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Neuromodulatory influences of vitamin C on D-galactose induced neuronal metabolic dysfunction model

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ABSTRACT

Aging is a physiological, non-pathological process that occurs mostly as passive, gradual and slow procedure affected by interplay of frequent environmental and genetic influences. Different ways have been tried to postponement aging and extent life span in animals; dietary antioxidants are one of them. Depending on the metabolic and free radical theories of aging, this work was carried out to evaluate the use of vitamin C in male rats, we used tree groups, control which received no treatment, experimental group which received the aging promoter agent and then subdivided into two groups one of which received Vitamin C while the other left. This study was carried out shedding the light on aging mechanisms underlying the anti-aging variations on both the molecular and the physiological level. Aging was enhanced by injecting rats subcutaneously with D galactose in a dose of 100 mg for 8 weeks brain tissues were subjected to analysis determining the level of serotonin, dopamine and nor epinephrine. D galactose caused down regulation of both serotonin, dopamine and BCL2, while nor epinephrine and BAX went up, histopathological, d-galactose caused severe damage in brain tissues such alteration has been resisted in rats administrated vitamin C. Thus, we can conclude that antioxidants could prevent the age-related brain deterioration.

1. INTRODUCTION

Aging has been targeted by dietary management, drugs and antioxidants, to extend lifespan and promote healthy aging. The National Institute of Aging has studied plentiful treatments, including antioxidants and diets, with the ability to lengthen life spans and postpone illness and dysfunction in animals. The beneficial effect of lifestyle on ageing met these rigorous requirements from a pharmaceutical point of view: dietary antioxidants and exercise (Omayma et al., 2021). Aging has been studied by many scientists in the last 200 years; the cause why we age? the time when we start aging? What are the aging indicators, is there a boundary to how old can we live? Many theories have been planned to elucidate the procedure of aging, organ theories of aging are grounded on the common finding that as we age, increments or decrements occur in organ functions. The organs currently most preferred as the likely ultimate origin of all age alterations are the immune system and the brain or neuroendocrine system (Straub 2017). D-galactose is an aldohexose, which is a reducing sugar naturally occurring in the body and in many dietary foods such as butter milk, yogurt, cheese, honey, beets, figs, plums, cherries, and celery (Azman et al., 2019) it is metabolized then excreted out the body within 8 h after oral administration (Bai et al., 2019). But, at high levels, it can be converted into aldose and hydroperoxide under the effect of galactose oxidase, resulting in the liberation of reactive oxygen species (ROS) (Homolak et al., 2021). Increased ROS may consequently result in inflammation, oxidative stress and mitochondrial dysfunction, and apoptosis (Kim et al. 2018). The free radical theory of aging is mostly back to free radical reaction injury. (Cadet and Davies 2017). Free radicals are molecules or atoms carrying an unpaired electron. Free radicals are normally highly reactive due to the affinity of electrons to pair. Furthermore, because the reaction of a free radical with a stable atom produces another radical, these actions result in multiple reactions in which a solitary free radical twitch a procedure which consumes many stable molecules. (Koltover 2017). Vitamin C amounts in tissues is vital for normal body function in addition to optimal health (Michels et al., 2013). Ascorbate is considered as a cofactor in many enzymatic reactions, including collagen production that, when D-galactose dysfunctional, reasons scurvy, though scurvy is considered a rare illness now, epidemiological studies recommend that large populations (between 5% and 30%) are be diagnosed with hypovitaminosis C (Gabbay et al., 2010). However, a hypovitaminosis C condition may not lead to scurvy, it places the individual in higher danger for metabolic abnormalities, cardiovascular diseases and malignancy (Frikke and Lykkesfeldt 2009). Also, it has newly been detected that ascorbate stops stress-induced harm on the brain through the decline of reactive oxygen species production in Gulo-/- mice depleted of ascorbate (Kim et al., 2013). Starting from the previously discussed theories of aging, and with the aid of age promoter D-galactose .this work was carried out to evaluate the anti-aging effect of Vitamin C, spotting the light on the age changes and deterioration in addition to the possible correction of the case using antioxidants

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2. MATERIAL AND METHODS

2.1. Chemicals

D-galactose was obtained in the form of powder from Medicinal Biochemistry Department, Faculty of Pharmacy, Assuit University.

Vitamin C: was obtained in the form of solution from Unipharm medical industrial company.

2.2. Animals:

The present study was carried out using thirty adult male albino rats (*Sprague Dawley strain*) weighing 200- 250 g and of 8 weeks age and obtained from the Nil company for pharmaceutical and chemical industries (Cairo-Egypt). Rats were housed in metal cages, ten rats per cage, under natural environmental laboratory conditions and diurnal cycle with free access water. All the ethical protocols for animal treatment were followed and supervised by the animal house, Faculty of Veterinary Medicine, Benha University.

2.3. Experimental design.

Rats were divided into two groups: 1- Control group: it consists of 10 rats and act as normal control. 2-Group (2): consists of 20 rats which injected subcutaneously with 50% D galactose at a dose of (100 gm/kg/d/8 week) (*Tao Tang and Bixiu He., 2013*). This group was divided into two subgroups (10 each). Subgroup (1): rats did not receive any treatment. Subgroup (b): rats will receive vitamin C at a dose of (150 mg/kg/d/6 week). At the end of experiment. Blood Samples were collected after overnight fasting from all animals blood was allowed to coagulate and centrifuged at 3000 rpm for 15 min. serum were aspirated and kept in Eppendorf till biochemical estimation then rats were sacrificed by cervical dislocation to collect brain.

2.4. Biochemical investigation:

was carried out by a fluorometric method according to method of ***(Liu et al., 2020)*** in addition to detection of BCL2 and BAX by ELISA technique. (**Guo and Li 2018**)

2.5. Histopathological results

Brain kept in formalin 10%. Sections from the brain were examined to evaluate the damage occurring due to D galactose and the possible modulatory effect of vitamin C. Stains 1-Haematoxylin and Eosin stain (***Bancroft and Gamble, 2008***). Method 1. The deparaffinization of the sections were done in xylol. Hydration was done in descending grades of alcohol. 2. The sections were stained in haematoxylin for ten minutes. 3. Washing of excess blue color under running tap water for five minutes. 4. Dryness was done on hot plate at 40°C until tissue appeared dried and stretched. 5. Counterstain in 1% solution of eosin for ten minutes was done. 6. Washing excess color under running tap water was carried out 7. Dehydration in ascending grades of alcohol, clearing in xylol and mounting were performed.

2.6. Statistical analysis.

The SPSS version 20 was used in data analysis. Data were analyzed using one-way analysis of variance followed by Duncan's multiple range test. A value of $p < .05$ was considered to indicate significance. The data were expressed as mean ± standard error.

3. RESULTS

Table (1) demonstrates the effect of vitamin C administration on Serotonin, Dopamine, Nor Epinephrine, BCL2 and BAX level in rats, D-galactose caused significant decrease in serotonin, dopamine and BCL2 while nor epinephrine and BAX increased. In Vitamin C group, the antioxidant resulted in significant increase in serotonin, dopamine and BCL2 while the levels of nor epinephrine and BAX decreased in comparison to the D galactose group.

Histopathological examination:

Figure (1) control group with normal pyramidal cells and different types of the neuralgia cells were scattered inside the neurotic matrix while figure (2) shows diffuse gliosis in the cerebral cortex due to the aging enhancer agent, on the other hand the group which fed vitamin C showed Few dark shrunken neurons with slight perineuronal edema and slight necrosis as shown in figure (3).

Table 1 Effect of vitamin C administration on serum Serotonin, Dopamine, Nor Epinephrine, BCL2 and BAX level in aging induced experimentally in rats.

		Control	D-galactose	Vitamin C
Serotonin	(ng/ml)	145.95±2.35	60.8±2.4$^{(a\downarrow)}$	108.05±1.75$^{(a\downarrow,b\uparrow)}$
Dopamine	(ng/ml)	65.9 ± 3.4	27 ± 4.6$^{(a\downarrow)}$	44.5 ± 2.4$^{(a\downarrow,b\uparrow)}$
Nor epinephrine	(ng/ml)	14.15±1.35	37.45±1.75$^{(a\uparrow)}$	26.55±2.35$^{(a\uparrow,b\downarrow)}$
BCL2	(pg/ml)	1.005 ± .005	.22 ± .01$^{(a\downarrow)}$	.72 ± .01$^{(a\downarrow,b\uparrow)}$
BAX	(pg/ml)	1.04±.02	6.7± .2$^{(a\uparrow)}$	2.91±.11$^{(a\uparrow,b\downarrow)}$

Data are presented as (Mean ± S.E). Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$). (a↑)= significant increase from control group. (a↓)= significant decrease from control group. (b↑)= significant increase from D-galactose group. (b↓)= significant decrease from D-galactose group

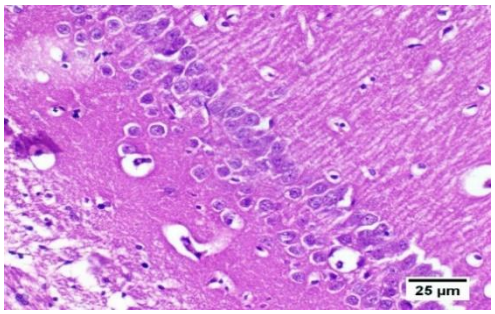

Figure 1 Photomicrograph of Brain of rat from group 1 showing apparently normal neuron in the hippocampus (H & E).

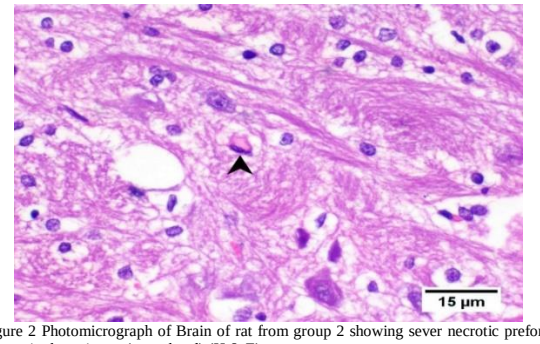

Figure 2 Photomicrograph of Brain of rat from group 2 showing sever necrotic preform neuron in the striatum (arrowhead) (H & E).

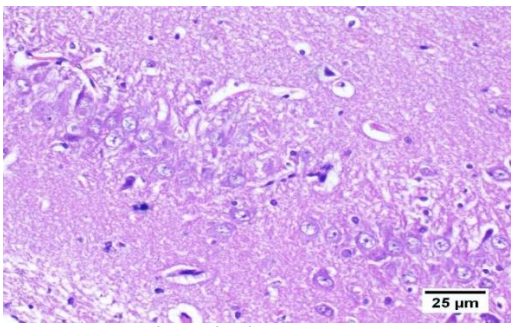

Figure 3 Photomicrograph of Brain of rat from group 3 showing slight decrease density of the neurons in the hippocampus (H & E).

4. DISCUSSION

Aging is an advanced increase of changes during time that are connected to the ever-increasing predisposition to sicknesses and death which attends to advancing age. These time-related variations are attributed to the aging procedure. The nature of the aging procedure has been the subject of considered theory (Golubev et al., 2018). According to (Omayma et al., 2020), oxidative chemicals result in alteration in nucleic acid repair system leading to excessive production of reactive oxygen species. rat models of accelerated aging indicate a good role of ascorbic acid in early aging. Supplementation of vitamin C appeared to pause cell growth, telomere erosion (decrease telomere In the present study, vitamin C showed antioxidant effect against D-galactose administration in rat, this is agree with (Chen et al., 2018) who found that antioxidants can increase life span. Additionally, administration of vitamin C led to antioxidant and anti-aging properties in rats this could be proved by the resistance of D galactose effect on nor epinephrine, this is agreed with (Akolkar et al., 2017) who stated that vitamin C can prolong the life span of rats through controlling this neurotransmitter. Serotonin plunges by aging, and thus deterioration in all cognitive function occurs, administration of antioxidant in ongoing search could improve the deterioration caused by D galactose, this is agreed with (Pullar et al., 2018) who noticed that mode is corrected by elevating vitamin C content due to increasing of serotonin in rat brains. Dopamine, in our study, was affected significantly by D-galactose administration as shown in table 1, this decrease is minimized in group treated with antioxidants, and such results are confirmed by another study carried by (Habtemariam2019) who stated that antioxidants can improve the cognitive function caused by D galactose. Aging is a complex process and require many ways to control or slow down the procedure, dietary antioxidants is one of them, so the current study tested the effect of vitamin C on enhanced aged rats using D galactose model. accumulating evidence proposes that administration of D galactose is associated decreasing the antioxidant content of the cell which is one of the most common signs of aging. Our results showed that vitamin C administration enhanced the deterioration in dopamine and serotonin caused by D galactose while norepinephrine levels was controlled. D galactose caused elevation in BAX and decreases in BCL2 in the ongoing trial while administration of Vitamin C caused correction to such levels, this is in approval with (Shahroudi et al., 2017) who stated that aging stimulation by D-galactose is scored by BAX increase and drop in BCL2.The balance between the pro and the anti-apoptotic proteins of the Bcl2 family is important in apoptosis progression (Lovato et al., 2020). Therefore, the Bax/Bcl2 ratio is a crucial predictor of apoptosis (Bai et al., 2020). Analyses of the proteins involved in apoptosis pathways (Bax and Bcl2) showed that the administration of D-galactose increased the Bax and decreased Bcl2 and this is a prove that apoptosis occurred in the tissues. (Wang et al., 2020). Morphologically, rats injected by D galactose showed severe changes in brain tissue cells noticed as necrotic areas as compared to control group. In similar study (Kalaz et al., 2014) reported that rats injected by D galactose showed similar effects on rat while vitamin C administration in group 3 corrected the state of histological findings and counterattacked the harmful effect of D-galactose.

length), disorganization of chromatin, oxidative stress and excessive secretion of inflammatory mediators, and thus extend lifespan (Monacelli et al., 2017) ascorbic acid (AA) was also proved to positively change inflamm-aging, one of the most important marks of biological aging. Moreover, vitamin C has been shown to epigenetically normalize genome stability and integrity showing a key role of nutrition during healthy adolescence (Pangrazzi et al., 2017). Antioxidants dietary materials, including vitamin C diminish oxidative stress made by oxidizing agents in the human body. So, these compounds can be employed to treat patients with diseases related to aging (Su KY et al., 2014).

5. CONCLUSION

From the results obtained from our study and discussed above, we can conclude that vitamin C administration could delay the aging accompanying deterioration in cognitive function through enhancing the brain neurotransmissions.

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