Antioxidant Activities of *Dichrocephala integrifolia* (Linn.f.) O. Kuntze (Asteraceae) in a Mice Model of D-Galactose-Induced Oxidative Stress and Accelerated Aging

Kouémou Émégam Nadège¹,²,a*, Fotio Lambou Agathe¹,b, Dongmo Mireille Sylviane³,c, Ouao Agnès Carolle⁴,d, Palé Simon¹,²,e, Kandeda Kavaye Antoine⁵,f, Taiwe Sotoing Germain¹,g, Ngo Bum Elisabeth²,⁶,h

¹Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon
²Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, Ngaoundéré, Cameroon
³Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Buea, Cameroon
⁴Department of Biological Sciences, University of Bamenda, Bamenda, Cameroon
⁵Department of Biology and Animal Physiology, University of Yaoundé I, Yaoundé, Cameroon
⁶Institute of Mines and Petroleum Industries of Maroua, University of Maroua, Kaële, Cameroon

a nadegekouemou@yahoo.fr, b agyfotio@yahoo.fr, c syldong@yahoo.fr, d aguyouaf@yahoo.fr, 
*e simonpale@yahoo.fr, f kandedakavaye@yahoo.fr,
⁹ taiwe_sotoing@yahoo.fr, h eli_bum@yahoo.fr

Keywords: *Dichrocephala integrifolia*, Antioxidant, Aging, D-galactose, Alzheimer’s disease

Abstract. *Dichrocephala integrifolia* is a plant widely used in folk medicine in Africa to treat central nervous diseases such as dementia. In the present study, we evaluated the effect of the leaf’s decoction of *Dichrocephala integrifolia* against D-galactose-induced neurotoxicity, oxidative stress and accelerated aging in mice. D-galactose (100 mg/ kg sc), was chronically injected daily to mice during 42 consecutive days after pretreatment with distilled water (10 ml/kg) or the decoction of *D. integrifolia* (35; 87.5; 175 or 350 mg/kg p.o) or vitamin C (100 mg/kg p.o). Following behavioral tests (Open Field, Elevated Plus Maze and Morris Water Maze), animals were sacrificed on day 43 and their brains were used to evaluate some biochemical parameters of oxidative stress (malondialdehyde, nitrite oxide and reduced glutathione) and for histopathological assessments. The results of this study showed that a pretreatment of animals with the decoction of *D. integrifolia* at the doses of 87.5 and 175 mg/kg significantly (p<0.05) reversed learning deficits, recall of memories and oxidative stress induced by D-galactose. The decoction of *D. integrifolia* also prevented neurogeneration in the dentate gyrus induced by D-galactose. These results indicated that *D. integrifolia* possesses neuroprotective effects against D-galactose-induced senescence, probably due to its antioxidant capacities and this can at least explain the wide use of this plant in traditional medicine in Cameroon in the prevention and treatment of dementia.

List of abbreviations: AD: Alzheimer’s disease; *D. integrifolia*: *Dichrocephala. integrifolia*; D-gal: D-galactose; MWM: Morris Water Maze; s.c.: sub cutaneous; p.o :per os
1. Introduction

Aging is a gradual and inevitable physiological process, which affects all living organisms. In humans, it is characterized by alterations in cellular homeostatic mechanisms leading to diverse health problems such as reduction in hearing and vision, increase in body weight, diabetes, hypertension and a range of central nervous disorders [1, 2]. The aging brain is characterized by a decrease in cortical gray matter, a reduction in neurotransmission, a massive loss of neuron and reduction of complexity of neuronal correlation. Alzheimer’s disease (AD), Parkinson’s disease and Huntington’s disease are among the most age related central nervous diseases [3]. Due to the increase life expectancy, age related central nervous disorders have become a major health concern. Although predetermined by genetics, different environmental factors can interfere with the normal process of aging [1]. There are cumulative evidences pointing to the involvement of oxidative stress in mitochondrial dysfunction, neuronal loss observed in the aging brain, and subsequent neurodegenerative diseases such as AD [3-6].

D-Galactose (D-gal) is a reducing sugar resulting from the hydrolysis of lactose (milk sugar) by intestinal lactase to produce glucose and galactose. Two enzymes normally metabolize D-gal in the intestinal tract: D-galactokinase and galactose-1-phosphate uridylyltransferase. In case of accumulation of D-gal in the body, due to its oversupply, D-gal is converted by galactose oxidase to galactitol, which in turn lead to the generation of reactive oxygen species (ROS) [3, 7, 8]. ROS generated by the accumulation of D-gal can lead to the damage of mitochondria and subsequent accelerated aging [9]. Systemic oversupply of D-gal in rodents have been widely used to study the effects of natural compounds against some pathological conditions such as neurodegenerative diseases in the elderly [3, 6-8].

Dichrocephala integrifolia (Linn.f.) O. Kuntze Asteraceae, has been frequently used in traditional medicine in Cameroon to treat malaria, pains, asthma, worms infections and schizophrenia. D. integrifolia has been shown to possess anti-inflammatory and analgesic properties and has been used to treat cattle for swelling infections, necrosis and oedema [10-13]. Despite the wide uses of D. integrifolia by traditional practitioners in Cameroon, the pharmacological studies of the effect of this medicinal plant on the central nervous system are scarce. Therefore, the aim of the present study was to evaluate the effects of D. integrifolia against D-gal induced oxidative stress, memory impairment and accelerated aging in mice.

2. Materials and Methods

2.1. Animals

This experiment was carried out on adult Swiss albino mice weighing 20-25 g. Animals were reared in the animal house of the Faculty of Science of the University of Buea under 12 h light/dark cycle. Food in the form of dried pellets and water were available ad libitum. The experiments were conducted in accordance with the International Guideline for the Care and Use of laboratory animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and the National Ethical Committee Guideline (NTFWA-IRB00001954). All efforts were made to minimize the number of animals used as well as their suffering.

2.2. Preparation of plant extract

The fresh leaves of D. integrifolia were collected in April 2014 in the locality of Buea (Cameroon). The botanical identification of the plant was done in the National Herbarium of Cameroon with 24276/SRFcam as identification number. The leaves of the plant were cleaned, shade-dried and ground. The D. integrifolia leave decoction was prepared daily in conformity with the traditional usage. Thus, 10 grams of the leaves’ powder were macerated into 75 ml of distilled water and were boiled for 20 min using a hot plate. The mixture was cooled and filtered using Whatman N°1 filter paper. The green filtrate obtained was diluted for less concentrated solutions. The doses of the decoction used in this study were 35; 87.5; 175 and 350 mg/kg.
2.3. Chemicals

D-galactose, Vitamin C, Trichloroacetic acid; Thiobarbituric acid, Sodium nitrite, naphthylethylenediamide and sulfanilamide were purchased from Sigma Aldrich, St Louis (USA) while Ellman reagent (5,5'-Dithiobis(2-nitobenzoic acid) were from Biochemica (China).

2.4. D-galactose mediated neurotoxicity

2.4.1. Treatment schedule

Animals were randomly divided into seven groups of 10 animals each and treated for 42 days (6 weeks). The distilled water group and the D-gal group received distilled water p.o 10 ml/kg, the test groups received one of the four doses of the decoction of D. integrifolia: 35; 87.5; 175 or 350 mg/kg and the positive control received vitamin C 100 mg/kg. D-gal was administered everyday to all groups except the distilled water group one hour after treatments (Table 1).

Table 1. Grouping and treatment of animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (42 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>DW oral + Saline (s.c)</td>
</tr>
<tr>
<td>D-galactose</td>
<td>DW oral + D-gal (100 mg/kg, s.c.)</td>
</tr>
<tr>
<td>Test group -I</td>
<td>DI (35 mg/kg) oral + D-gal (100 mg/kg, s.c.)</td>
</tr>
<tr>
<td>Test group -II</td>
<td>DI (87.5 mg/kg) oral + D-gal (100 mg/kg, s.c.)</td>
</tr>
<tr>
<td>Test group -III</td>
<td>DI (175 mg/kg) oral + D-gal (100 mg/kg, s.c.)</td>
</tr>
<tr>
<td>Test group -IV</td>
<td>DI (350 mg/kg) oral + D-gal (100 mg/kg, s.c.)</td>
</tr>
<tr>
<td>Positive control</td>
<td>Vit. C (100 mg/kg) oral + D-gal (100 mg/kg, s.c.)</td>
</tr>
</tbody>
</table>

DI = Dichrocephala integrifolia, Vit. C = Vitamin C; D-gal= D-galactose; n = 10 animals per group

2.4.2. Behavioral assessment

2.4.2.1. Open-Field Test

The Open-field adopted in this study was a square box made from plywood with dimensions (40 cm x 40 cm x 25 cm). The floor of the box was divided into 16 squares of 10 cm x 10 cm. This test was used to assess the locomotion of mice by counting the number of lines crossed by each animal in 5 min session as described earlier [14]. Observations were done weekly and started 30 min following D-gal administration.

2.4.2.2. Elevated Plus Maze Test

The Elevated Plus Maze (EPM) adopted comprised of two open arms (30 cm x 5 cm) and two closed arms (30 cm x 5 cm x 15 cm) that extended from a common central platform (5 cm x 5 cm). The entire maze was elevated to a height of 50 cm above the floor level. The EPM test is a behavioral test where the stimulus exists outside the body. This test was used to assess learning and memory of mice by recording initial transfer latency (ITL) on day 20 and retention transfer latencies (RTL) on day 21 and day 42 of drug treatment.

On the first day of the test (day 20 of treatment) learning of animals was recorded by experimenter researchers using stopwatches. Thus, each mouse was placed singly at the end of an open arm, facing away from the central platform. ITL was recorded as the time taken by the animal to move into any one of the covered arms of the EPM with all its four limbs. The cutting time was 120 s, which represented the time after which if the mouse did not enter into one of the covered arms, it was gently pushed into one and the ITL was assigned as 120 s. The mouse was then allowed to explore the maze for another 2 min. Memory retention was examined by recording RTL on day 21 and on day 42 of drug treatment [9, 15].
2.4.2.3. Morris Water Maze Test

The Morris Water maze (MWM) test was performed as previously described by Morris in 1984 with little modifications [16]. The MWM was a brown circular pool (100 cm diameter, 50 cm high) located in a room with diverse visual cues (shelters, curtains, lamps, etc.). The position of the pool and that of the cues were kept constant throughout the experiment. The pool was filled to a depth of 17 cm with milky water maintained at (25 ± 2°C). The water maze was virtually divided into four equal quadrants: North, South, East, and West, with a white platform (10 cm diameter, 16 cm height) centered in one quadrant, 1 cm below the water surface so that it was invisible. The position of the platform was kept constant throughout the training session.

The day before the test (day 37 of drug treatment), each mouse received an acclimatization trial during which it was placed inside the pool and allowed to swim in absence of the platform for 60 s. From day 38 to day 41 of drug treatment, each animal was given four training sessions per day during which the time taken to find the hidden platform (escape latency) was recorded. Once the animal reached the platform, it was allowed to stay there for 10 s. The cutting time for each trial was 120 s. The animal that did not find the platform during this period was gently guided to it and allowed there for 10 s while its escape latency was noted as 120 s. After each trial, every animal was returned to its cage and was allowed to dry up under a 60-watt bulb lamp. The retention trial was on day 42 of treatment. During this trial, the platform was removed from the quadrant and each animal was placed in the pool to swim for 120 s. The latency time to reach the place of the non-existing platform, the number of crossing of the formal platform quadrant as well as the time spent in the target quadrant was recorded using stopwatches.

2.4.3 Biochemical evaluation

2.4.3.1. Tissue preparation

At the end of the behavioral procedures, the animals were sacrificed on day 43 by decapitation following light ether anesthesia brains were rinsed with normal saline; weighted and then introduced in a mortar, containing Tris/ HCl buffer (50 mM) and ground. The homogenates were centrifuged at 3000 rpm for 15 min and the supernatant were separated and kept for the estimation of total proteins, malondialdehyde, nitrite oxide and reduced glutathione levels.

2.4.3.2. Total protein concentration

The total proteins content of homogenate was estimated using the method of Bradford with Bovine Serum Albumin as standard [17]. 5 μl of brain homogenate or standard were introduced in microplate wells. 250 μl of Bradford reagent were then added after agitation and 5 min incubation in the dark, the absorbance of the mixture was read at 595 nm using a microplate reader.

2.4.3.3. Brain lipid peroxidation

Malondialdehyde (MDA), a measure of lipid peroxidation was measured in brain’s homogenate using the thiobabituric assay as previously described by Nelson et al. [18]. Briefly, 1 ml of brain supernatant was mixed with 0.5 ml of thirchloroacetic acid (20%) and 1 ml of thiobarbituric acid (0.67%). The mixture was heated in a water bath at a temperature of 100°C during 60 min. After cooling with tab water, the mixture was centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 530 nm using a spectrophotometer. The amount of MDA is expressed as nmol/mg [19].

2.4.3.4. Brain nitrite level

The accumulation of nitrite in the supernatant, which is an indicator of the nitride oxide level, was evaluated using Greiss reagent. Sodium nitrite standard curve was used to determine the concentration of nitrite [19].
2.4.3.5. Brain reduced glutathione

The method originally described by Ellman in 1959 was used [20]. 20 μl of brains homogenate were added to 3 ml of Ellman reagent. After one hour of incubation in room temperature, the absorbance was read at 412 nm using a microplate reader. The amount of reduced glutathione was expressed in mmol/mg of protein [18].

2.4.4. Histopathological examination of brain tissues

In each treatment group, the brains of 3 to 4 animals were used for histopathological assessment. Thus, the entire brains were fixed in 10% formol for a week. 50 μm coronally sections of the brains were dehydrated in ascending concentration of ethanol and then embedded in paraffin. Brain sections were stained with Nissl stain. For the assessment of hippocampal lesions, slides were photographed and images were captured using a digital camera attached to a light microscope.

3. Statistical Analysis

Results are expressed as mean ± Standard Error of Mean (SEM). The differences among groups were analyzed using One way analysis of variance (ANOVA) using the software Graphpad Instat 3.10 for windows. When needed ANOVA was followed by Tukey or Dunnet for multiple comparisons group. The values of P≤0.05 were considered to be significant.

4. Results

4.1. Effects of D. integrifolia on locomotor activity of D-galactose treated mice

42 days of administration of D-gal did not significantly modify the locomotion of animal when compared to distilled water group. The administration of the decoction of D. integrifolia also did not caused any significant increase nor decrease in locomotory activities of D-gal treated mice (Fig. 1).

![Figure 1](image_url)

**Figure 1.** Effect of *D. integrifolia* on locomotor activity of D-galactose treated mice.

Results are mean ± SEM, n= 6 mice. Data were analyzed by one way ANOVA follow by tukey DW= distilled water; DI35; DI87.5; DI175 and DI350 = doses of *Dichrocephala integrifolia* (mg/kg); D-gal = D-galactose; Sal= Saline, Vit C: Vitamin C.
4.2. Effect of *D. integrifolia* on transfer latencies in an Elevated Plus Maze of D-galactose treated mice

There were no significant changes in ITL between distilled water group and D-gal -treated group on day 20. Meanwhile on day 21, the decoction of *D. integrifolia* at all the doses significantly reduced (F (6, 35) = 4.584; P = 0.021) the RTL of animals when compared to D-gal-treated group. This same observation was noted on day 42 with the second RTL (F (6, 35) = 15.491; P <0.001) (Table 2).

**Table 2.** Effect of *D. integrifolia* on transfer latencies in an Elevated Plus Maze of D-galactose treated mice.

<table>
<thead>
<tr>
<th>Treatment (42 days)</th>
<th>Day 20 (ITL)</th>
<th>Day 21 (RTL)</th>
<th>Day 42 (RTL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW + Saline</td>
<td>30.33 ± 1.22</td>
<td>11.33 ± 2.15***</td>
<td>10.66 ± 1.14***</td>
</tr>
<tr>
<td>DW + D-gal</td>
<td>59.50 ± 1.68</td>
<td>63.33 ± 12.82a</td>
<td>58.00 ± 7.67a</td>
</tr>
<tr>
<td>DI35 + D-gal</td>
<td>30.50 ± 6.90</td>
<td>12.50 ± 4.18***</td>
<td>13.33 ± 3.76***</td>
</tr>
<tr>
<td>DI87.5 + D-gal</td>
<td>50.50 ± 14.67</td>
<td>8.83 ± 1.35***</td>
<td>10.50 ± 2.61***</td>
</tr>
<tr>
<td>DI175 + D-gal</td>
<td>54.00 ± 11.48</td>
<td>10.66 ± 3.95***</td>
<td>14.50 ± 4.69***</td>
</tr>
<tr>
<td>DI350 + D-gal</td>
<td>56.33 ± 20.20</td>
<td>22.33 ± 4.47***</td>
<td>15.00 ± 3.22***</td>
</tr>
<tr>
<td>Vit C + D-gal</td>
<td>50.00 ± 14.49</td>
<td>17.50 ± 3.03***</td>
<td>16.00 ± 2.59***</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM, n= 6 mice. ***p<0.001, compare to D-gal treated group. a p<0.001 compare to distilled water group. Data were analyzed by one way ANOVA followed by Tukey-kramer multiple comparisons test. DW: distilled water; DI35; DI87.5; DI175 and DI350 = doses of *Dichrocephala integrifolia* (mg/kg); D-gal = D-galactose; ITL= Initial transfer latency; RTL= Retention transfer latency; Sal= Saline; Vit C; Vitamin C.

4.3. Effect of *D. integrifolia* on escape latencies in a Morris Water Maze of D-galactose treated mice

The results of the acquisition phase of the Morris Water Maze are presented in Table 3. Administration of D-gal significantly delayed the acquisition transfer latency to reach the invisible platform as compared to distilled water. The decoction of the plant at the doses of 87.5, 175 and 350 significantly reduced the acquisition transfer latency within all the four days of the acquisition.

**Table 3.** Effect of *D. integrifolia* on escape latencies in a Morris Water Maze of D-galactose treated mice.

<table>
<thead>
<tr>
<th>Treatment (42 days)</th>
<th>EL (Day 38)</th>
<th>EL (Day 39)</th>
<th>EL (Day 40)</th>
<th>EL (Day 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW + Saline</td>
<td>38.33 ± 5.46**</td>
<td>19.20 ± 3.03***</td>
<td>16.08 ± 3.13***</td>
<td>10.20 ± 4.98***</td>
</tr>
<tr>
<td>DW + D-gal</td>
<td>72.83 ± 7.59b</td>
<td>68.79 ± 12.37a</td>
<td>59.75 ± 4.13a</td>
<td>68.70 ± 6.01a</td>
</tr>
<tr>
<td>DI35+ D-gal</td>
<td>60.08 ± 4.75</td>
<td>40.35 ± 2.15*</td>
<td>40.45 ± 5.68c</td>
<td>30.19 ± 9.66*</td>
</tr>
<tr>
<td>DI87.5 + D-gal</td>
<td>36.95 ± 5.62**</td>
<td>30.75 ± 1.43**</td>
<td>29.16 ± 3.31</td>
<td>20.00 ± 9.81***</td>
</tr>
<tr>
<td>DI175 + D-gal</td>
<td>35.04 ± 5.76***</td>
<td>20.50 ± 2.03***</td>
<td>30.75 ± 1.48**</td>
<td>25.20 ± 5.4**</td>
</tr>
<tr>
<td>DI350 + D-gal</td>
<td>32.75 ± 5.39***</td>
<td>31.85 ± 9.01**</td>
<td>30.12 ± 9.29**</td>
<td>25.10 ± 8.1**</td>
</tr>
<tr>
<td>Vit C + D-gal</td>
<td>26.83 ± 3.26***</td>
<td>16.75 ± 3.00***</td>
<td>14.05 ± 1.96***</td>
<td>15.50 ± 4.88***</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM, n= 6 mice. *p<0.05, **p<0.01, ***p<0.001 compared to D-galactose-alone group. c<0.05, b<0.01, a<0.001 compared to distilled water group. Data were analyzed by one way ANOVA followed by Tukey multiple comparison test. DW: distilled water; DI35; DI87.5; DI175 and DI350 = doses of *Dichrocephala integrifolia* (mg/kg); D-gal = D-galactose; EL= Escape latency ; Vit C; Vitamin C (100 mg/kg.)
4.4. Effect of *D. integrifolia* on retention parameters in a Morris Water Maze of D-galactose treated mice

The results of the retention phase of the MWM task is presented in Fig. 2. Administration of D-gal for 42 days significantly reduced the latency to reach the non existing platform (Fig. 2 A) as well as the time spent in the target quadrant. The decoction of *D. integrifolia* at the doses of 175 mg /kg significantly ([F (6, 35) = 4.054; P = 0.0035]) decreased the latency time to the non-existing platform (Fig. 2 A) and significantly ([F (6, 35) = 3.854; P = 0.0047]) increase the time the animal spent in the target quadrant (Fig. 2 B). There was no significant difference in the number of crossing of the target quadrant among groups (Fig. 2 C).
Figure 2. Effect of *D. integrifolia* on retention parameters of Morris Water Maze of D-galactose-treated mice. Latency to the non-existing platform (A); time spent in the target quadrant (B); Number of crossing of the target quadrant (C).

Results are mean ± SEM, n= 6 mice. Data were analyzed by one way ANOVA followed by Tukey multi comparisons test. * P < 0.05; ** P < 0.01; vs. D-galactose-alone treated group. c<0.05 compared to distilled water group.

4.5. Effect of *D. integrifolia* on the relative weight of brain of D-galactose treated mice

The relative weight of brain of D-gal treated mice is presented in Table 4.

Administration of D-gal during 42 days resulted in the increased of brain relative weight in comparison to the distilled water group. The decoction of *D. integrifolia* at the dose of 175 and 87.5 significantly reduced the relative weight of brain (Table 4).

<table>
<thead>
<tr>
<th>Treatment (42 days)</th>
<th>Relative weight of brain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW + Saline</td>
<td>1.24 ± 0.08**</td>
</tr>
<tr>
<td>DW + D-gal</td>
<td>1.63 ± 0.08</td>
</tr>
<tr>
<td>DI35+ D-gal</td>
<td>1.30 ± 0.06**</td>
</tr>
<tr>
<td>DI87.5 + D-gal</td>
<td>1.32 ± 0.04*</td>
</tr>
<tr>
<td>DI175 + D-gal</td>
<td>1.27 ± 0.06**</td>
</tr>
<tr>
<td>DI350 + D-gal</td>
<td>1.35 ± 0.06</td>
</tr>
<tr>
<td>Vit C + D-gal</td>
<td>1.26 ± 0.01**</td>
</tr>
</tbody>
</table>

Results are mean ± SEM, n= 6 mice. Data were analyzed by one way ANOVA followed by Dunnett multi comparisons test. * P < 0.05; ** P < 0.01; vs. D-galactose-alone treated group. DW= Distilled water; DI35; DI87.5; DI175 and DI350 = doses of *Dichrocephala integrifolia* (mg/kg); D-gal = D-galactose; Vit C = Vitamin C.
4.6. Effect of *D. integrifolia* on brain malondialdehyde, nitrite and reduced glutathione levels of D-galactose treated mice

The results of the assay of some biochemical parameters of oxidative stress are presented in table 5. Administration of D-gal for 42 days significantly (p<0.01) increased the brain malondialdehyde and nitrite levels and lead to reduction of the amount of reduced glutathione as compared to distilled water group. The decoction of *D. integrifolia* (87.5 mg/kg) significantly reversed the oxidative damage induced by D-gal evidence by reducing MDA and nitrite levels and increasing reduced glutathione when compare to D-galactose alone treated group (Table 5).

**Table 5.** Effects of *D. integrifolia* on brain malondialdehyde, nitrite and reduced glutathione levels of D-galactose treated mice.

<table>
<thead>
<tr>
<th>Treatment (42 days)</th>
<th>MDA levels (nmol/mg protein)</th>
<th>Nitrite levels (umol/mg protein)</th>
<th>Reduced glutathione (mmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW + Saline</td>
<td>0.14 ± 0.01***</td>
<td>55.89 ± 3.80***</td>
<td>34.75 ± 1.80**</td>
</tr>
<tr>
<td>DW + D-gal</td>
<td>0.39 ± 0.02c</td>
<td>197.84 ± 9.48c</td>
<td>16.25 ± 1.40b</td>
</tr>
<tr>
<td>DI35+ D-gal</td>
<td>0.24 ± 0.05**a</td>
<td>163.00 ± 7.72**c</td>
<td>28.47 ± 1.92b</td>
</tr>
<tr>
<td>DI87.5 + D-gal</td>
<td>0.21 ± 0.02***a</td>
<td>112.64 ± 9.74***c</td>
<td>33.83 ± 4.31**</td>
</tr>
<tr>
<td>DI175 + D-gal</td>
<td>0.24 ± 0.02**b</td>
<td>168.44 ±10.09c</td>
<td>32.93 ± 14.20**</td>
</tr>
<tr>
<td>DI350 + D-gal</td>
<td>0.27 ± 0.03*</td>
<td>76.76 ± 1.38***</td>
<td>20.84 ± 0.46a</td>
</tr>
<tr>
<td>Vit C + D-gal</td>
<td>0.16 ± 0.02***</td>
<td>56.25 ± 5.77***</td>
<td>35.11 ± 1.14**</td>
</tr>
</tbody>
</table>

Results are mean ± SEM, n= 6 mice. Data were analyzed by one way ANOVA followed by Tukey multi comparisons test. * p < 0.05; ** p < 0.01; vs. D-galactose-alone treated group. a p < 0.05; b P < 0.01; c P < 0.001 compare to distilled water group. D-Gal = D-galactose ; DI35; DI87.5; DI175 and DI350 = doses of *Dichrocephala integrifolia*. (mg/kg); Dw: distilled water; Vit C = Vitamin C (100 mg/kg).

4.7. Effect of *D. integrifolia* on the histological structure of the brain of D-galactose treated mice

The results of the histological analysis are presented in Fig. 3. Administrations of D-gal for 42 days lead to the reduction of the density of neuron in the dentate gyrus. The density of neuron in the granular cell layer was quite reduced (Fig. 3 B). The doses 87.5 and 175 mg/kg of *D. integrifolia* decoction as well as the distilled water group showed similar effects on the architecture of the dentate gyrus (Fig. 3 C, 3D and 3A).
Figure 3. Results of histopathological studies; distilled water group (A), D-galactose group (B), *D. integrifolia* (87.5 mg/kg (C) and 175 mg/kg (D) and vitamin C (E). g: granular cell layer of the dentate gyrus. Arrows indicate the reduction of the number of cell of the dentate gyrus of D-galactose group.

5. Discussion

Alzheimer’s disease the most common form of dementia in the elderly is a neurodegenerative disease characterize by a progressive memory loss associated with cognitive dysfunctions. Many hypotheses have been postulated to explain the etiology of this disease , amongst them the oxidative hypothesis of AD [21, 22]. The AD brain is characterize by a pronounce oxidative stress status evidence by an increase in advanced glycation end products, lipid peroxidation, free protein carbonyls and nitration [22]. D-gal-induced oxidative stress and accelerated aging in rodents is an animal model widely used to test the beneficial effects of plants and/or their secondary metabolites against oxidative damages as those found in AD [6, 7]. In the present study, which aim was to evaluate the effect of the decoction prepare from the leaves of *D. integrifolia* against D-gal induced oxidative stress and accelerated aging in mice. Administration of D-gal (100 mg/kg; s.c) to animal for 42 days led to behavioral abnormalities and cognitive deficits as previously described [3, 9]. In fact in the Elevated Plus Maze task, D-gal alone treated mice had higher retention transfer latencies on days 22 and 42. This increase of retention transfer latencies was significantly (p<0.01) reduced in the group treated with the decoction of the plant at the dosage of 87.5 and 175 mg/kg as well as in the group treated with vitamin C (100 mg/kg). These results suggest that *D. integrifolia* improve memory in an EPM apparatus [23]. Furthermore, in the MWM task, the D-gal-treated mice spent a longer time to find the invisible platform during acquisition phase, whereas during the retention phase they spent a lesser time in the target quadrant compared to the distilled water group. The decoction of *D. integrifolia* at all the doses used in this study significantly reversed the learning deficit induced by a chronic administration of D-gal in a MWM paradigm. The MWM is a behavioural task used to assess spatial long-term memory in rodent. The improvement by *D. integrifolia* of the performance of animal in the MWM suggests that the decoction of the plant improve spatial long-term memory, which is one type of memory affected in AD [16]. In our study, the relative brain weight of the mice treated with D-gal alone was significantly higher than, those of
the distilled water group. This increase of brain relative weight could be the results of inflammatory process generated by a chronic administration of D-gal. Earlier reports had demonstrated that chronic administration of D-gal can lead to the production of inflammatory mediators like proinflammatory cytokines, prostaglandins and platelet activation [24]. The decoction of the D. integrifolia significantly decreases the relative brain weight of mice. The evidence of an inflammatory process in the brain of D-gal – alone-treated mice was observed with the assay of brain nitrite oxide level. In fact, it has been reported that, due to an increase of astrocyte activation, the inflammatory processes in the brain can lead to an increase in nitrite oxide production [25]. In the present study administration of D-gal for 6 weeks in mice resulted to an increase in brain nitrite level. The decoction of the plant significantly reduced this increase of brain nitrite level. These results suggest that this D. integrifolia can have antinflammatory properties [19]. This property of the plant has been earlier reported by Ngueguim et al. in 2016 [12].

In line with literature, administration of D-gal to animals for a period of 42 days also resulted in an increase in brain oxidative stress status. The brain level of malondildehyde of animals treated with D-gal alone was higher than those of the distilled water group; whereas the amount of reduced glutathione was smaller. The decoction of the D integrifolia at the doses of 87.5 and 175 mg/kg significantly reverses these markers of oxidative stress induced by a chronic administration of D-gal. The results of the histopathological analysis revealed that in the hippocampus of the D-gal-alone -treated mice there was a reduction in the density of neurons in the dentate gyrus. This observation has been previously mentioned by Cui et al. in 2006 which indicated that D-gal can impair neurogenesis in the dentate gyrus [26]. The decoction of the D. integrifolia like Vit C have an architecture of the dentate gyrus smilar to that of the distilled water group indicating that D. integrifolia as well as Vit C reversed the neurodegeneration induced by D-gal. From this observations it can be clearly observed that the decoction of D. integrifolia protect mice against D-gal -induced neurodégénération, memory loss and learning deficits. The mechanism by which D. integrifolia exerts its effects can be its antioxidant and anti inflammatory activities as previously mentioned [12].

6. Conclusion

The results of the present study showed that the decoction of D integrifolia prevents neurodegeneration learning and memory deficits induced by a chronic administration of D-galactose. The beneficial effects of this plant against neurotoxicity could be attributed to its antioxidant activities. Theses results can explain the wide uses of this D. integrifolia in folk medicine in central Africa to prevent and treat dementia and diverse others central nervous disorders such as epilepsy.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This research was supported by the Ministry of higher education of Cameroon. The authors are very thankful to Mr. Mbaabe Felix Akumcha of the life science laboratory of the University of Buea for his technical assistance during this work.
References


