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The Effects of Centella asiatica Aqueous Extract on some Blood Parameters in Rat Model of Alzheimer's Disease

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Abstract

Introduction: Alzheimer's disease (AD) is a progressive and irreversible neuropsychiatric disorder of the brain with a deadly outcome and unknown etiology in which the concentration and function of some brain transmitters, particularly acetylcholine, reduce in AD in the cerebral cortex, e.g., the hippocampus. This study aimed to evaluate the effects of aquatic extract of *Centella asiatica* (CeA) *leaf* on hematological parameters in NBM (Nucleus Basalis of Mynert)- lesioned rats induced by Ibotenic acid (IA).

Methods: Thirty-two male Wistar rats (20- 24 months old; weighing 330±30 g) were selected and randomly divided into four equal groups (n=12) including 1. Control group (which were intact rats), 2. L group (NBM-lesioned rats), 3. CeA group and 4. L+CeA group. NBM was bilaterally lesioned. Seven days after stereotaxic surgery, the total number of white blood cells (WBCs), the total number of red blood cells (RBCs), hemoglobin level (Hb), and hematocrit level (Hct) were assessed. For statistical analysis of data Mann Whitney U- test ($p \leq 0.05$) was used.

Results: IA treatment induced a significant decrease of WBCs, followed by significant decrease in RBCs, Hb and Hct compared with control group ($p \leq 0.05$). Also, the lesion of the NBM after oral administration of *Centella asiatica* extract had no effect on the above blood parameters and even all of the parameters in two groups of CeA and CeA+L compared with the control group showed a non-significant increase ($p \geq 0.05$).

Conclusion: It appears that some blood parameters decrease in rat model of Alzheimer's disease. Also, this research shows the important role of the cholinergic neurons of central nervous system in the regulation of erythropoiesis, also *Centella asiatica* extract can stimulate hematopoiesis.

Keywords: Centella asiatica, Hematological Parameters, Rat, Alzheimer

Introduction

Alzheimer's disease (AD) is a progressive and irreversible neuropsychiatric disorder of the brain with a deadly outcome and unknown etiology (1). Patients with dementia of the Alzheimer type (DAT) reportedly have reduced concentration and function of some brain transmitters, particularly acetylcholine and somatostatin, not only in the cerebral cortex, but also in subcortical structures (2). The brain substrates of learning and memory include several areas, one of which is the hippocampus (3). AD leads to a progressive deterioration of cognitive function with memory loss and injury to hippocampal neurons (4). The decline in cognitive functions can be largely related to cholinergic dysfunction arising from disruption of basal forebrain cholinergic pathways (5), impaired balance between several neurotransmitters has been implicated in the pathogenesis of progressive supranuclear palsy (PSP) (6), with serotonin playing a pivotal role (7). The basal forebrain provides the major source of cholinergic input to the neocortex and hippocampus (8) and the cholinergic neurons in the nucleus basalis of mynert (NBM) are markedly degenerated in AD (9). *Centella asiatica* (CeA) is an Ayurvedic herb used to enhance memory and nerve function (10). The treatment with the water extract of *Centella asiatica* improves learning and memory deficits in Tg 2576 mice, an animal model of Aβ accumulation (2). Hematopoiesis, the dynamic process of blood cell production and

development, is characterized by a continuous, robust turnover of cells. The hematopoietic system, which consists of the bone marrow (BM), liver, spleen, lymph nodes and thymus, provides leukocytes, erythrocytes and platelets through a complex network of tissues, organs, stem cells and regulatory factors (11). The processes of hematopoiesis in the bone marrow are regulated by a variety of factors including those produced by the stromal elements, the developing hematopoietic cells themselves and by exogenous substances, such as hormones and neurotransmitters, like acetylcholine. In particular, cutting nerves which enter the hip bones influenced erythropoiesis, while injecting nicotine inside the bone induced changes in the processes controlled by the autonomic nervous system (12). These data demonstrated the role of the bone marrow innervation in hematopoiesis and the presence of nicotinic acetylcholine receptors within the bone marrow. This view was further supported by the recent studies of nicotine-stimulated changes in hematopoiesis (13, 14). There are restriction studies on the effect of this plant extract on blood parameters. Therefore, this study was conducted to investigate the effect of *Centella asiatica* on some of hematological parameters in rats with NBM lesion. In summary, the aim of this study was to evaluate whether nucleus basalis magnocelluralis (NBM) neuron lesion may induce hematological disorders and whether *Centella aciatica* may have effects on hematological disorders induced of NBM lesion.

Method

In the present study, *Centella asiatica* plant was collected during spring from Anzali wetland region, Iran. The samples were then authenticated for their correct botanical identity by the Chief Botanist, Department of Biology, in the Faculty of Science, Yasouj University, Iran (*Herbarium code: 8645*). The whole plant was dried and coarsely ground with a grinder. For the preparation of *aqueous extract* of Centella *asiatica leaf*, the coarse powder of the plant (5g) was extracted with 8 parts of double-distilled water under boiling for 5h and cooled. The supernatant was then filtered through a 400-mesh cloth to collect the extract and rotary evaporated at 40 ˚C for 30 min. Later the product after flash evaporation was lyophilized to yield a greenish brown powder (totally yielding, 1.5 g) (14).

Healthy male Wistar rats used in this study were fed food and water ad libitum and maintained in a 12:12 h dark and light cycle. The room temperature was kept constant at 25˚C. Behavioral test (Morris water maze) were performed between 9:00 am and 13:00 pm. Only the minimum required numbers of rats were used and they were handled in a humane way. Animals were randomly divided into four groups of 12 rats in each group, including: Group I (Control): Aged control rats (above 24-months-old weighing 380±30 g, intact rats), Group II (Lesion): rats with NBM lesion + normal diet; The lesion was induced bilaterally by using the Ibotenic acid (0.5 µg/site/5 min) infusion into NBM, Group III (CeA): The control + CeA diet (6 ml/kg, 6 Weeks, orally), Group IV (CeA+L): aqueous extract of CeA dieting (6 weeks, 100 mg/kg, orally) followed by NBM lesioning in aged rats. Extract of *Centella asiatica* (100 mg/kg daily) (15) was dissolved in 0.89% physiological saline and administrated orally. Control animals received physiological saline alone. On completion of experimental period, animals were killed by decapitation. The rats were not found to have other causes of anemia, the nutritional state was adequate and no evidence of hemorrhagic and thrombotic disorder was observed. For NBM lesioning, on the day of surgery, the animals were anesthetized with intraperitoneal (IP) injections of ketamine (100 mg/kg) and xylazine (10 $mg.kg^{-1}$) (Alfasan, Woerden-Holland). NBM lesion was induced by the method of Wang et al (16). With some modifications (17). After fixing the head of animals in a steriotaxic instrument (Narishige, Japan), the lesion was induced by injection of IA (0.5 µg/0.1µl for 5 min in each side, Sigma-Aldrich Chemical Co., USA) dissolved in distillated water into NBM bilaterally (AP; -1.3, L; ±2.3, V; -6.6). Coordinates were chosen based on a rat brain atlas (18). Injection was made through 2 µl Hamilton syringe connected to a short piece of polyethylene tube and an injection needle (gage 27). All animals were allowed to have a recovery period (7-10 days). Hematological parameters were assayed one week after the neurosurgery (Figure 1). One week after neurosurgery, blood samples were withdrawn from lateral tail vein via the Biotrol sampling catheter from 15 sham operated and 15 Ibotenic acid-treated rats. Blood samples (0.5mL approximately/sample) were collected in vials containing EDTA for hematological investigations. Hematological parameters were assayed by a COULTER® Ac•T

5diff™ CP-precision instrument for hematology research. In order to rule out the iron deficiency anemia, the standard discrimination indices were calculated by using red blood cell indices as in our previous study (19). All describe data were expressed as mean ± standard deviation. Data were analyzed by SPSS (version 25). Because the data were not normally distributed, the non-parametric statistic Mann-Whitney U-test was employed ($p \leq 0.05$).

Results

Changes in some of blood parameters were studied in four groups: 1) Aged healthy male rats [Control], 2) rats with NBM lesion that did not receive any drug [Lesion], 3) Aged male rats that received extract (6 weeks, 100 mg/kg) [CeA], and 4) Aged male rats that received extract of *Centella asiatica* during 6 weeks prior to the destruction of NBM [CeA + L]. In this study, all measurements obtained from blood parameters are shown in Table 1. Figure 2 shows mean number of red blood cells in groups I to IV. As seen in Figure 2, the average number of RBCs in the group that received only the extract of *Centella asiatica* (CeA) and also in the group that received *Centella asiatica* extract at a dose of 100 mg/kg for 6 weeks prior to the lesion of the NBM in comparison with the group that had just been exposed to the destruction of NBM showed a significant increase ($p < 0.01$). Figure 3 shows average percentage of Hematocrit in groups I to IV. As seen in Figure 3, the average percentage of hematocrit in the group that received only the extract of *Centella asiatica* (CeA) and also in the group that received *Centella asiatica* extract at a dose of 100 mg/kg for 6 weeks prior to the destruction of the NBM (CeA+L) in comparison with the group that had just been exposed to the destroyed NBM (L) showed a significant increase ($p < 0.01$). Figure 4 shows mean hemoglobin levels in groups I to IV. As seen in Figure 4, the average amount of hemoglobin in the group that received only the extract of *Centella asiatica* (CeA) and also in the group that received *Centella asiatica* extract at a dose of 100 mg/kg for 6 weeks prior to the destruction of the NBM (CeA+L) in comparison with the group that had just been exposed to the destroyed NBM (L) showed a significant increase ($p<0.01$). These data suggest that Ibotenic acid reduced the rate of hemoglobin. On the other hand, oral administration of *Centella asiatica* extract increased hemoglobin levels in healthy aging rats, but this increasing was not significant. Figure 5 shows average number of white blood cells in groups I to IV. As seen in Figure 5, the average number of white blood cells in the group that received only the extract of *Centella asiatica* (CeA) and also in the group that received *Centella asiatica* extract at a dose of 100 mg/kg for 6 weeks prior to the destruction of the NBM (CeA+L) in comparison with the group that had just been exposed to the destroyed NBM showed a significant increase ($p < 0.001$). Also, the mean number of white blood cells in the CeA and control groups did not show a significant change.

Table1. Demographic variables of the study groups

group / Variable	Control	Lesion	Ce	CeA+L
	Mean ± Standard Deviation			
Hemoglobin (gdl^{-1})	14.12±0.15	11.09±0.31	14.8±0.42	14.6±0.20
Red Blood Cell (10^6 mm^{-3})	9.85±0.10	7.04±0.23	9.3±0.19	9.2±0.45
White Blood Cell (10^3 mm^{-3})	8.7±0.50	4.36±0.27	8.33±0.32	8.33±0.19
Hematocrit (%)	43.4±0.18	36.6±0.28	42.1±0.19	43.4±0.10

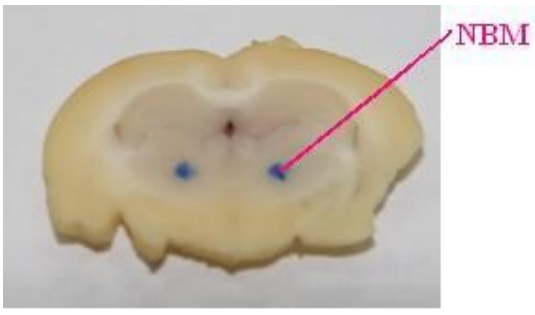

Figure 1. The location of the NBM destruction

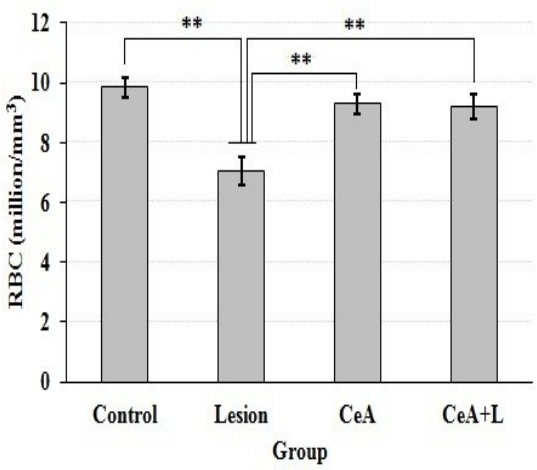

Figure 2. Effect of *Centella asiatica* extract on RBCs (*p < 0.05, **p < 0.01).

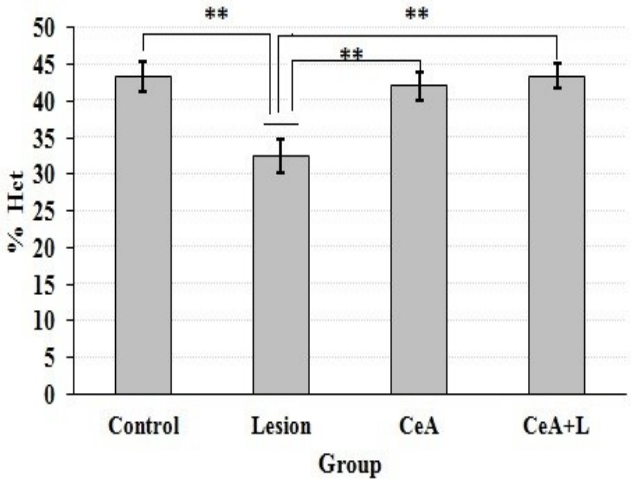

Figure 3. Effect of *Centella asiatica* extract on hematocrit (*p < 0.05, **p < 0.01).

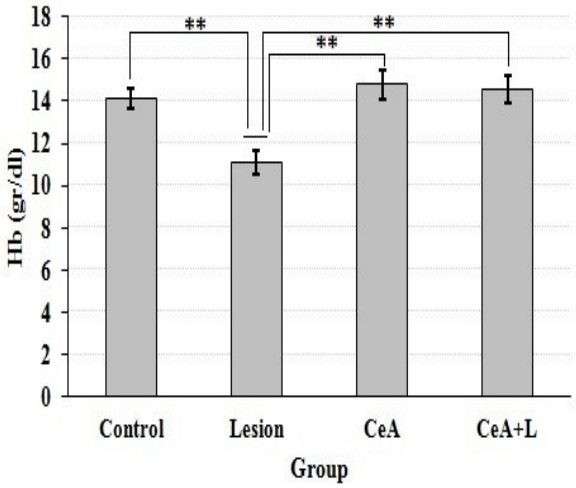

Figure 4. Effect of *Centella Asiatica* extract on hemoglobin (*p < 0.05, **p < 0.01).

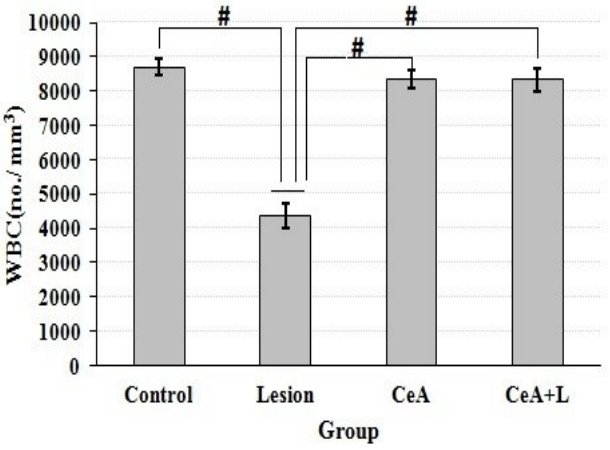

Figure 5. Effect of *Centella asiatica* extract on WBCs (#p < 0.001).

Discussion

The results showed that the mean of erythrocyte has been significantly decreased in aged male rats with Ibotenic acid injection. On the other hand, results have shown that the destruction of the NBM after oral administration of *Centella asiatica* extract at a dose of 100 mg/kg for 6 weeks had no effect on RBC decreasing. Considering that the average time to the maturity of red blood cells of proerythroblast ancestral state to the erythrocyte (mature red blood cell) is about 9-10 days, so this 25-day period can provide a sufficient opportunity for the effects of extracts of *Centella asiatica* in possible activities of hematopoiesis and erythrocytosis. RBC removal saved in the center of the body storage and its entry into the peripheral circulation under stresses is from unknown cases and in fact it is a kind of pseudo-polycythemia that can be related to this increase in the average. Possible increase of cortisol and other glucocorticoids that usually occur in many stresses can also be of help to this increase (20). Increase or decrease in erythrocyte is associated with increasing or decreasing in its volume percent (hematocrit). Reduction of the aforementioned percentage in the group in which only the lesion of NBM is created fits with a significant reduction of mean erythrocyte. On the other hand, results have

shown that the lesion of the NBM after oral administration of *Centella asiatica* extract at a dose of 100 mg/kg for 6 weeks had no effect on the decrease in RBC and hence this plant has prevented the effect of Ibotenic acid on hematocrit reduction. This issue is also related to the mean gram of hemoglobin percent like hematocrit. The mean number of white blood cells has been significantly decreased in aged rats with NBM-lesioned. On the other hand, results have shown that the lesion of the NBM after oral administration of *Centella asiatica* extract at a dose of 100 mg/kg for 6 weeks had no effect on WBC reduction and even the number of white blood cells increased somewhat in these two groups than in the control group which is not significant. This increase in the number of WBC is consistent with increasing the number of RBC, which can be a sign of hematopoiesis under the stimulatory effect of *Centella asiatica* extract. Study on the effect of *Centella asiatica* on blood parameters is very little and limited research is reported and included. Pang *et al.* studies in 2010 showed that high dose administration of CeA in the treatment of rats with adenine-induced chronic renal failure has significant increasing in the number of red blood cells, number of white blood cells, hemoglobin and hematocrit levels (21). Rahim *et al.* (2010) studied blood disorders caused by Ibotenic acid in rats with animal model of Alzheimer's disease. The results of this study showed that Ibotenic acid reduces significantly number of red blood cells, number of white blood cell and hemoglobin and hematocrit levels in animal models of Alzheimer's disease (22). Maneewan *et al.* (2014) studied the effects of *Centella asiatica* blood composition in piglets; 32 nursery pigs were fed 0.0, 0.5, 1.0 and 2.0% dietary C. asiatica from 15 to 90 kg BW. Hematological parameters were checked at 40 and 80 kg BW. On hematological values, at 40 kg hematocrit, total white blood cells, neutrophils, eosinophils, basophils, monocytes and lymphocytes were higher at the 2.0% level. Most of these values except basophils and monocytes continued until at 80 kg, at which total white blood cells, neutrophils, eosinophils and lymphocytes were higher even at 1.0%; neutrophil-to-lymphocyte ratio tended to be higher at 2.0%. The results showed that C. asiatica increases values of serum hematocrit and white blood cells, and has the potential to enhance innate immunity (23). Pang *et al.* (2010) investigated the pharmacological effects of compound *Centella asiatica* enema on chronic renal failure (CRF) rats. Rats were divided into control group, CRF model group, Niaoduqing positive control group, compound *Centella asiatica* enema high, middle and low three group's kidney coefficient. CRF rats fed with adenine due to the long feed, could result in decreased food intake, inadequate intake of raw materials erythropoietin, which led to hematocrit, red blood cell count and hemoglobin levels decreased markedly. After treatment, they could see that the positive control group and the compound *Centella asiatica* enema high-dose group was markedly elevated HCT, RBC and HGB levels. The *Centella asiatica* enema middle-dose group and low-dose group of the RBC also has a significant role in increased. into control group, CRF model group, Niaoduqing positive control group, compound *Centella asiatica* enema high, middle and low three group's kidney coefficient, hematocrit, red blood cell counts, hemoglobin content were observed after 30 days treatment (21). The results of these studies confirm the results reported in the present research.

Conclusion

In conclusion, this study confirms earlier observations of reduced RBC, WBC, Hb and Hct and increased mentioned parameters by CeA in rats with the diagnosed AD. Thus, in present study sample, low hematological parameters induces by Ibotenic acid may have increased susceptibility to AD. The potential role of these changes in hematological parameters in increasing risk of AD should be explored prospectively, in both developed and developing countries, considering its high prevalence in older adults. Also, this research show the important role of the cholinergic neurons of central nerves system in the regulation of erythropoiesis and also *Centella asiatica* extract can stimulate hematopoiesis.

Ethical issues

This research was approved by the ethical committee of Ahvaz University of Medical Sciences.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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