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Aloe Vera aqueous extract and yogurt enhance peripheral immune cells, and brain astrocytes response in rats

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Abstract

Aloe Vera (AV) is a reputable herbal medical and feed additive. The gel extract of AV contains mucopolysaccharides that stimulate the innate immune response. The reaction to *Aloe Vera* administration might involve beneficial or drastic responses depending on the dose and extraction method. In the current investigation, two different concentrations of *Aloe Vera* gel extract at 10% and 20% were orally administrated for one week either alone or as a supplement to yogurt. Wistar albino rats were randomly distributed into6 groups and administered orally : 1 Distilled water (DW) group, 2) *Aloe Vera* gel extract 10% (AV10) group, 3) *Aloe Vera* gel extract 20% (AV20) group, 4) Plain yogurt group (Y), 5) *Aloe Vera* 10% fortified yogurt group (Y + AV10), and 6) *Aloe Vera* 20% fortified yogurt (Y + AV20) group . The effects of different treatments were investigated on body weight, feed intake, the proliferation of brain astrocytes, differential count of peripheral immune cells, and serum proteins. AV20 neutrophilia with a lymphocytopenia. Glial fibrillary acidic protein (GFAP), a marker of astrocytes, was significantly enhanced in brain tissues that received Aloe Vera-fortified yogurt with non-significant enhancement in other groups. No prominent changes were observed in serum proteins and antioxidant activity. In conclusion, *Aloe Vera* could enhance the proliferation of brain astrocytes and innate peripheral immune cells without further changes in proteins and antioxidant activity and the response of astrocytes to *Aloe Vera* in the brain is augmented by yogurt.

Keywords: *Aloe Vera*, Astrocytes, GFAP, Leukocytes, Yogurt

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Introduction

Aloe Vera is a succulent herb that is widely used as a therapeutic agent. It is rich in polysaccharides, mainly acemannan, which stimulate the immune response (Radha and Laxmipriya, 2015).

Aloe Vera has a protective effect against bacterial pathogens (Lawrence *et al.*, 2009). The administration of *Aloe Vera* could enhance immune response and potentiate complement system activity during bacterial infection. The aqueous extract of *Aloe Vera* promotes secondary humoral immunity mediated by B and T lymphocytes (Zanuzzo *et al.*, 2017). Moreover, high concentrations of *Aloe Vera* were found to enhance the proliferation of polymorphonuclear cells including neutrophils, eosinophils, and basophils during an inflammatory response (Soesilawati *et al.*, 2021). The action of *Aloe Vera* on immune cells is type-specific since it can enhance the relative count of neutrophils without further change in the total leukocytic count (Bala *et al.*, 2019).

Immune response in the brain tissue could be mediated by astrocytes which contribute to antigen presentation (Dong and Benveniste, 2001) and undergo several changes during trauma, ischemia, and neurodegenerative diseases known as astrogliosis (Iglesias *et al.*, 2016). Immunostimulation by lipopolysaccharides leads to the release of tumor necrosis factor-alpha which mediates astrocytes proliferation (Rodgers *et al.*, 2020). Moreover, IL-8 and IL-6 enhance the glial fibrillary acidic protein (GFAP); a marker of astrocytes in brain tissue (Azzolini *et al.*, 2022). However, the effect of *Aloe Vera* or its component consumption on brain astrocytes is not well documented.

The concentration of *Aloe Vera* and the method of extraction are important factors in its biological effect. A high concentration of *Aloe Vera* causes diarrhea and reduces body weight, and feed intake. However, this effect is mostly related to whole leaf extracts or unfiltered extracts of *Aloe Vera* which contains the latex toxic portion (Herlihy *et al.*, 1998; Sehgal *et al.*, 2013).

Aloe Vera encompasses antioxidant components such as ascorbic acid, flavonoids, tannins, and vitamin c (Aburjai and Natsheh, 2003; Eshun and He, 2004; Radha and Laxmipriya, 2015). *Aloe Vera* could enhance antioxidant activity during oxidative stress, but it has no impact on antioxidant capacity in reluctant animals (Prueksrisakul *et al.*, 2015). Administration of *Aloe Vera* could modulate the level of serum protein in a species-related manner. For instance, supplementation of *Aloe Vera* in the fish diet can enhance serum albumin and globulin (Nku *et al.*, 2018). On contrary, it does not affect the serum proteins of rodents (Prueksrisakul *et al.*, 2015).

Aloe Vera is often used as a supplement to dairy products such as yogurt. Manufacturing of yogurt involves fermentation of milk using lactic acid-producing gram-positive bacterial cultures such as *lactobacillus bulgaricus* and *Streptococcus Acidophilus* which possess a cell wall with polysaccharides, peptidoglycan, and teichoic acid that have immunostimulatory effects (W. J. Lee and Lucey, 2010).

Although the immune-stimulatory effect of yogurt and *Aloe Vera* extract was investigated previously, the literature concerned with the immune-stimulation effect of *Aloe Vera* and yogurt mixture is

scarce. Therefore, the current study aims to investigate the effect of yogurt *Aloe Vera* gel extract mixture on the blood hematological and biochemical parameters related to the innate immune response in albino rats. Moreover, the study focused on the influence of *Aloe Vera* on the astrocytes as an important maintainer and modulator of immune response in brain tissue.

Materials and methods

Ethics and consent to participate

All animal experiments were following institutional animal care and use committee guidelines (IACUC#: 12-214). The experiments were also following the Egyptian welfare laws. Protocols for animal experiments were approved by the Ethical animal use committee at the Faculty of Veterinary Medicine, South Valley University

Animals

A total of Seventy-two Wistar male adult albino rats (250 – 300g) were randomly divided into six groups. Rats were acclimatized to the laboratory conditions for two weeks before the experiment. Rats were housed in clean plastic cages with a 12 h light/dark cycle at 25 ± 2 °C and *ad libitum* access to food and water. Body weight changes of all groups were measured as the difference between initial and final body weights after one week of treatment. At the end of the experiment, animals were sacrificed, the spleen was removed and weighed, and the relative organs' weights were calculated. (Loveless *et al.*, 2008).

Experimental Design

Rats were distributed randomly into six groups (n = 12). All treatments were given orally at a volume of 2ml/100 g b. wt. daily. The groups were: Distilled water (DW) group, Plain Yogurt(Y) group, *Aloe Vera* gel 10% group (AV10), *Aloe Vera* 10% fortified group (Y + AV10), *Aloe Vera* 20% group (AV20), and finally Yogurt + *Aloe Vera* 20% (Y + AV20) group.

Preparation of *Aloe Vera* gel

Aloe Vera was collected from Qena governorate, Egypt, and the preparation of *Aloe Vera* gel was performed manually; Clean *Aloe Vera* leaves were sliced into square shapes then the outer membrane was removed to extract *Aloe Vera* gel which was rapidly washed in distilled water to be cut into small pieces, blended, and rapidly diluted by distilled water to get the final concentration of gel 10% and 20%.

Preparation of yogurt *Aloe Vera* gel Mixture

Raw Cow Milk was pasteurized at 90 C° for 20 min and then cooled to 42 C°, followed by adding 3% yogurt's starter culture (YC-X11, Thermophilic yogurt culture - YoFlex®) then pasteurized 10g and 20g of *Aloe Vera* gel were added to 80g and 90g of yogurt respectively to reach the final concentration of 10% and 20% *Aloe Vera* . The extract was added immediately after preparation and kept in clean plastic cups for incubation at 42 C° till pH reach 4.6 and complete coagulation occur, finally both plain yogurt and *Aloe Vera* yogurt samples were kept in the refrigerator at 4C° (Mukhekar and Desale, 2018).

Samples collection

Individual blood samples were collected from retro-orbital venous plexus from each animal at the 7th day and kept on test tubes without anticoagulant just before slaughtering of animals. A small portion of the collected blood sample was used directly for the preparation of blood film for differential leukocytic count

(DLC), then blood was centrifuged at 3000 rpm for 15 minutes then the sera were separated and collected in Eppendorf tubes**.** Sera were kept after collection at -80˚C until assay of serum total protein, serum globulins, and total antioxidant capacity. Rats were anesthetized with sodium thiopental (50 mg/kg B.W, I.P) then sacrificed then brain and spleen were collected for histopathological examination.

Differential leukocyte count (DLC)

Blood films were fixed with absolute ethyl alcohol and stained with Giemsa stain. Stained blood film was examined under a light microscope 100X oil lens. One hundred leukocytes were counted, and the percentage of each cell type was calculated (Laposata and McCaffery, 2022).

Biochemical analysis

All biochemical parameters were assessed calorimetrically using commercial kits. Total serum protein (g/dl) (Kaplan and Rhona, 1995), albumin (g/dl) (Doumas *et al.*, 1971), and total antioxidant capacity (T-AOC) (Koracevic *et al.*, 2001) were analyzed using commercial kits (Spectrum, Egyptian Company for Biotechnology, Obour City, and Cairo, Egypt). Globulin level (g/dl) was calculated by subtracting the albumin value from the total protein value.

Glial fibrillary protein (GFAP) Immunohistochemistry

The distribution of GFAP, a marker of brain astrocytes, was estimated through immunolabeling of paraffin-embedded brain tissues. Sections were treated with the UltraVision hydrogen peroxide block for 10 min. Afterward, Rabbit monoclonal antibody against GFAP protein was used (Roche, USA, Cat. No. # 05269784001). Sections were incubated with 100–150 µL of the antibody–buffer mixture. The sections were incubated with the secondary antibody [ultraView Universal HRP Multimer contains a cocktail of HRP labeled antibodies (goat anti-rabbit) for 1 h, sections were rinsed, reacted with the avidin-biotin complex (ABC) reagent at room temperature (RT) for 45 min, and colorized with 0.05 M Tris-HCl (pH 7.4) containing 0.006% H2O2 and 0.02% 3-3′-diaminobenzidine tetrahydrochloride. Counterstaining was performed using hematoxylin. Control staining was made with PBS which replaced the primary antibody (Awad *et al.*, 2019). For each group, GFAP staining intensity was calculated from at least three images with the same magnification scale and same size. The color of GFAP staining was masked using a custom python 3.8 scripts and the average color intensity was calculated.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and statistical significance between means was analyzed by one-way analysis of variance (ANOVA) using Graph-Pad Prism 8 (Graph Pad Software, San Diego, CA, USA). Results were considered significant at $P < 0.05$.

Results

Body weight and spleen weight/body weight ratio

Spleens were excised from sacrificed animals for assessment of spleen weight change to total body weight. There were no significant changes in the relative spleen weight in response to any of the treatments (Table 1).

Table (1): Body weights (g) and spleen weight/body weight ratio of different groups one week after the onset of treatment

Group	Body weight (g)	Spleen weight/body weight ratio
DW	287.37 ± 10.79[a]	0.383 ± 0.041 [a]
Y	267.57 ± 13.88 [a]	0.419 ± 0.044 [a]
AV10	268.81 ± 8.03 [a]	0.492 ± 0.079 [a]
Y+AV10	283.48 ± 9.58 [a]	0.356 ± 0.023 [a]
AV20	277.36 ± 10.96 [a]	0.379 ± 0.079 [a]
Y+AV20	273.48 ± 13.07 [a]	0.405 ± 0.023 [a]

Data presented as mean ± SEM (n = 12). Digits were rounded to two decimal places. Groups with different letters indicates a significant difference in means.

Differential leukocyte count (DLC)

Blood samples were collected from animals one week after treatment and differential leukocytic counts for each cell type were calculated (table 2). (AV20) group showed a significant increase in Neutrophils count (29.33 ± 3.84) when compared to DW (10.57 ± 1.09) $P < 0.01$and (Y + AV20) (10 ± 2.29) (AV10) (16.83 + 3.96) groups at $P < 0.01$. The lymphocytic count was also significantly decreased in the(AV20) group (60.33 ± 4.18 when compared to the DW group (80.43 ± 1.96) at $P < 0.01$ and (Y+AV20) group (83.17 ± 2.21). However, the differential cell counts of other cell types including Basophils, monocytes, and eosinophils were not significantly different among all the treatment groups.

Table (2): Differential leukocytic count (DLC) in different groups one week after the onset of treatment

Group	Differential leukocytic count (%)				
	Neutrophils	**Eosinophils**	**Basophils**	**Monocytes**	**Lymphocytes**
DW	**10.57 ± 1.09[a]**	**1.43 ± 0.37**	**0.86 ± 0.55**	**6.71 ± 1.96**	**80.43 ± 1.96[a]**
Y	**7.00 ± 1.65[a]**	**2.50 ± 0.89**	**0.17 ± 0.17**	**7.17 ± 3.35**	**83.17 ± 2.70[a, b]**
AV10	**13.33 ± 2.40[a]**	**0.33 ± 0.33**	**0.33 ± 0.33**	**9.00 ± 1.15**	**77 ± 3.21[a, b]**
Y±AV10	**16.83 ± 3.96[a, b]**	**2.17 ± 0.40**	**0.33 ± 0.33**	**3.83 ± 1.78**	**76.83 ± 4.07[a, b]**
AV20	**29.33 ± 3.84[b]**	**4.67 ± 1.20**	**0.33 ± 0.33**	**5.33 ± 2.73**	**60.33 ± 4.18[b]**
Y± AV20	**10.00 ± 2.29[a]**	**1.67 ± 0.92**	**1.50 ± 0.92**	**3.67 ± 1.26**	**83.17 ± 2.21[a]**

Data were presented as mean ± SEM. Digits were rounded to two decimal places. Groups with different letters indicate a significant difference ($P < 0.05$)

Total Antioxidant Capacity and serum proteins

The total antioxidant capacity and serum total proteins, albumin, and globulin were estimated in sera samples one week after the onset of treatment. No significant changes were observed among all treatment groups. However, serum globulin showed a tendency toward elevation in Y+AV20 when compared to the DW group (table 3).

Table (3): Total antioxidant capacity serum total protein, serum albumin, and serum globulin levels one week after treatment of different groups.

Group	T-AOC	Total protein	Serum Albumin	Serum Globulin
DW	1.23 ± 0.15	6.54 ± 0.19	4.81 ± 0.54	1.73 ± 0.41
Y	1.40 ± 0.03	6.59± 0.03	4.39 ± 0.58	2.21 ± 0.61
AV10	1.35 ± 0.05	6.64 ± 0.21	4.54± 0.51	2.1 ± 0.38
Y+AV10	1.39 ± 0.12	6.81± 0.19	4.73 ± 0.55	2.07 ± 0.39
AV20	1.45 ± 0.02	6.83 ± 0.23	5.12 ± 0.19	1.71 ± 0.22
Y+AV20	1.46 ± 0.05	7.23± 0.23	4.6 ± 0.57	2.63 ± 0.63

Data represented as mean ± SEM. Digits were rounded to two decimal places. T-AOC = total antioxidant capacity

GFAP expression in brain tissue

Selected immunohistochemical micrographs of brain tissue (Fig. 1) from different treatment groups showing the expression of GFAP as a marker of astrocytes in brown color, and counter-stain hematoxylin. DW group showed a moderate expression of GFAP when compared to other groups (Fig.1, a).

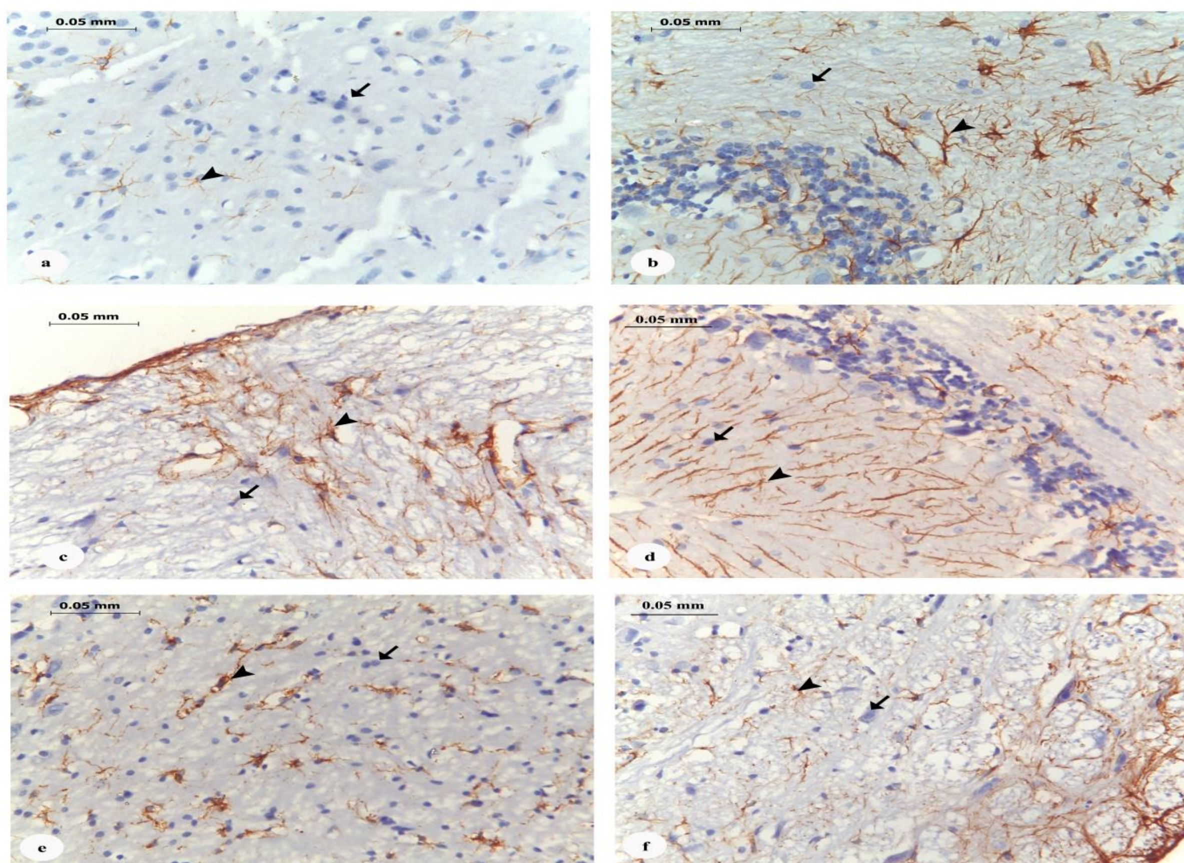

Fig.(1): Immunohistochemical micrographs of the brain showing expression of astrocyte marker (GFAP) in brown color (arrowheads), and nuclei marker (DAPI) in blue color (arrows) for (a) DW group, (b) Y group, (c) AV10 group, (d) Y+AV10 group, (e) AV20 group, and (f) Y+AV20 group.

However, there was an enhanced expression of GFAP in (Y, AV10, Y+AV10, AV20) and (Y+AV20) groups (Fig. 1, b-f). Quantification of GFAP brown color intensity showed that (Y + AV10) and (Y + AV20) groups are significantly higher than the DW group (Fig.2, c).

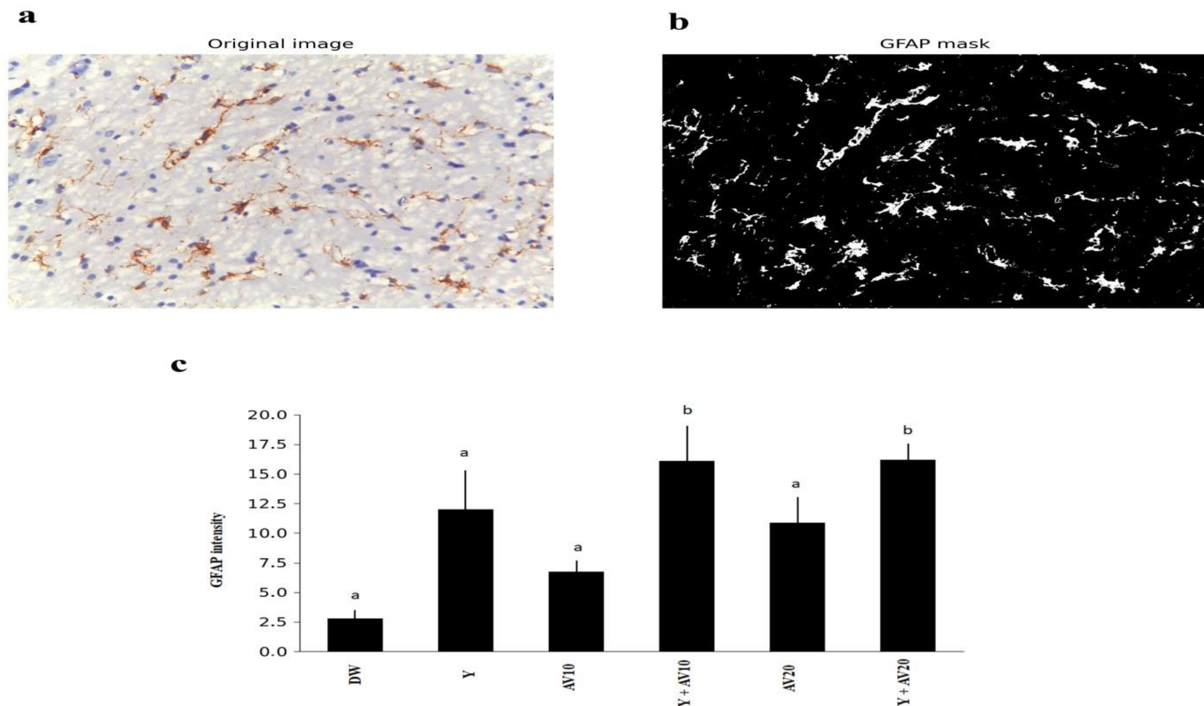

Fig (2): Quantification of GFAP intensity. a) a sample histochemical brain micrograph showing GFAP staining in brown color, b) GFAP staining masked image for calculation of color intensity, and c) Average GFAP staining intensity in different treatment groups. Groups with different letters have significantly different means.

Discussion

Herbal components and plant extracts are utilized for various therapeutic and cosmetic purposes. The health impacts of such products are dependent on the way of manufacturing and preparation. The method of extraction, filtration, and dose of *Aloe Vera* is a crucial factor in its pathophysiological implications (Sehgal *et al.*, 2013; Herlihy *et al.*, 1998). In this study, trials were made to assess the impact of two different concentrations of aqueous extract of *Aloe Vera* at 10% and 20% on peripheral blood immune cells, serum proteins, and brain astrocytes as the main maintainers of the neuronal environment, and modulators of the immune response.

Type-specific peripheral leukocyte proliferation was assessed through the differential leukocytic count. The findings revealed that Neutrophils count was significantly enhanced in the higher concentration of *Aloe Vera* (20%) when compared to the control, the lower dose of *Aloe Vera* (10%), and the *Aloe Vera* (20%) fortified yogurt group. The enhanced neutrophil count was opposed by a relative reduction in lymphocyte count in the same group. Likewise, similar results were achieved by a previous investigation that showed an enhanced polymorphonuclear cells count by higher concentrations of *Aloe Vera* (Soesilawati *et al.*, 2021). The neutrophil count is relatively increased without further change in the total leukocytic count (Bala *et al.*, 2019). The results might be related to the role of *Aloe Vera* components with innate immunostimulant effects such as

acemannan that enhance the innate immune response (Djeraba and Quere, 2000; Stuart *et al.*, 1997).

However, the same results were not obtained using 10% *Aloe Vera* extract which indicates a dose-dependent response to *Aloe Vera* administration. Previous reports showed that only high concentrations of acemannan could induce a significant induction in immune response on its own, while low doses can only enhance other immunostimulant agents (Ramamoorthy *et al.*, 1996).

No significant change was obtained in the differential count of monocytes, eosinophils, and basophils. *Aloe Vera* might promote other aspects of immune response including phagocytosis in macrophages, and proliferation of dendritic cells (J. K. Lee *et al.*, 2001; Liu *et al.*, 2006)

Current findings did not reveal any significant change in antioxidant capacity, total serum protein, globulin, or albumin. These findings might be a result of short-term treatment, Likewise, previous investigations showed that *Aloe Vera* has no effect on serum protein, globulin, or albumin in normal subjects, nevertheless, it could recover reduced serum proteins and antioxidants capacity in animals subjected to oxidative stress (Prueksrisakul *et al.*, 2015). Moreover, the obtained findings were supported by a previous literature which showed that *Aloe Vera* could not induce the activity of SOD, GSH, or CAT in sedentary animals, but it can recover the reduced activity of these enzymes in stressed animals (Bala *et al.*, 2019). However, experiments made using crud *Aloe Vera* extract revealed a reduction in total serum proteins and an elevation of ALT and AST levels (Akpan *et al.*, 2014).

Most of the reported undesirable effects of *Aloe Vera* resulted from whole leaf or the outer leaf rind extracts and the filtration of *Aloe Vera* extract helps the exclusion of the latex portion of *Aloe Vera* with toxic effects (Sehgal *et al.*, 2013) which justifies the absence of drastic effects of *Aloe Vera* gel extract on serum proteins in the current study. The preparation of *Aloe Vera* products is an essential step for the reduction of side effects. Filtration of *Aloe Vera* extract using charcoal was shown to enhance the useful impact of *Aloe Vera* and reduce its irritation (Herlihy *et al.*, 1998). (Sehgal *et al.*, 2013).

In the current study, an investigation of the immunohistochemical expression of GFAP in the brain showed an enhanced expression of GFAP in animals that received *Aloe Vera* treatment at both high and low concentrations. This indicates that *Aloe Vera* could stimulate the proliferation of astrocytes in the brain. These results are supported by a previous report which showed a similar effect of *Aloe Vera* extract components such as emodin which could enhance GFAP expression in the brain (Mijatovic *et al.*, 2005). Moreover, the action of *Aloe Vera* on astrocytes is suggested to be an improvement in the activity of astrocytes rather than a drastic side effect on brain tissue (Tornero-Martínez *et al.*, 2022). Yogurt groups showed enhanced expression of GFAP in brain tissue. The proliferation of astrocytes by yogurt administration might be a result of immune response augmentation by yogurt components. Literature showed that yogurt can enhance the level of interleukin-6 suppressed in stressed animals. Moreover, yogurt can recover the stress-suppressed proliferation of

monocytes, neutrophils, and lymphocytes (Lollo *et al.*, 2013).

Conclusion

In the current study, oral administration of *Aloe Vera* is a natural enhancer of neutrophils as an innate immune cell. It also can enhance proliferation of Astrocytes in brains tissue which are important maintainers of neurons and modulators of immune response. Action of *Aloe Vera* on brain astrocytes is enhanced by yogurt. Both yogurt and *Aloe Vera* has no further impact on serum proteins and antioxidant activities.

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Conflict of interest

The authors declare that there is no conflict of interest.

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