage of the tympanic membrane. Head

was fixed in such a position that lambda and bregma sutures were in the same horizontal plane by introducing the incisor bar properly attached to the mouth. For aseptic surgery, absolute alcohol or rectified spirit was applied. The scalp was incised and retracted. An incision was made in the scalp and two holes were drilled in the skull for placement of the injection cannula into the lateral cerebral ventricles. The stereotaxic coordinates for intracerebroventricular injection were: 0.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 3.6 mm below the cortical surface (Veerendra Kumar and Gupta, 2002). Subjects were infused through a 10 μL Hamilton syringe with 15 μg of colchicine (Wako chemicals) in 5 μL of artificial cerebrospinal fluid (ACSF; in mM: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 2.2 Dextrose and 1.7 $CaCl_2$) in lateral cerebral ventricle bilaterally. A total volume of 10 μL was delivered to the injection site and the injection cannula was left in place for 2-3 min following infusion.

Postoperative care: After surgery, all aseptic measures and care were taken for feeding until recovery from surgical stress. Penicillin was given post operatively to all animals for 3 consecutive days by intramuscular route. After 3 days of surgery, experiment was started and continued routinely until sacrificed. Similar procedure was repeated thrice, each at an interval of two days.

Biochemical estimation

Tissue preparation: Rats were sacrificed by cervical dislocation on day 14 immediately after behavior study. The Cerebral cortex (CC), Cerebellum (CB), Caudate nucleus (CN), Pons and Medulla (PM) and Midbrain (MB) were dissected out. The tissues were weighed and homogenized in ice-cold phosphate buffer and prepared for biochemical estimation.

Estimation of SOD, catalase and LPO activity: Catalase activity was estimated by the method of Cohen *et al.* (1970) and Roy *et al.* (2007), superoxide dismutase (SOD) was estimated by the method of Misra and Fridovich (1972) and Roy *et al.* (2007) and Lipid Peroxidation (LPO) was estimated by the method of Bhattacharya *et al.* (2001) and Roy *et al.* (2007).

Statistical analysis: The data was processed by one-way analysis of variance (ANOVA) followed by multiple comparisons t-test.

RESULTS

Behavioural study: BH (100-450 mg kg^{-1}) dose dependently increased the number of correct choices out of 10 daily trials and decreased the latency time of the rats. At doses (100, 200 and 300 mg kg^{-1}), the number of correct choices increased and the latency time decreased but this increase or decrease was not statistically significant. At the dose of 350 mg kg^{-1}, the change of the number of correct choices was not statistically significant but the latency time significantly ($p<0.001$) decreased. The effect of BH was most effective at the doses of 400 mg kg^{-1}. At 450 mg kg^{-1} dose, the latency time was also decreased and the number of correct choices increased but the value was not statistically significant with respect to control.

In BH treated colchicine induced Alzheimer's rat model, at doses of 100, 200 and 300 mg kg^{-1}, BH did not elicit any significant changes on latency time and the number of correct choices. At 350 mg kg^{-1} dose, the latency time significantly decreased in BH treated colchicine group as compared to colchicine group. But the changes of the number of correct choices were not statistically significant. At 400 mg kg^{-1} dose the latency

Table 1: Dose dependent effect of BH on behaviour study (Values are mean±SE from 6 animals in each group)

	Acquisition		Re-acquisition	
Groups	No. of trials	Latency period (sec)	No. of trials	Latency period (sec)
Group 1 (Control)	8.59±0.19	105.50±3.890	8.54±0.12	111.00±5.050
Group 2 (BH-100 mg kg^{-1})	8.33±0.33	115.00±2.240	8.67±1.22	108.00±1.180
Group 3 (BH-200 mg kg^{-1})	8.83±0.16	110.25±7.810	8.83±1.07	107.83±1.180
Group 4 (BH-300 mg kg^{-1})	7.33±0.98	120.00±18.82	9.00±0.91	107.00±1.370
Group 5 (BH-350 mg kg^{-1})	8.50±0.43	107.33±5.950	9.00±0.91	79.00±0.470
Group 6 (BH-400 mg kg^{-1})	7.17±1.20	96.67±12.57	9.83±0.15*	73.00±0.330*
Group 7 (BH-450 mg kg^{-1})	7.17±1.20	96.67±12.57	9.83±0.15*	73.00±0.330*
Group 8 (Colchicine)	9.00±0.47	106.00±4.540	1.64±0.03*	210.50±10.34*
Group 9 (BH-100+Col)	8.50±0.34	146.33±17.97	1.74±0.20	205.83±3.290
Group 10 (BH-200+Col)	7.33±0.98	88.33±3.640	3.00±1.13	172.33±23.69
Group 11 (BH-300+Col)	8.17±0.31	68.67±7.870	1.83±0.28	202.67±4.400
Group 12 (BH-350+Col)	6.33±0.84	96.67±7.870	1.83±0.19	142.50±0.390#
Group 13 (BH-400+Col)	7.33±0.98	91.67±7.550	7.50±0.20#	127.00±0.330#
Group 14 (BH-450+Col)	7.33±0.98	91.67±7.550	7.50±0.20#	127.00±0.330#

p-values: *$p<0.001$, when compared with control group; #$p<0.001$, when compared with colchicine treated group

Table 2: Dose dependent effect of BH on SOD activity (Values are mean±SE from 6 animals in each group)

	SOD (% inhibition unit)				
Groups	CC	CB	CN	MB	PM
Group 1 (Control)	10.13±0.10	12.68±0.20	11.86±0.18	10.45±0.12	12.37±0.110
Group 2 (BH-100 mg kg^{-1})	9.95±0.40	11.82±0.97	10.53±0.88	9.45±1.15	11.42±2.240
Group 3 (BH-200 mg kg^{-1})	9.97±0.50	11.85±1.02	10.73±6.55	9.25±0.65	10.41±1.060
Group 4 (BH-300 mg kg^{-1})	8.60±0.29***	8.10±0.19*	9.91±0.70**	9.60±1.05	11.57±1.410
Group 5 (BH-350 mg kg^{-1})	8.58±0.16*	8.92±0.06*	8.97±0.61***	9.45±1.12	10.42±1.150
Group 6 (BH-400 mg kg^{-1})	8.71±0.09*	8.04±0.20*	8.33±0.13*	7.59±0.10*	9.32±0.130*
Group 7 (BH-450 mg kg^{-1})	8.71±0.09*	8.04±0.20*	8.33±0.13*	7.59±0.10*	9.32±0.130*
Group 8 (Colchicine)	21.79±0.26*	21.77±0.22*	19.75±0.32*	21.06±0.21*	21.53±0.300*
Group 9 (BH-100+Col)	21.40±0.78	20.46±2.00	18.60±2.02	18.60±2.02	20.40±1.860
Group 10 (BH-200+Col)	21.78±0.67	19.67±2.98	19.60±1.72	18.60±2.02	20.47±2.000
Group 11 (BH-300+Col)	21.45±0.72	18.30±1.99	17.17±1.37	17.62±2.36	19.66±2.980
Group 12 (BH-350+Col)	15.03±0.10#	14.93±0.13#	18.22±1.59	18.62±2.04	18.34±1.670
Group 13 (BH-400+Col)	15.13±0.08#	15.00±0.12#	13.61±0.18#	15.24±0.09#	14.96±0.110#
Group 14 (BH-450+Col)	15.13±0.08#	15.00±0.12#	13.61±0.18#	15.24±0.09#	14.96±0.110#

p-values: *p<0.001, **p<0.05, ***p<0.01 when compared with control group; #p<0.001, when compared with colchicine treated group

Table 3: Dose dependent effect of BH on LPO activity (Values are mean±SE from 6 animals in each group)

	LPO (nmol of TBARS/g mol of tissue)				
Groups	CC	CB	CN	MB	PM
Group 1 (Control)	4.13±0.12	4.16±0.02	3.18±0.09	3.27±0.12	3.25±0.07
Group 2 (BH-100 mg kg^{-1})	4.02±0.08	4.04±0.26	3.20±0.27	3.21±0.40	3.20±0.34
Group 3 (BH-200 mg kg^{-1})	3.95±0.26	3.79±0.29	3.11±0.27	3.19±0.50	3.25±0.26
Group 4 (BH-300 mg kg^{-1})	3.90±0.16	3.76±0.27	3.04±0.53	3.17±0.54	3.12±0.35
Group 5 (BH-350 mg kg^{-1})	2.14±0.05*	2.78±0.07*	3.07±0.28	3.09±0.51	3.09±0.33
Group 6 (BH-400 mg kg^{-1})	2.09±0.04*	2.34±0.05*	1.76±0.07*	1.68±0.07*	1.82±0.08*
Group 7 (BH-450 mg kg^{-1})	2.09±0.04*	2.34±0.05*	1.76±0.07*	1.68±0.07*	1.82±0.08*
Group 8 (Colchicine)	9.60±0.10*	9.89±0.06*	10.05±0.02*	10.42±0.09*	10.04±0.04*
Group 9 (BH-100+Col)	9.40±0.22	9.58±0.24	9.34±0.48	9.58±0.24	9.66±0.42
Group 10 (BH-200+Col)	9.09±0.46	9.34±0.48	9.34±0.51	9.45±0.43	9.31±0.49
Group 11 (BH-300+Col)	9.02±0.31	9.49±0.31	9.16±0.66	9.36±0.55	9.19±0.53
Group 12 (BH-350+Col)	5.53±0.09#	5.49±0.07#	6.33±0.08#	9.14±0.42##	5.76±0.08#
Group 13 (BH-400+Col)	5.68±0.03#	5.41±0.05#	6.33±0.08#	6.05±0.03#	5.58±0.08#
Group 14 (BH-450+Col)	5.68±0.03#	5.41±0.05#	6.33±0.08#	6.05±0.03#	5.58±0.08#

p-values: *p<0.001, when compared with control group; #p<0.001, ##p<0.05, when compared with colchicine treated group

time significantly decreased (p<0.001) and the number of correct choices significantly increased (p<0.001) in BH treated colchicine group compared to that of colchicine group. Higher dose (450 mg kg^{-1}) failed to produce any further change (Table 1).

Measurement of parameters of oxidative stress: BH (100-450 mg kg^{-1}) dose dependently increased the SOD, CAT, GSH and decreased the LPO activity of the rats. At low doses (100 and 200 mg kg^{-1}) BH increased SOD, CAT and GSH activity and decreased LPO activity with respect to control but this alteration was not statistically significant. At 300 mg kg^{-1} doses, the SOD activity was significantly increased in CC (p<0.01), CB (p<0.001) and CN (p<0.05) rather than control group. BH significantly increased the SOD level in CC (p<0.001), CB (p<0.001) and CN (p<0.01) with respect to control at 350 mg kg^{-1} dosage. However, BH increased the SOD level in MB and PM at 300 and 350 mg kg^{-1} dose but this increase was not statistically significant. The effect of BH was most effective at 400 mg kg^{-1} dose. Higher dose (450 mg kg^{-1}) failed to produce any further change.

In BH treated colchicine induced Alzheimer's rat model, at doses (100, 200 and 300 mg kg^{-1}), the changes of SOD activity was not statistically significant compared to that of Alzheimer's model. At 350 mg kg^{-1} dose, BH significantly increased SOD activity (p<0.001) in CC and CB of BH pretreated colchicine group as compared to colchicine group, but the result of SOD in CN, MB and PM at 350 mg kg^{-1} dose was not statistically significant. The effect of BH was most effective at 400 mg kg^{-1} dose (p<0.001). Higher dose (450 mg kg^{-1}) failed to produce any further change (Table 2).

At doses 100-300 mg kg^{-1} BH decreased the LPO activity with respect to control but the value was not statistically significant. At 350 mg kg^{-1} dose, BH significantly decreased LPO activity in CC and CB (p<0.001) with respect to control. However, BH decreased LPO activity rather than control at 350 mg kg^{-1} dose, but this decrease was not statistically significant. The BH was most effective at 400 mg kg^{-1} dose. Above 400 mg kg^{-1} dose, there was no significant change in LPO activity. At doses 100-300 mg kg^{-1}, the LPO activity decreased in BH pretreated colchicine group rather than colchicine

group but the value was not statistically significant. At 350 mg kg^{-1} dose, the LPO activity significantly decreased in BH pretreated colchicine induced Alzheimer's group (where, $p<0.001$ in case of CC, CB, CN, PM and $p<0.05$ in MB) as compared to colchicine treated group. The LPO activity significantly decreased in different parts of the brain ($p<0.001$) of BH pretreated colchicine induced Alzheimer's group rather than colchicine treated group. Higher dose (450 mg kg^{-1}) failed to produce any further change (Table 3).

At doses 100-300 mg kg^{-1}, BH increased the CAT activity with respect to control but the value was not statistically significant. At 350 mg kg^{-1} dosage, BH significantly increased CAT activity in CC, CB and PM ($p<0.001$) and MB ($p<0.05$) with respect to control but the result in CN was not statistically significant. The effect of BH was most effective at 400 mg kg^{-1} dose ($p<0.001$). Above 400 mg kg^{-1} dose, there was no further change in CAT activity. At doses 100-300 mg kg^{-1}, the CAT activity was increased BH pretreated colchicine induced Alzheimer's group rather than colchicine treated group but the value was not statistically significant. At 350 mg kg^{-1} dosage, the CAT activity was significantly increased in CC, CB and PM ($p<0.001$) of BH pretreated colchicine induced Alzheimer's group as compared to the colchicine treated group but the result in CN and MB was not statistically significant. The effect of BH was most effective at 400 mg kg^{-1} dose ($p<0.001$). Higher dose (450 mg kg^{-1}) failed to produce any further change (Table 4).

At doses 100-300 mg kg^{-1}, BH increased the GSH level with respect to control but the value was not statistically significant. At 350 mg kg^{-1} dosage, BH significantly increased GSH activity in CC and PM ($p<0.001$) region with respect to control but the result in CB, MB and CN at this dose was not statistically significant. The effect of BH was most effective at 400 mg kg^{-1} dose ($p<0.001$). Above 400 mg kg^{-1} doses, there was no further change in GSH activity. At doses 100-300 mg kg^{-1}, the GSH activity increased in BH

Table 4: Dose dependent effect of BH on CAT activity (Values are mean ± SE from 6 animals in each group)

	CAT (% inhibition unit)				
Groups	CC	CB	CN	MB	PM
Group 1 (Control)	14.37±0.08	13.35±0.19	13.21±0.15	13.20±0.10	13.05±0.03
Group 2 (BH-100 mg kg^{-1})	13.81±0.84	13.32±1.40	12.64±1.59	13.30±0.92	12.50±0.80
Group 3 (BH-200 mg kg^{-1})	13.39±1.52	13.25±1.51	12.60±1.38	11.42±2.24	12.43±1.70
Group 4 (BH-300 mg kg^{-1})	12.52±1.43	12.32±1.63	11.47±1.35	10.43±2.84	12.39±1.20
Group 5 (BH-350 mg kg^{-1})	11.19±0.11*	10.19±0.13*	10.37±1.84	10.49±0.99**	9.67±0.13
Group 6 (BH-400 mg kg^{-1})	11.51±0.07*	10.28±0.06*	10.48±0.09*	10.38±0.05*	9.07±0.04*
Group 7 (BH-450 mg kg^{-1})	11.51±0.07*	10.28±0.06*	10.48±0.09*	10.38±0.05*	9.07±0.04*
Group 8 (Colchicine)	22.84±0.10*	22.42±0.14*	21.79±0.19*	21.25±0.16*	20.86±0.17*
Group 9 (BH-100+Col)	19.10±1.97	21.30±1.58	21.39±1.80	20.25±1.49	19.57±1.43
Group 10 (BH-200+Col)	18.30±1.99	21.27±1.74	19.52±1.85	20.40±1.86	19.36±1.69
Group 11 (BH-300+Col)	18.42±1.99	20.31±1.03	18.36±2.02	20.31±1.03	19.21±1.60
Group 12 (BH-350+Col)	15.91±0.10#	15.43±0.09#	17.41±1.82	20.24±1.17	15.46±0.08#
Group 13 (BH-400+Col)	15.13±0.07#	15.32±0.06#	15.50±0.08#	15.37±0.03#	14.99±0.03#
Group 14 (BH-450+Col)	15.13±0.07#	15.32±0.06#	15.50±0.08#	15.37±0.03#	14.99±0.03#

p-values: *$p<0.001$, **$p<0.05$, when compared with control group; #$p<0.001$, when compared with colchicine treated group

Table 5: Dose dependent effect of BH on GSH activity (Values are mean±SE from 6 animals in each group)

	Reduced glutathione or GSH (μg g^{-1} of tissue)				
Groups	CC	CB	CN	MB	PM
Group 1 (Control)	40.51±0.14	43.05±0.24	30.56±0.40	28.31±0.30	31.48±0.50
Group 2 (BH-100 mg kg^{-1})	40.70±0.38	43.51±1.28	30.75±0.82	29.33±1.27	32.46±1.45
Group 3 (BH-200 mg kg^{-1})	41.18±0.83	43.48±1.39	30.75±1.02	29.55±1.49	32.62±1.06
Group 4 (BH-300 mg kg^{-1})	42.35±0.83	44.35±0.98	31.41±0.94	29.28±1.52	33.15±1.31
Group 5 (BH-350 mg kg^{-1})	47.67±0.16*	44.45±1.27	32.32±1.02	30.18±1.49	39.58±0.19*
Group 6 (BH-400 mg kg^{-1})	48.40±0.12*	49.29±0.04*	38.54±0.05*	37.33±0.07*	39.52±0.03*
Group 7 (BH-450 mg kg^{-1})	48.40±0.12*	49.29±0.04*	38.54±0.05*	37.33±0.07*	39.52±0.03*
Group 8 (Colchicine)	1.48±0.22*	2.16±0.10*	2.67±0.12*	1.62±0.08*	2.12±0.05*
Group 9 (BH-100+Col)	1.76±0.22	2.21±0.43	3.33±0.59	2.06±0.47	2.35±0.43
Group 10 (BH-200+Col)	1.75±0.55	2.22±0.44	3.35±0.54	2.20±0.59	2.58±0.42
Group 11 (BH-300+Col)	2.11±0.48	2.73±0.41	3.42±0.53	2.24±0.49	2.50±0.60
Group 12 (BH-350+Col)	11.54±0.17#	2.66±0.45	5.50±1.04##	2.48±0.43	9.36±0.10#
Group 13 (BH-400+Col)	13.34±0.09#	14.75±0.09#	8.81±0.07#	8.27±0.05#	10.13±0.05#
Group 14 (BH-450+Col)	13.34±0.09#	14.75±0.09#	8.81±0.07#	8.27±0.05#	10.13±0.05#

p-values: *$p<0.001$, when compared with control group; #$p<0.001$, ##$p<0.05$ when compared with colchicine treated group

pretreated colchicine model compared to that of colchicine model but the value was not statistically significant. The GSH activity significantly increased in CC and PM ($p<0.001$) and CN ($p<0.05$) of BH pretreated colchicine model compared to that of colchicine model but the result in CB and MB was not statistically significant. The effect of BH was most effective at 400 mg kg^{-1} dose ($p<0.001$). Above 400 mg kg^{-1} doses, there was no significant change in GSH activity (Table 5).

DISCUSSION

The present study evaluates the dose dependent activity of aqueous pulp extract of BH on the behavioral study in colchicine infused Alzheimer's rat model with the possible involvement of the antioxidant. It is evident from the results of the present investigation that treatment with aqueous pulp extract of BH significantly decreased the latency period and significantly increased the number of correct choices in a dose dependent manner. These findings can be explained by alterations of the lipid peroxidation activity and antioxidant activity such as SOD, CAT and GSH activity in different brain regions.

Intracerebroventricular infusion of colchicines causes it to bind with tubulin which is the structural and functional protein of microtubule and thereby generates more and more Reactive Oxygen Species (ROS) leading to neurodegeneration and ultimately produces a condition akin to AD or produces experimental AD model which is histopathologically characterized by the extracellular deposition of senile plaques and the intracellular deposition of neurofibrillary tangles. Free radicals play a crucial role in the pathogenesis of AD. Lipid peroxidation can be used as an index for measuring the damage that occurs in membranes of tissue as a result of free radical generation (Dianzani, 1985; Husain and Somani, 1997). In our present study, ICV infusion of colchicine, it significantly increased the LPO level. The results of significant elevation of LPO level in colchicines treated experimental Alzheimer's group is possibly due to the generation of free radicals via auto-oxidation or through metal ion or superoxide catalyzed oxidation process. This is possibly due to the generation of free radicals via autooxdidation or through metal ion or superoxide catalyzed oxidation process. In the present experiment, BH significantly decreased LPO level in a dose dependent manner compared to other groups. So, from the result of LPO levels it may be concluded that the protection by BH may be due to vitamin E and beta carotene which is present in BH pulp extract.

Endogenous antioxidant status in colchicines induced experimental Alzheimer's rat model was evaluated here by noting the activities of CAT, SOD and GSH as these are the important biomarkers for scavenging free radicals (Venkateswaran and Pari, 2003). Colchicine induced oxidative stress is further supported here by the study of antioxidant scavenger enzyme activities.

The reduction of hydrogen peroxide is catalyzed by CAT that protects the tissues from highly reactive hydroxyl radical. The primary role of CAT is to scavenge H_2O_2 that has been generated by free radicals or by SOD in removal of superoxide anions and to convert it to water (Sivasankaran *et al*., 2007).The destruction of superoxide radicals is catalyzed by SOD, is an important defense system against oxidative damage. From our experimental results of the aforesaid antioxidant enzyme activities in brain tissues colchicine significantly decreased SOD, CAT, GSH activities in colchicine treated experimental Alzheimer's group rather than control, BH treated group and BH co-treated colchicine treated experimental Alzheimer's groups. BH containing vitamin E and beta-carotene significantly increased SOD, CAT, GSH, number of correct choices along with significantly decreased the latency time in a dose dependent manner rather than other groups.

Glutathione is an endogenous antioxidant, which is present majorly in the reduced form within the cells. It prevents the hydroxyl radical generation by interacting with free radicals. During this defensive process, reduced glutathione is converted to oxidized form under the influence of the enzyme glutathione peroxidase (GPX).

The decreased level of reduced glutathione in colchicine treated experimental group seen in our study indicates that there was an increased generation of free radicals and the reduced glutathione was depleted during the process of combating oxidative stress (Reiter, 2000; Schulz *et al*., 2000). This has probably been possible either from the low level of ROS production or through a rapid dissolution of ROS that has further been strengthened from the elevated activities of important antioxidant defense enzymes CAT and SOD, studied in this experiment. Literature study has shown that the BH contains high level of Vitamin E and beta-carotene which protects rat neurons against oxidative stress possibly through the presence of both vitamin E and beta-carotene. Because Vitamin E (alpha tocopherol and other tocopherol) is the most potent antioxidant that can break the propagation of free radical chain reactions in the lipid part of biological membranes. It may be inferred from the present results BH protects rat neurons against oxidative

stress as is evidenced from our results of LPO, CAT, SOD and GSH activities possibly by Vitamin E and beta carotene which is present in BH.

At low doses (100 and 200 mg kg^{-1}) BH did not reveal any significant change in lipid peroxidation, SOD, CAT, GSH activity as evidenced by the number of correct choices out of 10 daily trials and the latency time. It might be that, the antioxidant property of BH is very low at lower doses (100, 200 and 300 mg kg^{-1}) in colchicine-induced Alzheimer's rat model. But BH elicits the highest antioxidant activity at a dose of 400 mg kg^{-1} as compared to other doses on colchicine-induced Alzheimer's rat model. Further increase of the dose (450 mg kg^{-1} b.wt.) of BH did not reveal any further changes. Thus, the dose of 400 mg kg^{-1} b.wt. was chosen throughout the experiment. Thus the findings are that the aqueous pulp extract of this plant results significant protection in the level of antioxidant status in CC, CB, CN, MB and PM after a certain period of co administration on colchicine induced oxidative stress without causing any general and metabolic toxicity. From this point of view, it may be proposed that further research on this field is essential to find out other active ingredients present in the BH pulp extract and their specific role by which the therapeutic importance may be evaluated and the outcome of which can be utilized in the protection of AD.

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