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Original Article

The effects of oral administration of *Aloe vera [barbadensis]* on rat central nervous system: An experimental preliminary study*

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ABSTRACT

Aloe vera [barbadensis] (Av) is widely used for both commercial and therapeutic purposes. It has been used for an array of ailments since ancient times as a medicinal plant. There are more than 360 different species of Av. Its products have been used in health foods for medical and preservative purposes.

The objective of this study was to investigate the effects of Av on the rat's central nervous system; since there are limited studies on that issue. Gel form of Av is used in the study. It is commercial, preserved but otherwise untreated form of Av. Female Wistar Albino rats were divided into three study groups. Tissue specimens from cerebrum, cerebellum, hippocampus and ventricular area were processed for the microscopic examination. All sections from each group were stained with hematoxylin eosin and cresyl violet.

Our results indicate that Av did not have any clear toxic effects on both neurons and glial cells of the central nervous system in different areas. Cytoplasmic features of the neurons, Nissle bodies, axonal hillock, and nuclei of neurons were the same after the treatment. However; the relationship between the Purkinje cells and the surrounding cerebellar tissue was decreased in the treated group. The other important finding was the change of ependymal cells at the ventricular zone: The number and the height of these cells were obviously increased. The single layered epithelium changed into the stratified epithelium in certain areas. It was also evident that microvilli and the cilia on the apical side of these cell increased dramatically. The capillaries in the region of choroid plexus were also dramatically increased.

We believe that further studies related with these morphological changes will be helpful to understand the mechanism(s) of the similar transformation of the cells in different conditions. © **Neuroanatomy. 2008; 7: 22–27.**

Key words [Aloe barbadensis] [rat] [central nervous system] [histologic examination]

Introduction

Aloe vera (Av) is a very popular plant which has been used for alternative medicine. It has more than 360 types. Gel form of Av is mostly preferred [1]. Its chemical and therapeutic properties have been recently investigated [2]. Av contains anthraquinone, polysaccharide and carbohydrate. Anthraquinone is extracted from the plant before use [3]. *Aloe barbadensis (Ab)*, one of the Av types, is the most common used form for commercial and also therapeutic purposes in North America, Europe, and Asia [4]. Plants containing Ab have been used as anti-inflammatory agents, for the treatment of ulcer, hepatitis and neoplasms, and also for wound healing [5]. It has been reported that it stimulates macrophages and has antiviral effects [6]. Antioxidative effects of Ab has been shown in several studies [4,7]. Antigenotoxic and chemopreventive effects of this drug was described in several studies [4,8]. It has been reported that Av gel has angiogenic effects and causes new vessel formation in cingulated cortex and septal areas in rats after ischaemia/reperfusion injury [9]. Depressive effects on neurotransmission, block formation of axonal reflex, anti-inflammatory and analgesic effects of Ab was stated in another study [10].

The aim of this study was to examine the morphological effects of systemic administration of Ab on neurons and glial cells in different parts of central nervous system at light microscopic level.

Material and Methods

After having the ethical consent from the Ethical Committee of Zonguldak Karaelmas University; 18 healthy adult (60-65 days old) female Wistar Albino rats were recruited for the experiment. All animals were kept in the laboratory conditions which provided 28±2 °C temperature, daily 12 hours day-night cycle and 40% of humidity. The weight of the rats were between 170-200 g (mean 185.76±12.93 g).

Rats were divided into three groups.

Ab was given to Group I (n=6) in a daily dose of 25 mg/kg (100 mg/kg) orally by gavage for 3 weeks. As was in capsules containing 500 mg soybean oil.

Group II (n=6) received 500 mg soybean oil via gavage every day.

Rats in Group III (n=6) constituted the control group. All of the three groups were fed with normal feed and water ad libitum.

At the 21st day; animals were anaesthetized with thiopental sodium and then perfused with 10% formaldehyde. Skulls were opened and brains were removed under dissecting microscope. All specimens were weighed freshly and put into 10% formaldehyde solution.

Tissue specimens from cerebrum, cerebellum, hippocampus and ventricular area were processed for microscopic examination. They were dehydrated in alcohol series, processed in xylene and then embedded in paraffin. Sections from each specimens in 0.4-0.6 micron

* This study was presented as an oral presentation in 6th National Neuroscience Congress (2007, Safranbolu–Turkey).

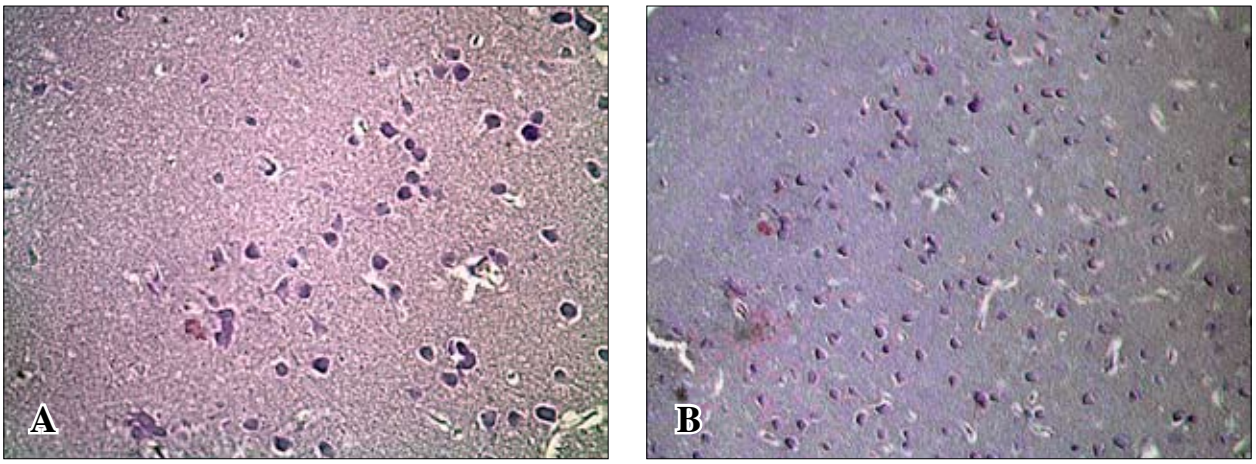

Figure 1. Sections from cerebrum of animals in control group (Group III), (***A***) and in Aloe Barbadensis treated group (***B***). There is no significant microscopic difference. Color version of figure is available online.

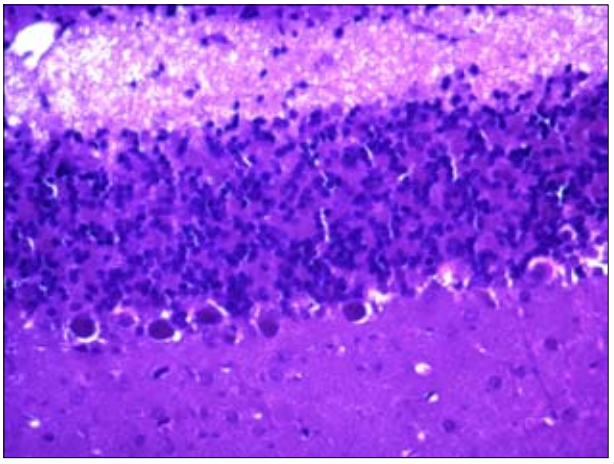

Figure 2. Figure shows the cerebellum of a rat from control group (Group III). Color version of figure is available online.

of thicknesses were stained with haematoxylin - eosin (HE) and cresyl violet. All sections were comparatively evaluated with Olympus BX51 light microscope at 20x, 40x, 100x magnification and photographs were taken.

Diameter of nucleus and number of nucleus for all preparations were measured using Image-pro plus 5.1 analysis system, and statistically compared. Diameter of nucleus and cilia lengths of ependymal cells were measured with the same system. ANOVA was used to analyze the relationship between groups. Kruskal Wallis test was used to assess the relationship between groups' brain weights. The confidence interval was 95% for all tests. For cell analyses, all the sections were evaluated with 40x of magnification.

Angiogenesis was evaluated with stereological method. Using the point-counting grids, we estimated the total number of points hitting any part of choroid plexus and the total number of points hitting vessels. The point-counting grid contacting to choroid plexus and vessels were counted on 100x images. With these data % proportion of angiogenesis was calculated [11].

Results

The weight of brains was about 1.79±0.11 g in Group I, 1.61±0.05 g in Group II and 1.63±0.13 g in Group III. The difference between Group I and II, I and III was statistically significant ($p<0.05$). Brain weights of the

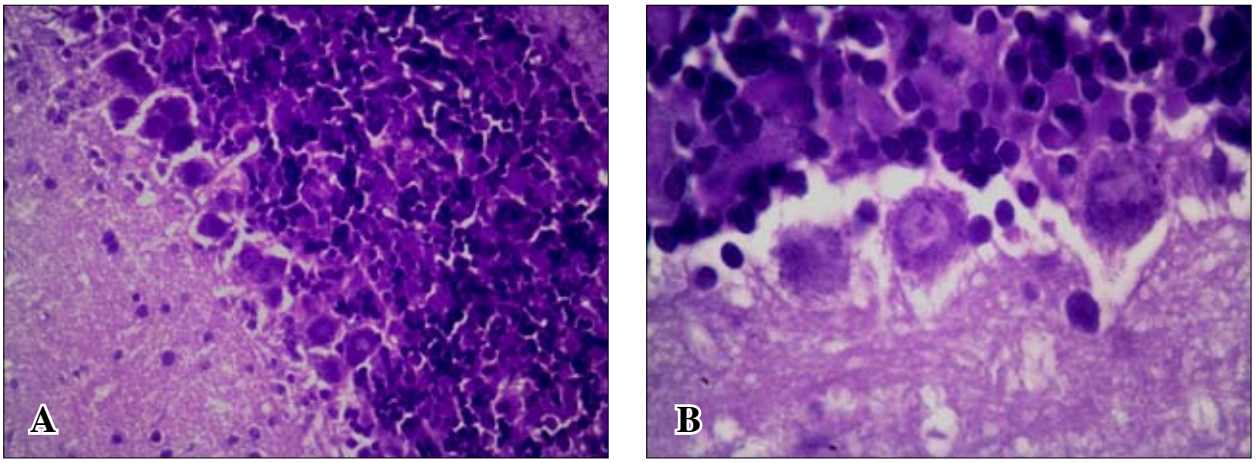

Figure 3. Cerebellar section of a rat in Group I (***A***). Please note the changes in Purkinje cells and the surrounding area after administration of Aloe Barbadensis (***B***). Color version of figure is available online.

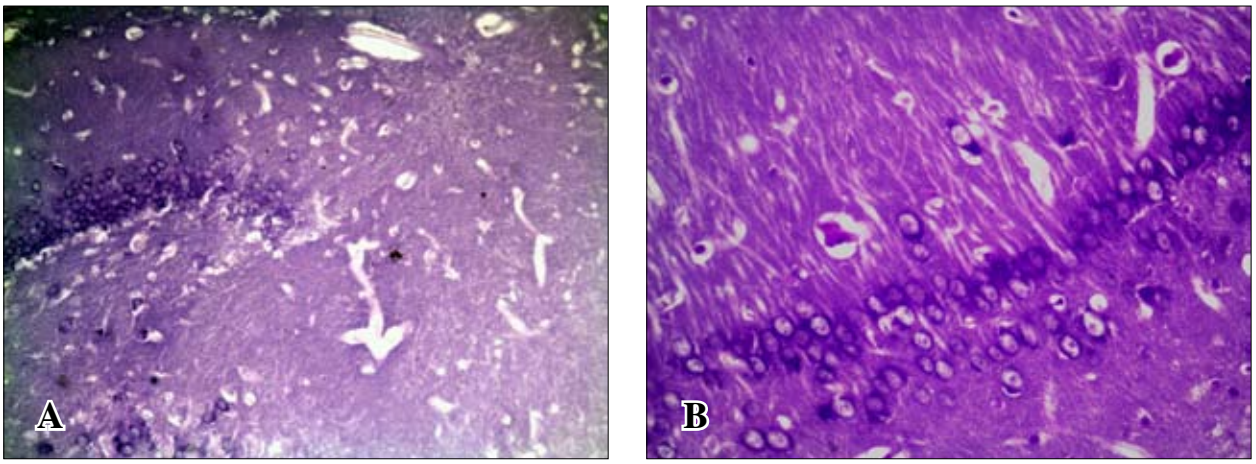

Figure 4. There is no histologic changes on the hippocampus after Aloe barbadensis therapy. *(**A:** Group III (control group); **B:** Group I (Av group).* Color version of figure is available online.

rats in the group treated with Ab were significantly increased ($p<0.05$).

No histological difference was observed between Group II and Group III (control group). No change was found in the group treated with soybean oil, the findings were of similar to the control group (Group III).

Cerebrum

There were no significant difference on the morphology of neuronal and glial cells between the Group III and the Group I in cerebrum (Figure 1A, B). Common histopathologic findings due to toxic agents, such as axonal swelling and oedomatous changes, were not observed in the specimens from any group. Diameter of nucleus of the cells were measured. No significant difference was observed between groups.

Cerebellum

There was no significant difference on the molecular and granular layers of the cerebellar sections between the control group (Figure 2) and the Group I (Figure 3A) at light microscopic level. However; the relation between the Purkinje cells and the surrounding brain tissue seemed to be decreased, since some gaps between these cells and adjacent tissue were observed (Figure 3B). Through the measurements with cell analysis system; we concluded that the size of Purkinje cells significantly increased in the group treated with Ab.

Hippocampus

Morphological findings of hippocampus from Ab treated (Group I) and control groups (Group III) were similar (Figure 4A, B).

Cytoplasmic features of neurons from different regions of brain were also investigated with 100x magnification. Cytoplasmic character of neurons and axonal hillock were not changed after the treatment. Structure of Nissle bodies were not affected (Figure 5A, B).

Ventricular area and plexus choroiedus

Ependymal cells were increased in number and there was also increase at the height of these cells after treatment with Ab (Figure 6A, B and 7). Microvilli and cilia on the apical side of these cells were significantly intensified. In some ventricular areas, single layer of ependymal cells was transformed to multiple layer. Cytoplasm of choroidal

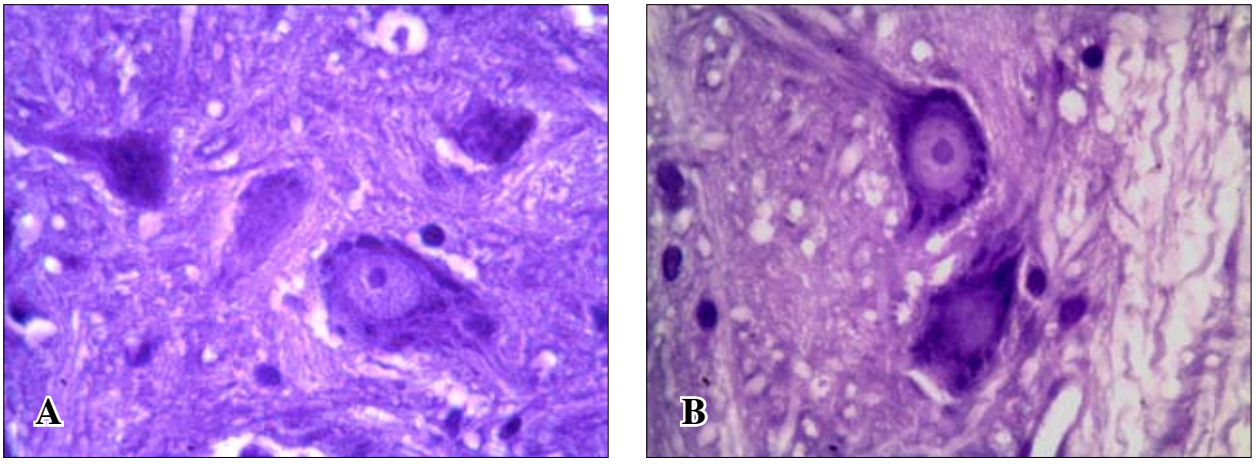

Figure 5. Two pictures demonstrating the neurons before (***A***) and after the therapy (***B***) in higher magnification. No changes on the Nissle bodies have been observed. Color version of figure is available online.

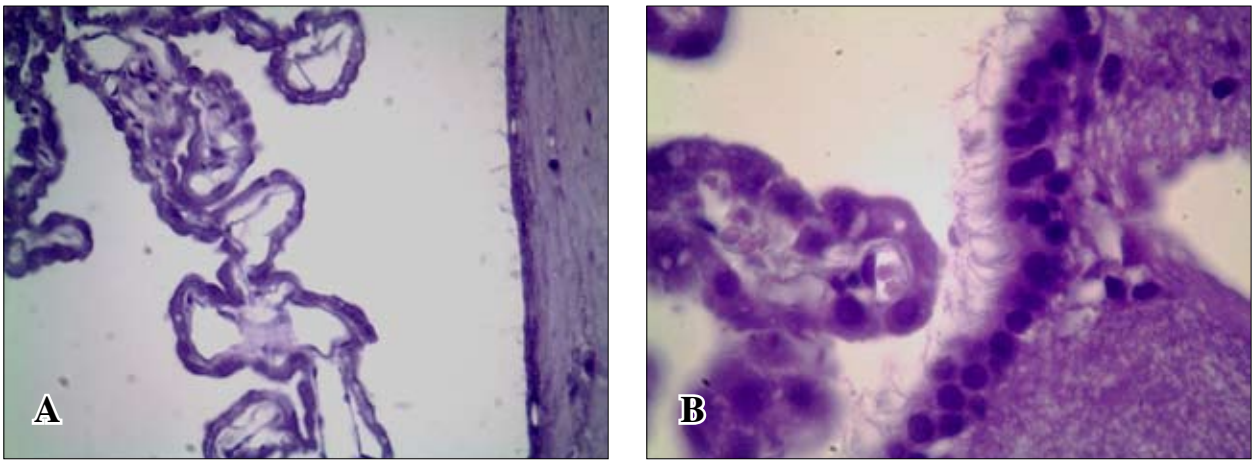

Figure 6. Figure ***A*** shows the ventricular area of a rat from control group (Group III). Significant changes on ependymal cells were observed after the treatment with Ab (B). Please note the apical side of ependymal cells, cilia height, size of the cells and multiple layers in some areas. Color version of figure is available online.

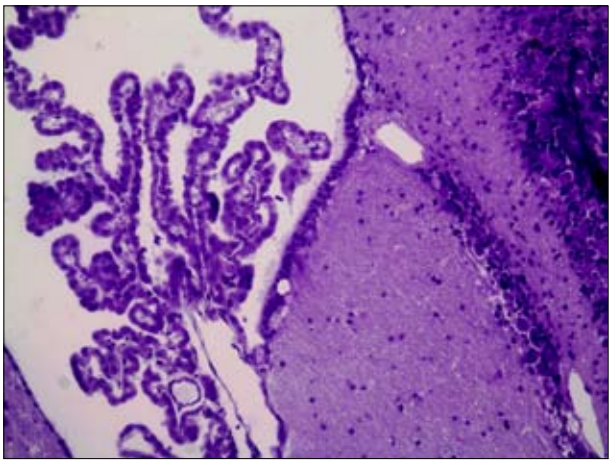

Figure 7. The histologic changes in both choroid plexus and ependymal cells were seen at low magnification in a section from a different Ab treated animal (Group I). Color version of figure is available online.

cells seemed more basophilic after administration of Av. Capillaries were also grown at this area in Group I (Figure 7, 8A, B).

Heights of ependymal cells, diameter of nucleus, amount and cilia heights of ependymal cells were compared using cell analysis system. Diameter of nuclei was greater in Group I, with an increase in the cell number and cilia height. Cells with long cilia have big nucleus. Measurement method and histograms of nucleus diameter and cilia heights are given in the figures. The difference between nucleus diameter and cilia height was statistically significant ($p<0.01$) (Figure 9, 10, 11, 12).

According to evaluation with stereological method, the ratio of the total number of points hitting to vessels and that hitting to choroid plexus, the difference for angiogenesis between Group I and Group III was 0.075. In Ab administrated group, vessels were developed 7.5% more.

Discussion

We conclude that administration of Ab do not cause significant morphological changes on neurons and glial cells in many regions of central nervous system, including cerebrum, cerebellum and hippocampus at light microscopic level. These results support the hypothesis suggesting that this chemical agent is not toxic to these cells. The only microscopic finding related with the neurons was that there was a decrease in the relation between the Purkinje cells and surrounding brain tissue. We also suppose that neurons were not adversely effected from this treatment since there was no changes at the cytoplasm and Nissle bodies of these cells. An interesting finding was observed around the ventricular area: morphology of ependymal and choroid plexus cells was changed clearly.

It has been recenly reported that Ab damages cell membranes. Several studies showed that it decreases neurotransmission [10]. We hypothesize that it might cause changes at the connection of the cells and might effect some physiologic parameters; but not morphology of neurons or glial cells. Inhibitor effects of Ab on endothelial cells were shown in another study [12]. We did not observe any light microscopic changes on endothelial cells; except the presence of more capillaries in choroid plexus. Stimulation of epidermal tissue and cellular proliferation were reported in an other study [13]. This is consistent with our findings in the ventricular area. Proliferation of fibroblasts and increase in angiogenesis were described in another article [14]. The changes at the microscopic anatomy of ventricular area and Purkinje cells after treatment of Ab were reported for the first time in our study. Further electron microscopic studies might show if either microvilli or cilia were increased on the apical side of ependymal cells and define ultrstructural changes of both ependymal and choroid plexus cells.

There were more capillaries in the choroid plexus area in the current study. However; we did not observe any microscopic changes on the vessels in other brain regions. There are studies showing angiogenetic effects of Ab. We

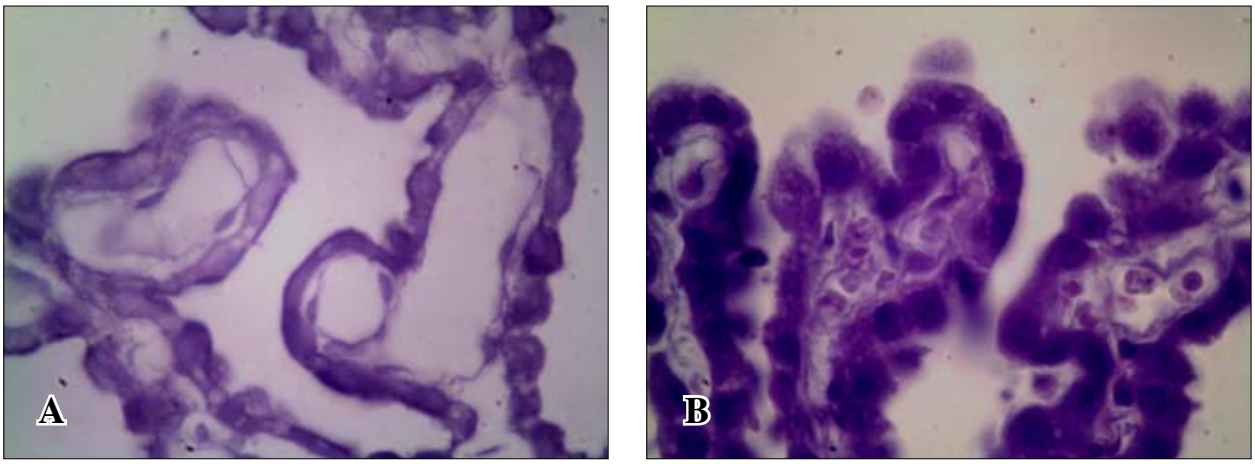

Figure 8. Photograph (***A***) is taken from a section of a rat from in control group. Photograph (***B***) shows plexus choroideus region after the Av treatment. Please note that the choroideal cells are more basophilic and sections contain more capilleries in both Figure 6B and Figure 8B than the Figure 6A and Figure 8A. Color version of figure is available online.

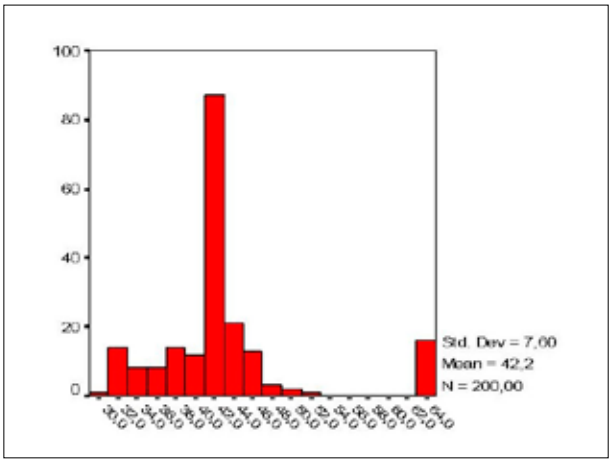

Figure 9. Cilia height after Aloe Barbadensis. Color version of figure is available online.

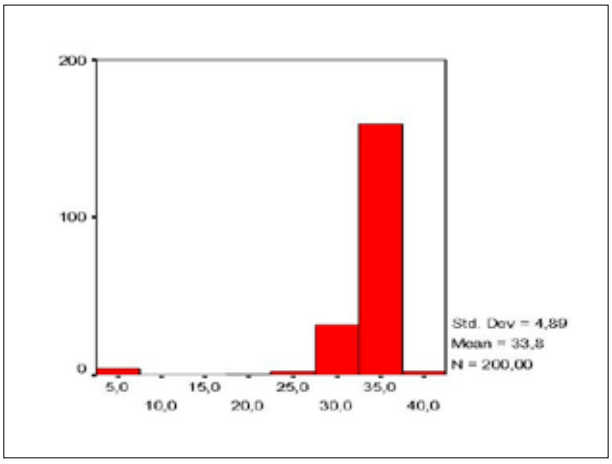

Figure 10. Cilia height of control group. Color version of figure is available online.

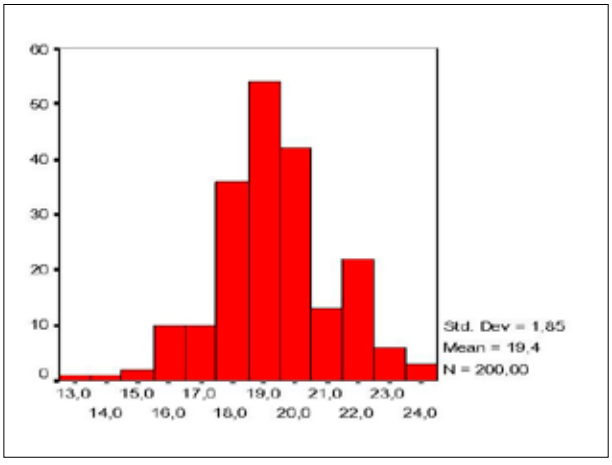

Figure 11. Diameter of nucleus after Aloe Barbadensis. Color version of figure is available online.

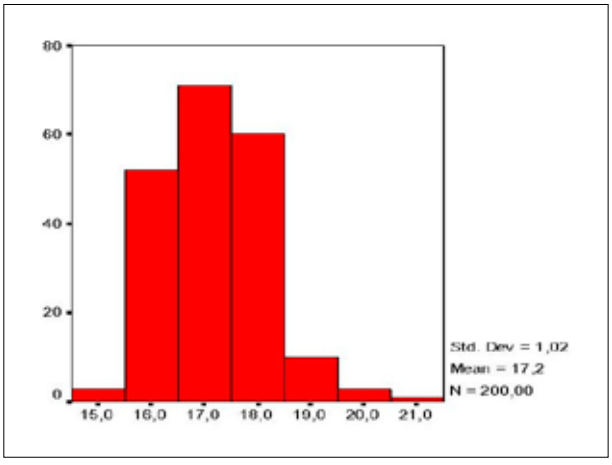

Figure 12. Diameter of nucleus of control group. Color version of figure is available online.

believe that further studies might provide more detailed information and answer these raised questions. The mechanism of the action of this agent needs to be further studied.

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