Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.085

RESEARCH ARTICLE

Neuro-Glial Modulatory Roles of Black and Red Grape Seed Extract-Derived Polyphenols (*Vitis vinifera*) in Normal Aged *Albino* Mice's Brain

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ARTICLE HISTORY (21-378)

Received: September 22, 2021
Revised: December 09, 2021
Accepted: December 13, 2021
Published online: January 16, 2022

Key words:
Black GSE
CD68pk1
Glial cell
Neuron
Red GSE
S100

A B S T R A C T

The underlying glial-neuromodulating ability of grape-derived polyphenolic extracts and chemicals is highlighted in this study. Thirty healthy-aged (10 weeks) *BALB/c* strain mice were simply classified into five groups (6 albino mice per group); Group I (the aged group) was fed a balanced diet, whereas Group II received 200 mg/kg of Black GSE, Group III received 400 mg/kg of Black GSE, Group IV received 200 mg/kg of Red GSE, and Group V obtained 400 mg/kg of Red GSE. The study lasted roughly four weeks, and all treatments were administered as a single daily dose via oral gavage every two days, with the third day off. The brains were carefully dissected and preserved in a 10% paraformaldehyde solution. Morphometric assessment and the immunohisto-chemical analysis were performed for the Primary S100B and CD68KP1 antibodies. Mean values for cell number and nucleus size were calculated for each type of cell including; astrocytes, microglia, and oligodendrocyte with small and large neurons by S100 and CD68pk1 expression. All treated groups, more specifically 400mg/kg of Black and Red GSE significantly played an impact role in reducing the aging damaging effect of S100 in each type of glial (astrocyte, microglia, and oligodendrocyte), and neuron, while increased the scavenging activity of CD68pk1in microglia and neuron that delayed aging and improved brain health in comparison to brain section in the aged group. We concluded that GSE possessed neuroprotective and anti-aging properties by minimizing the adverse effect of S100 and upregulating CD68pk1.

To Cite This Article: Hassan SN, Hassan SMA, Saleh N and Maarof NNN, 2022. Neuro-glial modulatory roles of black and red grape seed extract-derived polyphenols (*Vitis vinifera*) in normal aged *Albino* Mice's brain. Pak Vet J, 42(2): 185-193. http://dx.doi.org/10.29261/pakvetj/2021.085

INTRODUCTION

The functioning capacities of the brain, like those of other organs and systems, diminish as we become older. As we become aged, our learning capacities, memory, consideration, dynamic speed, sensory awareness (vision, hearing, contact, smell, and taste), and engine coordination all degenerate (Mattson and Arumugam, 2018). The major cause of age-related illnesses (ARDs), such as neurodegenerative disorders, is aging (Franceschi *et al*., 2018).

The majority of previous studies focused on neurons into aging-related brain changes. In any case, non-neuronal glial cells are the principal cells to react to "stress" in the central nervous system (Palmer and Ousman, 2018). Glial cells perform a variety of vital functions, putting them in a position to be affected by brain aging. Interestingly, glial cells, not neurons, showed the majority of the variations in gene expression with age, according to a transcriptional investigation of the aging brain (Soreq *et al*., 2017). The three primary types of glial cells in the central nervous system are astrocytes, microglia, and oligodendrocytes (CNS). Astrocytes are star-like cells that deliberate closely with neurotransmitters, veins, and other glial cells to arrange neurotransmitter development and work and give satisfactory nourishment to neurons (Allen, 2014). Microglia, which are resident immune cells in the CNS, are responsible for monitoring the brain environment and responding to injury and disease (Salter and Stevens, 2017). Oligodendrocytes are the main cells responsible for the myelination of neuronal axons in the CNS. It's crucial

for a neuronal function that they can appropriately ensheath axons (Young *et al*., 2013).

Polyphenols are normally found in vegetables, tea, espresso, chocolate, grains, and homegrown drinks. For instance, grapes, berries, apples, cherries, and pear can contain up to 200-300 mg poly-phenols per 100 g new weight (Ganesan and Xu, 2017).

The grape (*Vitis vinifera L.)* is the world's second-most generally developed organic product, and it tends to be eaten crude, dried, or matured into wine (Pari and Suresh, 2008). The grapefruit is utilized in natural cures due to its dietary benefit and high polyphenolic content, just as nutrients, minerals, and natural acids (Benmeziane *et al*., 2014). Epicatechins, procyanidins, and catechins, which are monomeric phenolic chemicals, are abundant in grapes, grape seeds, grape leaves, and grape pomace (Surai, 2014). In light of their different (pleiotropic) natural activities and potential health advancing advantages, polyphenols have been considered as neuroprotective, calming, hostile to cholinesterase, anti-amnesic, hypolipidemic, and anti-aging agents (Zhang *et al*., 2015).

Neurons and glia can change their morphology in response to CNS injuries, in addition to modifying their gene expression. It's still up for debate if and how neuron and glia morphology is affected in the aging brain. Immunohistochemistry studies are a common way to characterize changes in neuron and glia morphology (Ferrer, 2017).

The most commonly expressed S100 proteins in the brain are S100B, S100A8, and S100A6, which are all enhanced by age and neuronal injury. Secondary Ca2+ binding sites in the EF-hand motifs of these proteins can also bind $Zn2^{+}$/$Cu2^{+}$. This is an intriguing aspect, considering that dyshomeostasis of neurometals is a well-known phenomenon throughout neurodegeneration (Cristóvão *et al*., 2016).

S100B is quite possibly the most common protein in the brain (0.5%), and it is produced in unassuming amounts by astrocytes. Astrocyte activation and expanded expression of S100B, just as extracellular delivery and commitment of RAGE-mediated signaling and microglial enactment, happen because of brain harm and neurodegeneration (Leclerc *et al*., 2010).

In both normal and pathologic settings, CD68 has been frequently utilized to identify cells of the monocyte/macrophage lineage. KP1 and PG-M1 are the most widely used monoclonal antibodies that identify the CD68 antigen in diagnostic pathology (Cho *et al*., 2013), also found the expression of CD68KP1 in white matter microglia and astrocytes (Cho *et al*., 2013). While its biological purpose is uncertain. CD68 serves as a scavenger receptor for oxidized low densitylipoprotein and may be associated with cell communication (Kunisch *et al*., 2004).

Our goal was to investigate age-related differences in brain sections that were treated with the Black and Red GSE analyzing glial and neuronal cell numbers with their nucleus size by expression of S100 and CD68Kp1 and how they impact in delay aging and improving brain health.

MATERIALS AND METHODS

Grape seed sampling and extraction: The procedure was completed using two types of grape which were Red and black *Vitis vinifera*. The vine leaves were physically taken from sulaimani (Kurdistan Region) in the area of Sharbazher. This manual procedure was done in middle of July. The method of preserve used was open sun drying followed by using an electrical machine to grind it into a powder form.

The powder was stored before its use in freezing temperature (-20ºC). Using a 500mL conical flask, 72.2g of red grapes and 40.73g of black grape seed powder was mixed with 202mL and 114mL of 70% aqueous acetone solution. The mixture was placed onto a magnetic stirrer for 24 hours at room temperature. Using a Buchner funnel, the product was filtered. Acetone was then extracted using a rotatory evaporator. Finally, product was freeze dried for 24 hours to remove the water (Hassan *et al*., 2021).

GC-MS analysis: The chemicals found in purified samples of red and black grape seed (*Vitis vinifera*) from Sulaimania (Sharbazhear-Kurdistan region) were determined using gas chromatography-mass spectrometry (GC-MS). Total polyphenol accounted for >90% of net dry weight, as previously stated (Hassan *et al*., 2021).

Animals model and experimental design: Thirty healthy and normal aged (10 weeks) *BALB/c* strain mice were simply classified into five groups (6 albino mice per group, at approximately 30-35 gm each mouse);
Group, I (aged group) was fed on a balanced diet and was used as a control negative.
Group II was administered 200 mg/kg of Black GSE.
Group III was administered 400 mg/kg of Black GSE.
Group IV was given 200 mg/kg of Red GSE.
Group V was given 400 mg/kg of Red GSE.

All of the animals were purchased from the Animal House of the College of Veterinary Medicine in Slemani/ Iraq and were housed under lighting control (light and dark phases for 12 hours each) at a temperature of 25 degrees Celsius and 60% humidity. The current study was done with the local animal ethics commission's permission. The rules for the care and use of laboratory animals issued by the College of Veterinary Medicine at the University of Sulaimnai in Iraq were followed in all protocols (01599). Every two days, all treatments were given as a single daily dose via oral gavage, with a free treatment on the third day, and the trial lasted roughly four weeks.

Morphometric and immunohistochemical analyses: The brains were sliced and fixed for 12 hours in a 10% paraformaldehyde solution in 0.1 M phosphate buffer (PB, pH 7.0-7.5) before being rinsed in PB. Serial sections of 4µm thickness were produced after the tissues were soaked in paraffin wax. To detect brain abnormalities, hematoxylin and eosin stains were utilized.

For morphometric evaluation and immunohisto-chemistry research, paraffin-embedded slides were dewaxed in xylene and hydrated. Primary S100B and CD68KP1 antibodies were recovered by heating tissue sections in a microwave oven for 20 minutes in 10 mM sodium citrate buffer (pH 6.0), then cooling the sections in

de-ionized water. Incubating the slices in 3 percent hydrogen peroxide for 5 minutes decreased endogenous peroxidase activity. Primary S100B and CD68KP1 antibodies were incubated overnight at 4ºC with S100 rabbit polyclonal antibody (1:600; DAKO, Denmark) and CD68KP1 rabbit polyclonal antibody (1:100; DAKO, Denmark). Staining was detected using biotin-labeled anti-rabbit secondary antibodies and streptavidin linked to horseradish peroxidase, as directed by the manufacturer (DAKO Cytomation, USA). To see the reaction products, diaminobenzidine was utilized (Sigma-Aldrich Co., St. Louis, MO, USA). The Cytoplasm-Nuclei were stained with S100B and CD68KP1. To evaluate slices under a microscope, computer-assisted image analysis software (Motic, Japan) was employed (Am Scope Version 2.5 software, Japan). From each brain segment (the cerebellum and cerebrum), 100 µm regions with 400X magnification, depending on the size of the area were taken and examined under a microscope. S100 and CD68pk1 expression were utilized to determine mean values for cell number and nucleus size for each type of cell, including astrocytes, microglia, and oligodendrocytes with small and large neurons.

Statistical analysis: IBM SPSS Statistics 22.0 was used to conduct statistical tests (IBM Corp., Armonk, NY). Student's t-tests were used to make comparisons between two groups. The mean SE was used to present all of the data. Statistical significance was defined as a value of $P<0.05$.

RESULTS

Glial Cell detection: Three cell populations, astrocytes, microglia, and oligodendrocytes were identified in the series of brain tissue sections stained for S100 specifically and CD68kp1 immunoreactivity.

Effect of Black and Red grape seed extract (GSE) on the Glial cells in the brain: Brain section expressed S100 and CD68kp1 significantly in large numbers of glial and neuron in groups that treated with Black and Red GSE with different intensity as seen in Fig. 1b and 2b, respectively as compared to aged groups that were immunopositive cells (S100 and CD68kp1) markedly-weaky expressed in few numbers of associated cells (Fig. 1a and 2a, respectively).

1- Astrocytes: Astrocytes remain intact with normal histological features; seen as star-shaped with oval nuclei that had numerous processes extending from them in all treated groups of Black and Red GSE, while abnormal cells detected in the aged group, astrocytes became smaller, with thicker and shorter branches.

The mean number of astrocytes significantly increased, while the intensity of S100 immunostaining was reduced in all treated groups concerning the aged group (Table 1 and Fig. 3), especially in the groups that treated by 400mg/kg Black GSE (5.66±0.61, P=0.01) and 400mg/kg Red GSE (5.33±0.42, P=0.009) as in figure 3b and 3c respectively in comparison to the aged group that showed strong S100 expression in few numbers of astrocytes (2.00±0.44, Fig. 3a).

2- Microglia: In all treated groups microglia were showed normal morphology; small, exhibit elongated-irregular nuclei with little cytoplasm. While aged microglia appeared relatively enlarged and had less cytoplasmic branching that altered its features.

Large numbers of microglia significantly expressed weak-moderate stained S100 in all groups of GSE more definitely in the high dose treated groups, for example, the expression for 400mg/kg Black GSE (10.83±0.40, *P=0.000*), while (10.16±0.54, *P=0.000*) for 400mg/kg Red GSE as in figure 4b and 4c respectively, in contrast to the aged group that reached only (2.50±0.50) strongly stained cells (Fig. 4a).

Additionally, in table 2, we found a high number of microglia that expressed strong CD68kp1 staining in the brain of mice with 400mg/kg Black GSE (11.66±0.80, *P=0.000*), while (11.16±0.54, *P=0.000*) for 400mg/kg Red GSE as in figure 4e and 4f correspondingly, relative to the age-matched group that reached only (1.66±0.21) weak-moderate stained cells (Fig. 4d).

3- Oligodendrocyte: In GSE-treated groups, normal oligodendrocytes had tiny nuclei surrounded by cytoplasm rings. They had long cytoplasmic projections extending from the soma, while older cells have dense inclusions in the cytoplasm of cell bodies and swellings along with their processes.

There was a noticeable increase in the number of oligodendrocytes that expressed S100 with weak immunostaining in the 400mg/kg Black GSE (1.83±0.16, P=0.001) as in Fig. 5b, and 400mg/kg Red GSE (1.50±0.22, P=0.01) as in figure 5c about the aged group that extended simply (0.50±0.22) with strong immunostaining (Fig. 5a and Table 1).

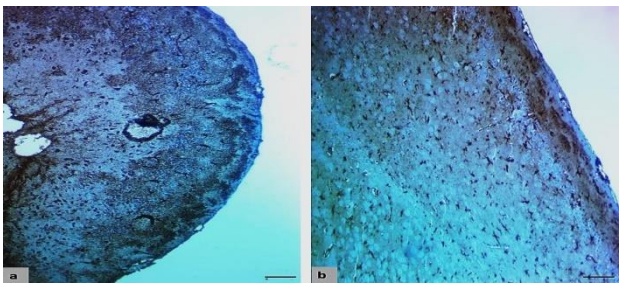

Fig. 1: Immunopositive S100 expression in brain section; a: Strong stained few cells in aged group and b: Weak-moderate stained large number of cells in the treated group, (50 scale bar).

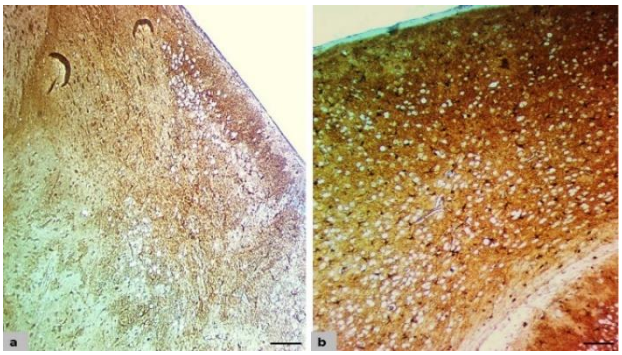

Fig. 2: Immunopositive CD68kp1 expression in brain section; a: Weak-moderate immunopositive few microglia and neuron in aged group and b: Strong stained large microglia and neuron in treated group, (50 scale bar).

Effect of Black and Red grape seed extract (GSE) on the neuron in the brain

1- Small neuron: Small neurons appeared normal as clear cell bodies with a nucleus and prominent axon in Black and Red GSE groups, while the aged small neuron had a small cell body with absence-short unclear axon.

Significant elevation in several small-sized neurons with weak immune positive S100 expression recorded in the 400mg/kg of Black GSE (12.50±0.71, P=0.005) as in Fig. 6b, and Red GSE (10.66±0.66, P=0.01) as in figure 6c, in comparison to the aged group that showed few numbers of strong immunostaining cells (6.33±0.61) as in figure 6a.

Regarding CD68kp1 expression, a significant strong stain was found in a maximum number of small neurons in 400mg/kg of Black GSE (12.66±0.42, P=0.000) as in figure 6e, and Red GSE (11.33±0.42, P=0.000) as in figure 6f, in comparison to the aged group that showed few numbers of weak immunostaining cells (5.33±0.42) as in Fig. 6d and Table 2.

2- Large neuron: Large neurons were characterized by normal histology showed relatively large cell bodies, nuclei with single prominent nucleoli, and long axons in comparison to small neurons in treated GSE groups and more specifically about aged large neurons that showed small cell bodies with short axons.

A significant strong positive S100 was found largely in high dose treated groups that recorded raising in their number; Black GSE (8.00±0.89, P=0.005) as in Fig. 7b and Red GSE (6.33±0.61, P=0.002) as in Fig. 7c if compared to the aged group that expressed a small number of weak positive cells (2.66±0.42) as showed in Fig. 7a and table1.

Although a large number of neurons expressed strong stained CD68kp1 more specifically in 400mg/kg of Black GSE (8.33±0.61, P=0.000) as in Fig. 7e, and Red GSE (7.33±0.66, P=0.002) as in Fig. 7f, in contrast to the aged group that trend towards lower numbers of expressed weak positive cells (2.16±0.40) as in Fig. 7d and table 2.

Effect of Black and Red grape seed extract (GSE) on the nucleus size of glial and neuron in the brain: The brain section treated by Black and Red GSE significantly had an impact on age-related glial and neuron nucleus size (Table 3). Astrocyte nucleus did not alter in high dose Black (1.98±0.04, P=0.003) and Red (1.73±0.20, P=0.01) GSE about the aged group in which their nucleus reduced in their size significantly (0.90±0.12). The same effect of GSE on the microglial nucleus in comparison to the aged group (1.02±0.23), in 400mg/kg of Black (1.50±0.21, P=0.005) and 400mg/kg Red (1.24±0.09, P=0.01). Improvement in oligodendroctye nucleus size detected in treated GSE groups for both types for example; in 400mg/kg

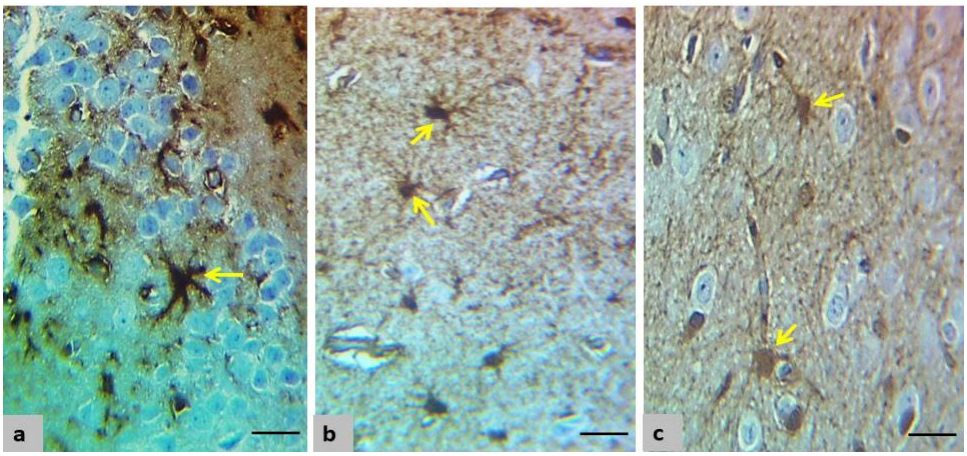

Fig. 3: Immunohistochemistry section in the brain for brown nucleus S100 expression in astrocytes. a; Strong intensity in aged group, b: Moderate intensity in 400 mg/kg Red GSE group, c: Weak intensity in 400 mg/kg Black GSE group (20 scale bar).

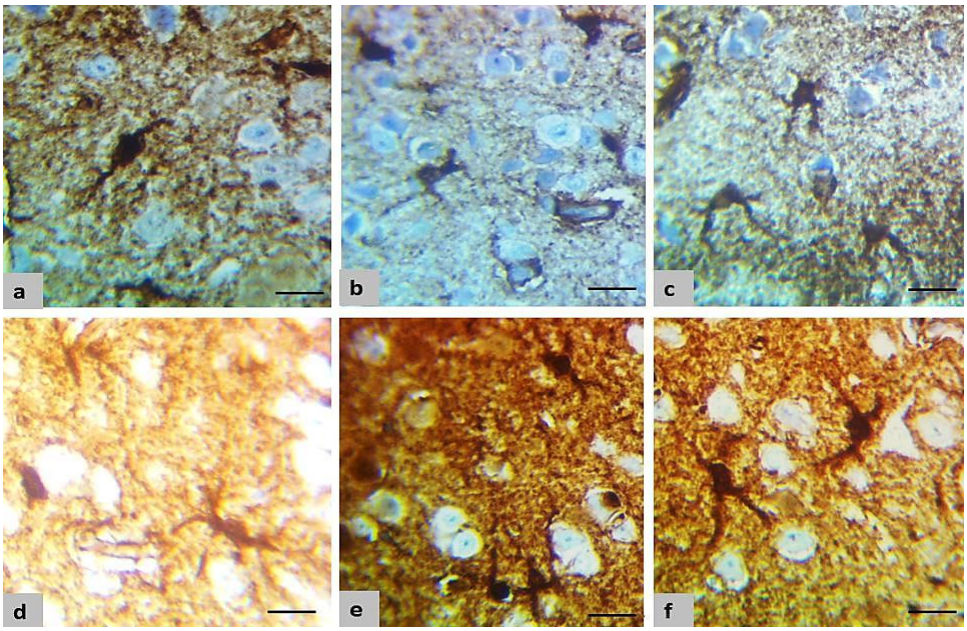

Fig. 4: Immunohistochemistry section in the brain for brown stained nucleus S100 and CD68kp1 expression in microglia. a; Strong stained S100 in aged group, b: Moderate-strong stained S100 in 400 mg/kg Red GSE group, c: Weak-moderate stained S100 in 400 mg/kg Black GSE group d; Weak-moderate expression of CD68kp1 in aged group, e: Moderate-strong expression of CD68kp1 in 400 mg/kg Red GSE group, f: Strong expression of CD68kp1 in 400 mg/kg Black GSE group (20 scale bar).

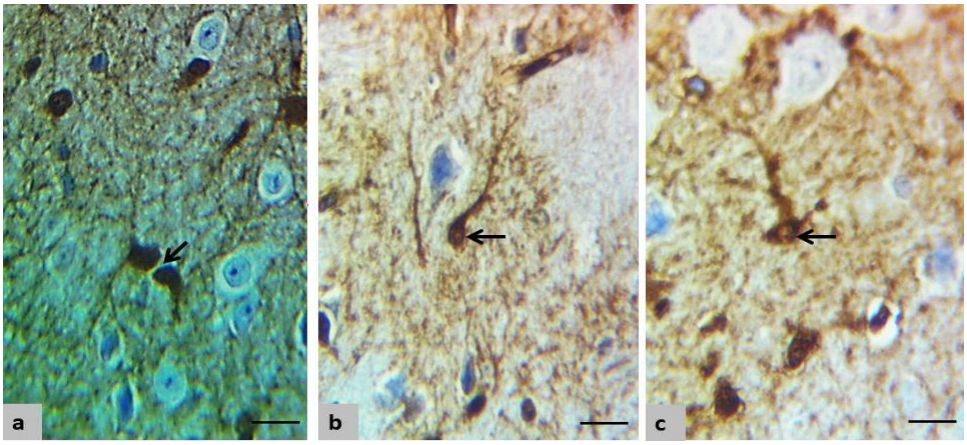

Fig. 5: Immunohistochemistry section in the brain for brown cytoplasmic-nucleus S100 expression in oligodendrocyte. a; Strong immunopositive in aged group, b: Moderate immunopositive in 400 mg/kg Red GSE group, c: Weak-moderate immunopositive in 400 mg/kg Black GSE group (20 scale bar).

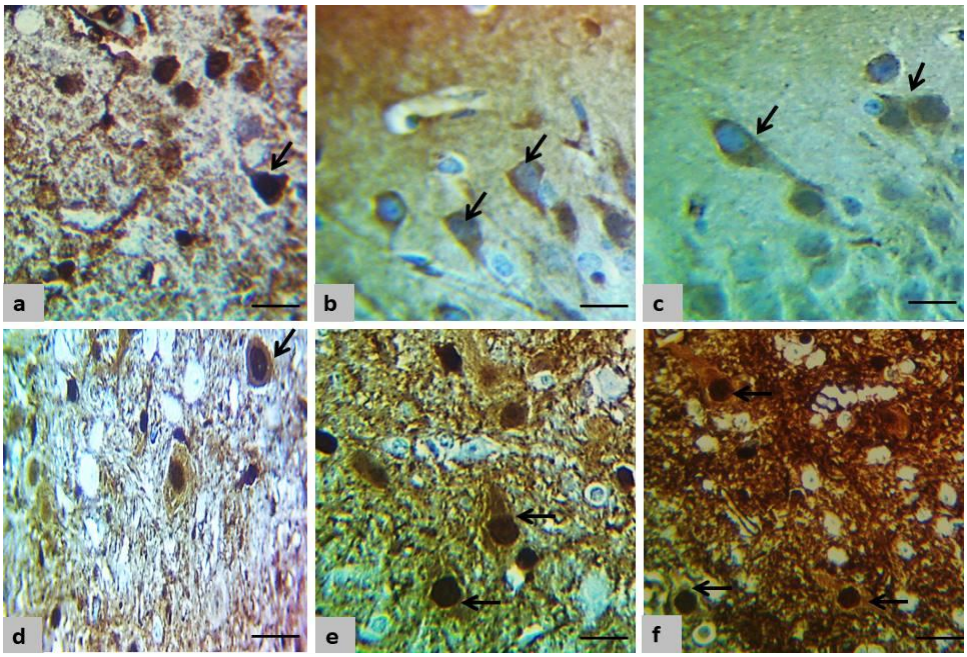

Fig. 6: Immunohistochemistry section in the brain for brown cytoplasmic-nucleus S100 and CD68kp1 expression in the small neuron. a; Strong cytoplasmic expression of S100 in aged group, b: Weak-moderate cytoplasmic expression of S100 in 400 mg/kg Red GSE group, c: Weak cytoplasmic expression of S100 in 400 mg/kg Black GSE group d; Weak-moderate cytoplasmic-nucleus expression of CD68kp1 in aged group, e: Moderate-strong cytoplasmic-nucleus expression of CD68kp1 in 400 mg/kg Red GSE group, f: Strong expression cytoplasmic-nucleus of CD68kp1 in 400 mg/kg Black GSE group (20 scale bar).

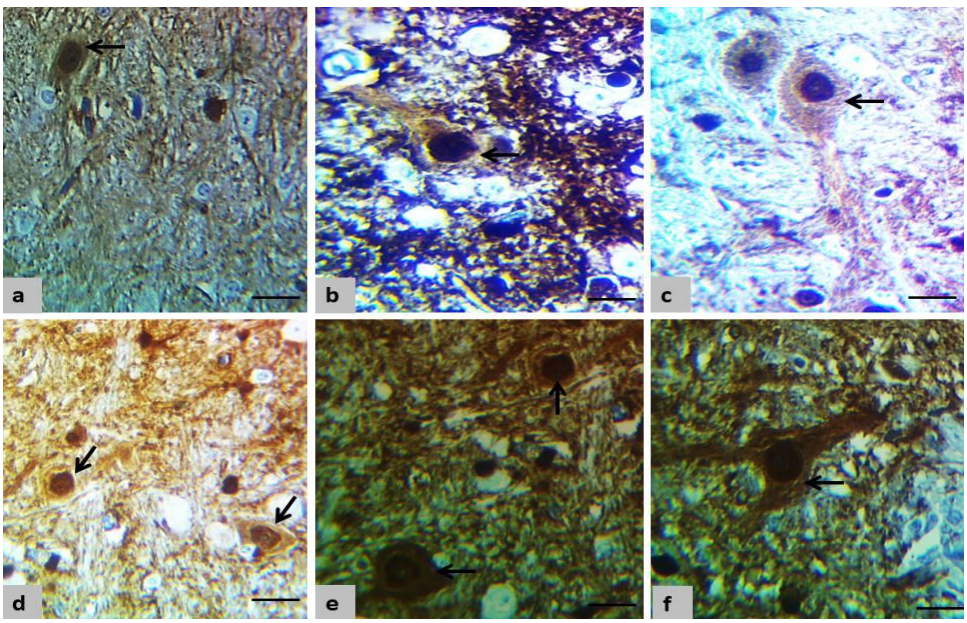

Fig. 7: Immunohistochemistry section of the brain for brown cytoplasmic-nucleus S100 and CD68kp1 expression in the large neuron. a; Strong cytoplasmic expression of S100 in aged group, b: Weak-moderate cytoplasmic expression of S100 in 400 mg/kg Red GSE group, c: Strong cytoplasmic expression of S100 in 400 mg/kg Black GSE group d; Weak-moderate cytoplasmic-nucleus expression of CD68kp1 in aged group, e: Moderate-strong cytoplasmic-nucleus expression of CD68kp1 in 400 mg/kg Red GSE group, f: Strong expression cytoplasmic-nucleus of CD68kp1 in 400 mg/kg Black GSE group (20 scale bar).

Table 1: Cell average at 100 µm of stained S100 glial and neuron cells in the brain of studied groups

Cell types	Aged group	200 mg/kg Black GSE	400 mg/kg Black GSE	200 mg/kg Red GSE	400 mg/kg Red GSE
Astrocytes	2.00±0.44	4.00±0.73	5.66±0.61	3.66±0.80	5.33±0.42
Microglia	2.50±0.50	6.33±0.61	10.83±0.40	5.33±0.84	10.16±0.54
Oligodendrocyte	0.50±0.22	1.33±0.21	1.83±0.16	1.00±0.25	1.50±0.22
Small sized neuron	6.33±0.61	11.00±0.44	12.50±0.71	10.00±0.51	10.66±0.66
Large sized neuron	2.66±0.42	6.00±0.51	8.00±0.89	5.33±0.42	6.33±0.61

The cell number is expressed as mean ± standard error. Within a row, the mean values vary from each other (P<0.05).

Table 2: Cell average at 100 µm of CD68kp1 stained microglia and neuron cells in the brain in studied groups.

Cell types	Aged group	200 mg/kg Black GSE	400 mg/kg Black GSE	200 mg/kg Red GSE	400 mg/kg Red GSE
Microglia	1.66±0.21	6.66±0.42	11.66±0.80	4.66±0.98	11.16±0.54
Small sized neuron	5.33±0.42	10.83±0.65	12.66±0.42	9.33±0.42	11.33±0.42
Large sized neuron	2.16±0.40	6.66±0.42	8.33±0.61	5.00±0.44	7.33±0.66

The cell number is expressed as mean ± standard error. Within a row, the mean values vary from each other (P<0.05).

Table 3: Nucleus size (µm) of each cell type in the brain of a different group.

Nucleus size	Aged group	200 mg/kg Black GSE	400 mg/kg Black GSE	200 mg/kg Red GSE	400 mg/kg Red GSE
Astrocytes	0.90±0.12	1.46±0.06	1.98±0.04	1.56±0.16	1.73±0.20
Microglia	1.03±0.23	1.47±0.19	1.50±0.21	1.51±0.31	1.24±0.09
Oligodendrocyte	0.86±0.15	1.09±0.14	1.45±0.14	0.94±0.15	1.39±0.10
Small sized neuron	1.69±0.19	2.00±0.25	1.98±0.25	1.80±0.22	1.65±0.24
Large sized neuron	2.41±0.18	3.53±0.24	3.70±0.26	3.30±0.27	3.55±0.31

The means of cell nucleus size are expressed as mean ± standard error. Within a row, the mean values vary from each other (P<0.05).

Black (1.45±0.14, P=0.01) and 400mg/kg Red (1.39±0.10, P=0.01) in contrast to the aged group in which its nucleus size declined (0.86±0.15). GSE could significantly improve neurons' size nucleus typically about the aged groups were significantly reduced in their nucleus size as seen in table 3 for instance; in small neuron for 400mg/kg Black and Red GSE (1.98±0.25 and 1.65±0.24, P=0.4) respectively, while in an aged group (1.69±0.19). However, for large neurons, the 400mg/kg Black GSE was (3.70±0.26, P=0.005) and 400mg/kg Red (3.55±0.31, P=0.01) GSE concerning the aged group was (2.41±0.18).

DISCUSSION

Several neurobiological changes accompany aging, all of which are linked to cognitive impairment. The term "inflammaging" was recently coined to describe the process of causing inflammation (Franceschi, 2007). During normal and pathological aging, the brain undergoes a variety of electrophysiological, structural, and morphological changes, all of which have a role in memory formation (Allen and Barres, 2009). In this work, we compared the number and nucleus size of neurons, microglia, astrocytes, and oligodendrocytes in the same region of the brain between old and Black with Red GSE treated groups during normal aging processes.

Astrocytes are important regulators of neuronal activities that are impaired in older brains, such as synaptic plasticity regulation and being a part of the blood-brain barrier structure (Matias *et al*., 2019). Our findings suggest that in older mice, S100 protein is highly expressed in astrocytes which are less in number (2.00±0.44). Additionally, astrocytes have distorted morphologies, becoming fewer, smaller, with thicker and shorter branches, or lower complexity, as seen in other study (Robillard *et al*., 2016). Because astrocytes adapt to brain aging by modifying their morphology as well as their gene expression, according to a prior study (Ferrer, 2017). From the Lam *et al.* (2001), astrocyte cell number does not change much with age, which is consistent with our findings (Lam *et al*., 2001). As revealed in previous work, high S100 expression in astrocytes is one of the pathogenic features of brain injury (Boom *et al*., 2004). Overexpression of this glial actuation marker in astrocytes advances the creation of proinflammatory cytokines, which might have a significant influence on neurodegenerative sickness, as per the past investigation (Mori *et al*., 2010). While S100 was expressed in large numbers with weak-moderate staining in 400mg/kg of Black and Red GSE groups (5.66±0.61 and 5.33±0.42, respectively) with normal histological features, the explanation for this result documented that oral administration of GSE had an impact on delayed aging features by saving glial morphology and proliferation or number, GSE's antioxidant capacity is connected to its immunomodulatory and anti-inflammatory characteristics, and polyphenols have demonstrated its adequate protection against age-related oxidative stress (Safwen *et al*., 2015).

Microglia are the brain's indigenous immune cells, accounting for 10% of all brain cells (Crotti and Ransohoff, 2016). The complexity of microglia and their varied activities, both pathogenic and reparative, are now better recognized (Wolf *et al*., 2017). We found that in the aged brain mice increased S100 expression was accompanied by a decreased number of microglia (2.50±0.50) that had abnormal histological features including enlarged and less cytoplasmic branching which is consistent with previous study (Hefendehl *et al*., 2014), According to some scientists, older mice have a higher amount of cortical microglia (Tremblay *et al*., 2012), others found no variations in the quantity of microglia in the hippocampus of young and aged rats (VanGuilder *et al*., 2011). Our findings revealed a high level of S100 in microglia, which is consistent with previous research (McQuade and Blurton-Jones, 2019). S100B drives microglia activation via a cytokine cycle that can cause harm by inducing inducible nitric oxide synthase upregulation and subsequent nitric oxide production, as well as stimulating NF-B in the inflammatory response, according to the study (Lam *et al*., 2001). Microglia are known for their capability to react to age-related neurodegenerative sicknesses like Parkinson's and Alzheimer's illness (McQuade and Blurton-Jones, 2019). While microglia's number increased in both treated groups by (10.83±0.40 and 10.16±0.54, respectively in Black and Red GSE groups) and expressed weak-moderate S100 immunopositive cells. This indicates that GSE reduced S100 protein expression and also had a reparative role on microglia, enhancing brain immunity, and delaying aging, which is consistent with our findings. As demonstrated by prior findings (Moosavi *et al*., 2016). Regular dosing of grape-derived polyphenolic extracts reduces neurodegeneration. Polyphenols, for instance, have been demonstrated to have outstanding anti-oxidant capabilities (Schaffer *et al*., 2016). Furthermore, it is highly linked to improved neurocognitive function in middle-aged and elderly people who are either

experiencing normal aging or are at risk for neurodegenerative illnesses (Kesse-Guyot *et al*., 2012).

The current study showed weak expression of CD68kp1 in microglia of the aged group in few numbers of the cell (1.66±0.21), CD68kp1 expression in the normal brain's microglia has been studied (Mittelbronn *et al*., 2001). In contrast to our findings, Perry *et al*. (1993) discovered an increase in CD68 cells across the parenchyma in both grey and white matter in the aged rat brain (Perry *et al*., 1993). To the best of the knowledge, our study is the only new result that demonstrated expression of CD68kp1 in the aged brain, while in both GSE treated groups showed the high intensity of CD68kp1 expression (11.66±0.80 and 11.16±0.54 for black and red GSE respectively), Microglia elevated CD68 in response to tissue injury, according to Fischer-Smith *et al*. (2004), since ageing plays a significant role in tissue injury (Fischer-Smith *et al*., 2004). Because CD68 kp1 is a member of the scavenger receptors, GSE increases the expression of CD68kp1 and the number of microglia due to its polyphenol content. GSE also expressed strong CD68 kp1 that plays an impact role in delaying aging and improving glial cells.

In the developing grown-up brain, oligodendrocyte antecedent cells (OPCs) bring about oligodendrocytes, the myelin-delivering cells. Grown-up oligodendrocyte antecedent cells (OPCs) stay in the brain, trusting that signals will form into new oligodendrocytes because of learning, injury, infection, or maturing (Bergles and Richardson, 2016). S100B is communicated by oligodendrocyte precursor cells (OPC) and is needed for oligodendrocyte separation and development into myelinating oligodendrocytes (OL) (Deloulme *et al*., 2004). S100B is also linked to microtubular structures in cultured OL, which are necessary for proper myelination (Lopresti, 2002). In our study, S100 was up-regulated but in a few remained oligodendrocytes (0.50±0.22), in agreement with other data in which needed for myelin production (Soreq *et al*., 2017). Other study, have discovered that the number of oligodendrocytes in the aging brain grows (Peters and Sethares, 2004). Also, the aged microglia altered morphology that contains dense inclusions in the cytoplasm of cell bodies and swellings along with their processes, following previous study (Peters, 2009). Excess S100B levels, on the other hand, halt oligodendrogenesis and OL maturation in vitro and prevent de novo myelination in an ex vivo model (Vickers, 2017). According to earlier research, the shape of old microglia has changed, with dense inclusions in the cytoplasm of cell bodies and swellings along with their processes (Santos *et al*., 2020). In comparison to brain sections that treated with Black and Red GSE in which S100 weakly expressed in the large numbers of oligodendrocyte (1.83±0.16 and1.50±0.22, in Black and Red GSE groups accordingly). We hypothesize that the observed differences in S100 protein levels and the number of positive oligodendrocytes are attributable to GSE, which normalized the expression of these genes, and prior research have revealed that GSE is a major source of polyphenols, which is compatible with our findings, Polyhydroxylated flavan-3-ols, in particular, are employed to regulate a variety of pathophysiological changes such as homeostasis, inflammation, and detoxification (Hasseeb *et al*., 2011), The flavonoids mediated attenuation of demyelination by inhibition of CNS inflammation, and oligomeric proanthocyanidin (OPC) is an effective component of GSE and part of a specific group of polyphenolic chemicals (Wang *et al*., 2019).

Both types of neurons (small and large, 6.33±0.61 and 2.66±0.42, correspondingly) in the aged mice expressed strong S100 immunopositive cells in reduced numbers of both types of neuron with abnormal histological structures. Overall, these findings suggest that apoptotic neurons are actively phagocytized throughout normal brain aging. These findings support the theory that S100B's neurotoxic effects in vitro are mediated through the stimulation of apoptosis in neurons (Sen and Belli, 2007), As noted in our findings, S100 is highly expressed in astrocytes and releases nitric oxide, which causes neuronal death (Donato, 2001). Our findings are also consistent with studies in which the expression of S100 proteins, which are all elevated by aging and neuronal injury, is upregulated (Cristóvão *et al*., 2016). Mice's brain treated with Black and Red GSE had a reduction in the intensity of S100 positive cells and act as neuroprotective, which increased the number of neurons with normal morphological features, we hypothesize that the polyphenol protects the neuron from being undergone atrophy therefore; the neuron did not decrease in comparison with the neuron in an aged group. As indicated by different studies, polyphenols apply neuroprotective impacts through a variety of mechanisms, including diminished neuroinflammation-initiated neural harm, changed ROS/RNS age, lessening of NFTs, and A-plaques testimony, and enactment of synaptic pliancy controlling flagging pathways (Balu *et al*., 2005). Direct consequences for flagging pathways to work on neuronal correspondence, changes in inflammatory gene expression, neuroprotection from excitotoxic stress, and a decrease in inflammatory mediators such as NF-B are some of the other strategies (Kaewkaen *et al*., 2012). In vitro study have demonstrated that grape extracts are excellent free radical scavengers (Bagchi *et al*., 2000), and prevent neurocytotoxicity in brain cells by lowering free radical production, protecting neuronal cells from oxidative stress and cellular DNA damage, and reducing neurocytotoxicity (Basli *et al*., 2012).

Regarding CD68kp1 expression in small and large neurons, the brain section in the aged group showed weak intensity in a few numbers of neurons (5.33±0.42 and 2.16±0.40 respectively for a small and large one), that showed abnormality in neuron histological structures. When we compared CD68-kp1 positive cell to aged group, in Black and Red GSE the CD68kp1expressed strongly in a large number of neurons, this finding suggested that GSE plays a neuroprotective and beneficial role in maintaining healthy neurons and slowing the aging of the brain also enhanced expression of CD68kp1 that acts as a scavenger receptor. Our findings are consistent with earlier research that suggests polyphenolics may be useful in slowing the progression of neuronal aging. They can protect organs and tissues from oxidative damage and modify the body's negative redox state process due to their remarkable antioxidant activity, such as scavenging free radicals. Improvements in dopamine release from striatal slices, as well as cognitive ability, were seen when

an elderly rat was given 10% grape juice (Shukitt-Hale *et al*., 2006). Supplementing with grape seed extracts (100 mg/kg b.wt.) for 30 days prevented the buildup of age-related oxidative DNA damages in brain tissue, according to further research (Balu *et al*., 2006).

Conclusions: We believe it is acceptable to conclude that;

- S100B protein is a good biomarker for astrocytes, microglia, oligodendrocytes, and neurons in older people.
- CD68pk1 candidate as a microglia and neuron diagnostic marker in the elderly brain
- GSE could be used as a therapeutic intervention to slow down the aging of the brain by raising glial and neuron numbers and conserving their shape.
- GSE demonstrated neuroprotective effects by lowering S100 adverse effects and increasing CD68pk1.
- However, additional research is needed to determine the most effective medicinal extracts and chemicals for brain aging.

Acknowledgment: This paper financially did not support by any sponsor.

Authors contribution: SN Hassan, wrote the paper checked the whole procedure and paper. SMA Hassan, supervised, conceived the original idea, did the research and analyzed the data, also wrote the paper and checked the Histopathological utilization and paper. N Saleh, performed the G-c mass test. NNN Maarof, checked the whole procedure and paper. All authors have read and approved the paper.

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