

ANTIOXIDANT PROPERTIES OF *DICHROCEPHALA INTEGRIFOLIA* (ASTERACEAE) IN A MOUSE MODEL OF MONOSODIUM GLUTAMATE-INDUCED NEUROTOXICITY

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Abstract

Background: In Africa, neurodegenerative diseases in the elderly have become a major health concern due to the increase in life expectancy. Glutamate mediated neurotoxicity is involved in neurodegenerative diseases such as Ischemia, Epilepsy, Alzheimer's and Parkinson diseases. Plants with antioxidant properties are reported to protect vital organs against glutamate toxicity. This study aims to assess the effect of *Dichrocephala integrifolia* against monosodium glutamate-mediated neurotoxicity and oxidative stress.

Methodology: The decoction prepared from the leaves of *Dichrocephala integrifolia* was evaluated against monosodium glutamate-induced neurotoxicity in mice. The animals were grouped in seven groups of 6 animals each. The animals received daily; distilled water (p.o) for the distilled water and the negative control groups, one of the four doses of the decoction of the plant (35, 87.5, 175 or 350 mg/kg p.o) for the tests groups and memantine (20 mg/kg p.o) for the positive control group. Monosodium glutamate (2.5 g/kg ip) was injected daily to animals except those of the normal control group all the seven days of the experimentation. Animals were observed for aggressiveness, locomotor and forepaws muscle grip activities 30 min after monosodium injections. Brain reduced glutathione and malondialdehyde levels were also assessed following the behavioral tests on day 8.

Results: The decoction of *Dichrocephala integrifolia* at the doses of 87.5 and 175 mg/kg significantly ($p < 0.01$) inhibited the aggressiveness of monosodium treated mice, significantly ($p < 0.01$) counteracted the reduction in locomotor and forepaws muscle grip capacity induced by monosodium glutamate. Furthermore, the decreases in reduced glutathione level and increases in lipid peroxidation level induced by monosodium glutamate were significantly ($p < 0.001$) reversed by *Dichrocephala integrifolia* at the doses of 87.5 and 175 mg/kg.

Conclusion: The results of this study reveal that *Dichrocephala integrifolia* is a medicinal plant that protects the brain against monosodium glutamate-mediated neurotoxicity. This can explain why this plant is intensively used in folk medicine in Cameroon to prevent and treat some central nervous system illnesses.

Keywords: Neurotoxicity; Glutamate; *Dichrocephala integrifolia*; antioxidant

Introduction

The brain as the main vital organ of a living organism can suffer from injuries due to endogenous or exogenous substances, a phenomenon known as neurotoxicity. Neurotoxicity can result in diverse central nervous system diseases such as mood disorders and diverse psychiatric disturbances (Han et al., 2011; Mason et al., 2014). Epilepsy, hypoglycemia, ischemia, Alzheimer's and Parkinson's diseases are among the diseases where neurotoxicity generated by glutamate

dysregulation plays a central role (Ganesan et al., 2013, Rajagopal et al., 2013; Swamy et al., 2013). Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system. Despite this key role, glutamate may be toxic to neuron in case it is in excess (Shivasharan et al., 2013). In the brain, it binds to its receptors, which are N-methyl D-aspartate (NMDA), α -amino-3-hydroxyl-5-methyl-4-isoxalepriopronic acid (AMPA), and Kainate receptors. Glutamate-mediated neurotoxicity can be mediated through all these above-mentioned receptors. Glutamate in the brain can also lead to the imbalance between prooxydant and antioxidant enzymes resulting in the production of reactive oxygen species and subsequent oxidative stress through an overstimulation of NMDA receptors or non-receptors pathway (Butterfield & Pocernich, 2003; Danysz & Parsons, 2012; Ganesan et al., 2013). Monosodium glutamate (MSG) or monosodium glutamate salt hydrate is a food additive used in the modern society gastronomy and which enhance food flavor, increase appetite and give a special food taste highly appreciated by its consumers (Neveen and Iman, 2010; Ganesan et al., 2013; Hassan et al., 2014). Despite the high consumption of MSG as a food additive, there are evidences suggesting the toxicity of oral or systemic administrations of MSG salt to rodents consecutively for 6 to 10 days from the dose- range of 0.5 g/kg to 3 g/kg (Ganesan et al., 2013, Hassan et al., 2014; Swamy et al., 2013; Shivasharan et al., 2013). In addition, MSG has been implicated in the mitochondrial oxidative status of different brain regions (Farombi & Onyema, 2006, Shivasharan et al., 2013). Many medicinal plant extracts or their secondary metabolites have been reported to prevent MSG-induced neurotoxicity in vivo and in vitro mainly due to their antioxydant capacities (Neveen and Iman, 2010; Ramanathan et al., 2007).

Dichrocephala integrifolia is a plant of the family Asteraceae. It is used in folk medicine in central Africa in general and in Cameroon in particular to treat humans as well as livestock. Alone or in addition with other medicinal plants, different parts of *D. integrifolia* are used to treat malaria, asthma, worm infections, schizophrenia, epilepsy, dementia, headache and for tooth extraction. In fact, fresh leaves of *D. integrifolia* associated with those of *Ageratum conyzoides* are used in Tanzania in the treatment of eye infections while in central part of Cameroon, same fresh leaves are use for tooth exraction (Moshi et al ., 2010; Agbor et al., 2011). Again, *D. integrifolia* is used by the population of west Cameroon to treat worm infections and amebiasis (Moudipa et al., 2005; Wabo et al., 2013).

There are no sufficient studies on the pharmacological action of *D. integrifolia* on the central nervous system. Therefore, for a better validation of the uses of *D. integrifolia* in folk medicine to treat neurodegenerative diseases, we in this study, evaluated the effect of the decoction of leaves of *D. integrifolia* on MSG-induced neurotoxicity, alteration in behavior, reduction in locomotion and forepaws muscle grip capacities. The effect of the plant was also evaluated against MSG -induced oxidative stress.

Materials and Methods

Animals

Wistar rats (180-200 g) and Swiss mice (25-30 g) of either sex obtained from the animal house of the Department of Zoology and Animal Physiology of the University of Buea were used in this study. All animals maintained on a 12 h light-dark cycle, were housed in standard cages in a number of 6 animals per cage with food and water available *ad libitum*. The experiments were carried in accordance with the International Guideline for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and the National Ethical Committee Guideline (NFWA-IRB00001954). All efforts were made to minimize both the suffering and the number of animal used.

Drugs and chemicals

Monosodium glutamate salt (MSG) (Alfa Aesar, Germany), Memantine hydrochloride (MT) (Cayman Chemical, USA), Ellman reagent (Biochemica, China), Trichloroacetic acid and Thiobarbituric (Sigma Chemical, St Louis USA) were used in this study.

Plant material and preparation of the decoction

D. integrifolia's fresh leaves were harvested in April 2014 in the locality of Buea (Cameroon), with coordinates: 4°15'30'' and 9°25'48''. The Botanical identification was done at the National Herbarium of Cameroon where a voucher specimen is conserved under the reference number: 24276/SRFcam. After collection, the leaves of the plant were carefully washed, shade dried and ground. The decoction of *D. integrifolia* was prepared daily according to the instructions of the herbalist. Ten (10) g of the leaves' powder of *D. integrifolia* were introduced in 75 ml of distilled water and boiled for 20 min. The decoction was then filtered using Whatman No1 filter paper and diluted 2; 4 and 10 time with distilled water. Twenty (20) ml of the filtrate was evaporated to dryness and the weight of the brown extract obtained was 700 mg. The yield of the extraction was 7%. The corresponding concentration of the stock solution was 35 mg/ml. The corresponding dose for the stock solution was 350 mg/kg and the different dilutions were 175; 87.5 and 35 mg/kg. The doses of *D. integrifolia* used in this study were determined based upon prescreening results (data not shown).

Phytochemical Screening

The Phytochemical screening of the decoction of *D. integrifolia* was done using the following method: Foam test for saponins, Meyer's test for alkaloids, Bornträger test for anthraquinones, Liebermann Buchard test for phytosterols and aqueous sodium hydroxide test for flavonoids (Trease and Evans, 2009).

Glutamate mediated neurotoxicity

Treatment schedule

The mice were grouped in seven groups of 6 animals each as follow: **Group I:** distilled water (distilled water p.o); **Group II:** Negative control (distilled water p.o); **Group III:** Decoction (35 mg/kg p.o); **Group IV:** Decoction (87.5 mg/kg p.o); **Group V:** Decoction (175 mg/kg p.o); **Group VI:** Decoction (350 mg/kg p.o) and **Group VII:** Positive control (memantine 20 mg/kg p.o). Animals of each group received the various treatments one hour before the injection of MSG (2.5 g/kg ip). The injection of MSG was done to all of the groups except the distilled water which received an intraperitoneal administration of saline. The treatments were done continuously during seven days. The dose of MSG was chosen according to literature (Olney et al., 1972; Ramanathan et al., 2007; Ganesan et al., 2013) and prescreening experiment (data not shown), while that of memantine was done according to literature (Danysz & Parson, 2012). The animals were observed for locomotion and forelimbs grip capacity on days 1, 3, 5 and 7 of treatment and for any sign of aggressiveness all the seven days of treatment and 30 min after MSG injection.

Behavioral tests

Agressivity

This test was used to evaluate the effect of *D. integrifolia* on MSG-induced agressivity and hyperactivity. Every day and 30 min after MSG administration, animals were observed for any sign of aggressiveness, hyperactivity and fighting or calmness. Mice were scored 5 when they exhibited aggressive and fighting behaviours and scored 0 for calmness (Neveen & Iman, 2010).

Locomotion

This test was used to evaluate the effect of *D. integrifolia* on MSG-induced alteration in locomotion using an open field apparatus. The open field was locally fabricated in ply wooden with the following dimensions 40 cm x 40 cm x 25 cm. The grid floor was further divided into 16 smaller squares of 10 cm x10 cm dimensions each. The locomotion or ambulatory behaviour of every single mouse was assessed every two days, 30 min after MSG administration by counting the number of lines crossed by the animal in five min time (Ngo Bum et al., 2012).

String suspension test

This test was performed as described by Barclay and coworkers in 1981 with little modifications (Barclay et al., 1981). This test was conducted every two days of treatment, immediately after the locomotor activity. And it was used to assess forelimbs grip capacity and strength of mice. For this purpose, each mouse individually were held by its tail and allowed to hang suspended with its fore arms, to a wire cable of 1 mm taut between two stands and 50 cm above a padded table. The time each animal remain suspended was recorded using a stopwatch for a period of 30 s.

Biochemical evaluation

Tissue preparation

The tissue preparation was done on day 8. The animals individually were sacrificed by decapitation after light ether anesthesia and their skull were gently opened to quickly remove the brains. The brains were rinsed with saline and weighed then introduced in a mortar with the corresponding volume of 50 mM Tris-HCl, buffer to prepare 10% homogenate. The homogenates were centrifuged at 3000 rpm during 15 min and the supernatant were separated and used for the estimation of total protein, malondialdehyde level and reduced glutathione levels.

Total protein concentration

The method describes by Bradford in 1976 was used for the estimation of total protein content, while using the bovine serum albumin as standard.

Brain reduced glutathione level

The brain reduced glutathione (GSH) was assessed in the brain supernatant using Ellman's reagent as described by Ellman in 1959. Twenty (20) μ l of brain homogenates were mixed with 3 ml of Ellmant reagent in room temperature. After one hour, the absorbance of the yellow compound was read at 412 nm using a microplatte reader. The amount of glutathione was calculated with the formula of Beer Lambert using the extinction coefficient value of 13,600/M/cm.

Brain Malondialdehyde level

The brain Malondialdehyde (MDA) level was measured in the supernatant using the thiobabituric assay as described by Nelson et al (1994) with little modifications. To 1 ml of brain supernatant was added 0.5 ml of Thrichloroacetic acid (20%) and 1 ml of thiobarbituric acid (0.67%). The mixture was allowed to heat in a water bath at 100C for an hour. After cooling with tab water, the mixture was centrifuged at 3000 rpm for 15 min and the absorbance of the supernatant was read at 530 nm. The amount of MDA was calculated with the formula of Beer Lambert using the extinction coefficient value of 1.56×10^5 M/cm (Fotio et al., 2009).

Acute toxicity

The acute toxicity of *D. integrifolia* was determined in Wistar rats according to the protocol of the Organization for Economic Corporation and Development (OECD) and principle of good laboratory Practice (OECD, 2001). Four (4) groups of 6 rats each (3 males and 3 females) received the following doses of the plant extract in a single oral administration: 0; 2000; 3000 and 5000 mg/kg. Prior to the administration animals were subjected to fasting. The general behavior (locomotion, sensitivity to noise, sensitivity to touch, aspect of their stools, feeding behavior, phonation etc) of the rats was observed for 2 hours after treatment. 3 hours after administration of the decoction, the rats were given food and water *ad libitum*. During one week, the rats were observed 2 hours per day for any sign of toxicity such as asthenia, piloerection, salivation or dead.

Statistical analysis

Graphpad Instat software 3.10 for windows was used for the statistical analysis. Results are expressed as Mean \pm SEM. One-way analysis of variance (ANOVA) was used to analyze the differences among groups. ANOVA was followed by Turkey for multiple comparisons group and Dunnet when needed. The values of $p < 0.05$ were considered to be significant.

Results

Results of phytochemical screening

The decoction of *D. integrifolia* contains alkaloids, saponins, tannins, flavonoids, anthraquinones and triterpenes (Table 1).

Table 1: Phytochemical components of the decoction of the leaves of *D. integrifolia*

Constituent	Inference
Alcaloïds	++
Flavonoïds	+++
Tannins	+++
Triterpenes	++
Anthraquinones	+
Saponins	+++

+ = Trace; ++ = Moderate; +++ = Abundance

Effects of *D. integrifolia* on the agressivity of glutamate treated-mice

The animals which received distilled water exhibited normal behaviour and they were not aggressive. The animal of the negative control exhibited fighting from day 4 upward. The score of aggressiveness was highest on days six and seven of treatment in negative control group. The plant decoction at the dosage of 87.5 mg/kg significantly ($p < 0.05$) reduced the aggressiveness of mice (Table 2).

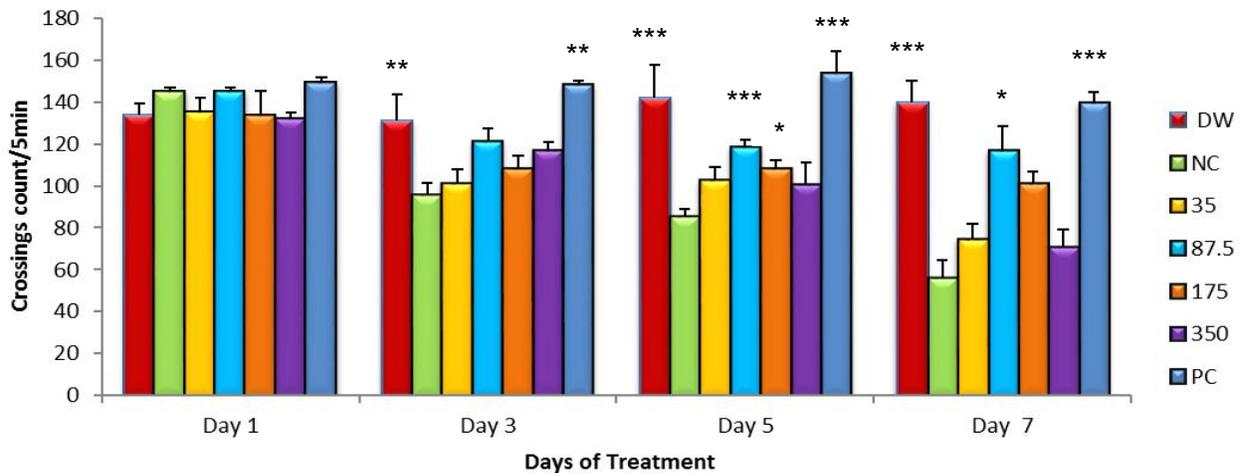
Table 2: Effects of *D. integrifolia* on the aggressivity of monosodium glutamate-treated mice

Treatment	Doses (mg/kg + g/kg)	Score day 4	Score day 5	Score day 6	Score day 7
DW + NaCl	-- + --	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00**	0.00 ± 0.00**
DW + MSG	-- + 2.5	2.50 ± 1.05	3.33 ± 1.05	4.16 ± 0.83	4.16 ± 0.83
DI + MSG	35 + 2.5	1.67 ± 1.05	1.67 ± 1.05	2.50 ± 1.05	3.33 ± 1.05
DI + MSG	87.5 + 2.5	0.00 ± 0.00	0.00 ± 0.00	0.83 ± 0.83*	0.83 ± 0.83*
DI + MSG	175 + 2.5	0.00 ± 0.00	0.83 ± 0.83	1.67 ± 1.05	1.67 ± 1.05
DI + MSG	350 + 2.5	0.00 ± 0.00	1.67 ± 1.05	1.67 ± 1.05	2.50 ± 1.05
MT + MSG	20 + 2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00**	0.00 ± 0.00**

Results are mean ± SEM, n= 6 mice. *p<0.05 or **p<0.01, vs negative control. Data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparisons group. DW= Distilled Water; MSG=Monosodium glutamate, DI= *Dichrocephala integrifolia*, MT= Memantine (20 mg/kg per os).

Effects of *D. integrifolia* on locomotor activity of monosodium glutamate-treated mice.

There were no significant changes in the locomotion of the animals of the distilled water group during the seven days of treatment. Administration of MSG resulted in significant decreased in animal locomotion in negative control starting from the third day of administration when compared to distilled water (F (6, 35) = 6.629; P<0.0001). The percentage of reduction of locomotion in MSG-treated mice from day 1 to day 7 is 61%. Pretreatment of animal with *D. integrifolia* (87.5 and 175 mg/kg) significantly improved locomotor activity when compared to negative control [F (6, 35) = 17.440; P<0.0001] (figure 1). MT also counteracted the reduction in locomotion induced by MSG (figure 1).

**Figure 1:** Effects of *D. integrifolia* on locomotor activity of monosodium glutamate treated-mice.

Results are mean ± SEM, n= 6 mice. *p<0.05, **p<0.01, ***p<0.001 vs negative control. Data were analyzed by one-way ANOVA follow by Tukey-kramer multiple comparisons group. DW= Distilled Water, PC= positive control (Memantine 20 mg/kg per os), NC=Negative control, 35; 87.5; 175 and 350= doses of *Dichrocephala integrifolia*.

Effects of *D. integrifolia* on forepaws muscle grip capacity of monosodium glutamate-treated mice.

There were no significant changes in forepaws muscle grip capacity of the animals of the distilled water group during the seven days of the treatment. On the other hand, seven days administration of MSG in animals led to a significant reduction of the time of suspension of the animal of negative control on a string taut from day 3 upward when compared to distilled water. *D. integrifolia* (87.5 mg/kg) significantly increased the time the animal remained suspended on the string on day seven when compared to negative control [F (6, 35) = 9.368; P<0.0001]. There were no changes in forepaws muscle grip capacity of MT group when compared to distilled water.

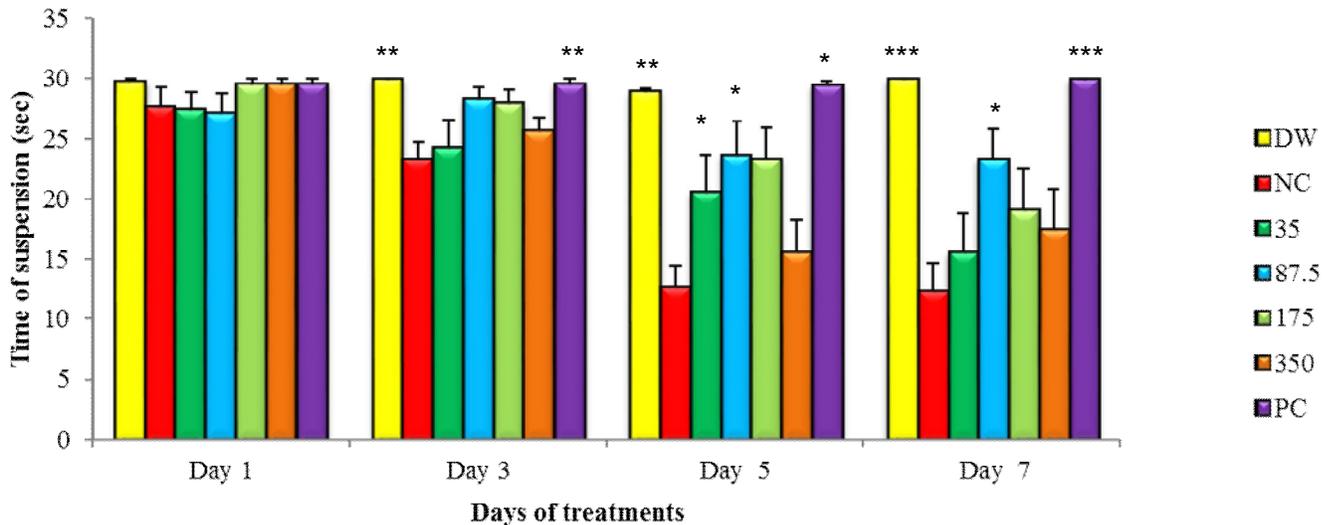


Figure 2: Effects of *D. integrifolia* on muscle grip capacity of monosodium glutamate-treated mice. Results are mean \pm SEM, n= 6 mice. *p<0.05, **p<0.01, ***p<0.001 vs monosodium glutamate alone treated mice. Data were analyzed by one-way ANOVA plus Tukey-kramer multiple comparisons group. DW= Distilled Water, PC= positive control (Memantine 20 mg/kg per os), NC=Negative control, 35; 87.5; 175 and 350= doses of *Dichrocephala integrifolia*.

Effects of *D. integrifolia* on brain reduced glutathione level of monosodium glutamate-treated mice

Figure 3 shows the brain level of reduced glutathione (GSH) of the MSG-treated mice. Whereas a significant decrease in brain glutathione level was observed with negative control, treatment with *D. integrifolia* (87.5 and 175 mg/kg) and standard drug MT as positive control significantly reversed the decrease of GSH level in the brain [F (6, 35) = 24.982; P<0.0001] (figure 3).

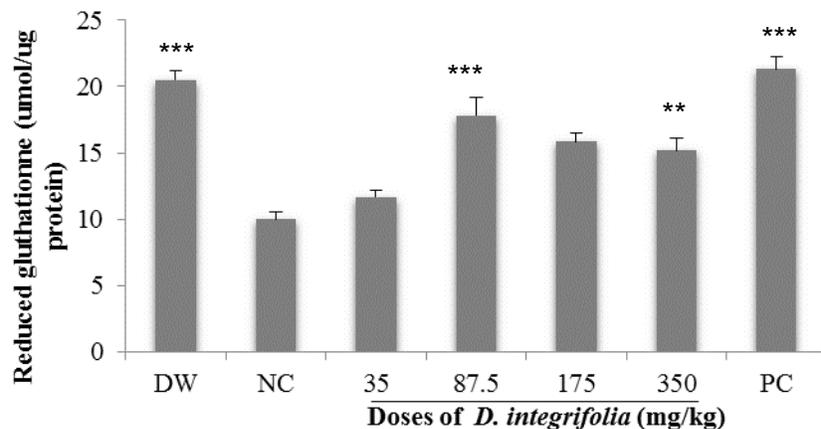


Figure 3: Effects of *D. integrifolia* on brain reduced glutathione level of monosodium glutamate treated mice. Values are mean \pm SEM, n= 6 mice. *p<0.05, **p<0.01, ***p<0.001 vs monosodium glutamate alone treated mice. Data were analyzed by one-way ANOVA plus Tukey-kramer multiple comparisons group. DW= Distilled Water, PC= positive control (Memantine 20 mg/kg per os), NC=Negative control.

Effects of *D. integrifolia* on malondialdehyde level of monosodium glutamate treated mice

Seven days of administration of MSG in mice resulted to an increase in brain MDA level in the negative control when compared to distilled water. The co administration of *D. integrifolia* (87.5; 175 and 350 mg/kg) and MSG (2.5 g/kg) reduced the level of brain MDA when compared to negative control ([F (6, 35) = 128.71; P<0.0001]) (Figure 4). MT (20 mg/kg) also reduced the level of MDA when compare to negative control (Figure 4).

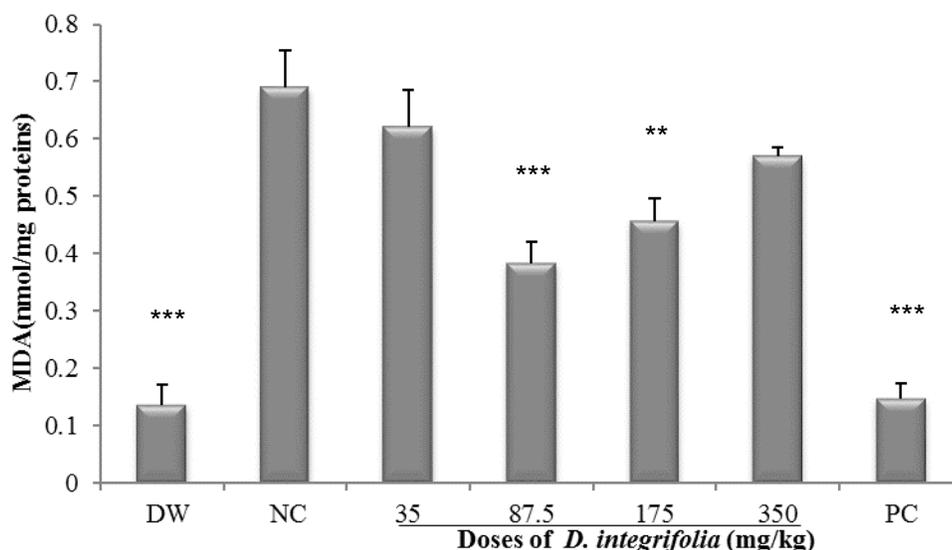


Figure 4: Effects of *D. integrifolia* on Malondialdehyde level of monosodium glutamate treated mice. Values are mean \pm SEM, n= 6 mice. *p<0.05, **p<0.01, ***p<0.001 vs negative control. Data were analyzed by one-way ANOVA plus Tukey-kramer multiple comparisons group. DW= Distilled Water, PC= positive control (Memantine 20 mg/kg per os), NC=Negative control, 35; 87.5; 175 and 350: doses of *Dichrocephala integrifolia*.

Results of acute toxicity study

There were no signs of toxicity or dead at the dose of up to 3000 mg/kg after a single administration of *D. integrifolia*. Nevertheless, at the maximum dose of 5000 mg/kg some adverse effects were recorded such as hyperactivity, asthenia and piloerection (Table 3).

Table 3: Acute toxicity of the decoction *D. integrifolia*

Treatment	Doses (mg/kg)	Sex	M/T	Toxic symptoms
Distilled water		Male	0/3	Absent
		Femelle	0/3	Absent
<i>D. integrifolia</i>	2000	Male	0/3	Absent
		Femelle	0/3	Absent
<i>D. integrifolia</i>	3000	Male	0/3	Absent
		Femelle	0/3	Absent
<i>D. integrifolia</i>	5000	Male	0/3	Hypoactivity, asthenia, piloerection
		Femelle	0/3	Hypoactivity, asthenia, piloerection

M/T=Mortality over the Total number of treated rats; Absent = No sign of toxicity observed during the experimentation period (7 days). The rats in control group received distilled water (10 ml/kg) while the others received the decoction of *D. integrifolia* as a single oral administration at the following doses: 2000, 3000 and 5000 mg/kg.

Discussion

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system where it is involved in 70 % of excitatory neurotransmission (Cekic et al., 2005; Danysz & Parsons, 2012). Oral or intraperitoneal administrations of MSG have widely been used as an animal model to evaluate the beneficial effects of medicinal plants against glutamate-induced neurotoxicity and oxidative stress (Ganesan et al., 2013). In this study, we evaluated the effect of the decoction of *D. integrifolia*, a medicinal herb used by indigenous population in Cameroon to treat central nervous system disorders, against MSG-induced neurotoxicity and oxidative stress in mice. Seven days intraperitoneal injections of MSG in mice at the dosage of 2.5 g/kg resulted in toxic effect evidenced by the aggressiveness, fighting behavior, reduction in locomotion and muscle grip strength activities in the animals of the negative control group. Pretreatment of animals with oral administration of the decoction of the plant at the doses of 87.5; 175 and 350 significantly prevented these toxic effects of MSG. Oral administration of *D. integrifolia* before the administration of MSG reduces the aggressiveness of mice, inhibited

the fighting behavior and restored the locomotion and the forepaws muscle grip capacities. These results showed that *D. integrifolia* has neuroprotective effect against MSG-induced neurotoxicity. This neuroprotection of the plant against MSG neurotoxicity was comparable to that of MT a standard drug used in the treatment of Alzheimer disease and which has beneficial effects against other neurodegenerative diseases (Danysz & Parson, 2012). The mechanism of action to explain neuroprotective action of MT is atypical and involves the blockage of the NMDA receptor channel without a total inactivation of the neurotransmission (Wenk et al., 2006; Danysz & Parson, 2012).

Apart from behavioral changes, one-week intraperitoneal administration of MSG glutamate in mice resulted in brain oxidative stress evidenced by the results of the dosage of reduced glutathione and malondialdehyde level. In fact, the brain level of GSH was reduced in the brain of animals of the negative control while the amount of malondialdehyde was elevated in the negative control when compared to those of animal in the distilled water group. Our results are similar with earlier works, which found that MSG administration in rodents could lead to the reduction of antioxidant enzymes such as glutathione and consequent accumulation of reactive oxygen species and neurodegeneration (Ramanathan et al., 2007, Rajagopal et al., 2013). The pretreatment of animal with the decoction of *D. integrifolia* at the doses of 87.5 and 175 mg/kg significantly inhibited the MSG induced oxidative stress. This is visible through the elevated amount of GSH in the brain; and the reduced level of MDA level is a marker of brain lipid peroxidation level. The effect of *D. integrifolia* was comparable to that of MT (20 mg/kg p.o). These findings are in line with literature, which reported that MT could prevent neurons against oxidative stress induced by MSG. In fact, in case of overstimulation of NMDA by glutamate, MT blocks the receptor channel. This prevents calcium influx to the postsynaptic receptor and subsequent excitotoxicity and oxidative stress (Danysz & Parson, 2012, Rajagopal et al., 2013). The results of the phytochemical analysis of *D. integrifolia* revealed the presence in the decoction of the plant of some metabolites such as flavonoids, tannins and anthraquinones. Therefore, it can be hypothesized that the beneficial effects of *D. integrifolia* against MSG-mediated neurotoxicity in mice could be explained by its antioxidant properties since these types of secondary metabolites are known to have good antioxidant capacities and have also been reported to be beneficial in neurodegenerative diseases (Nehlig, 2012; Swamy, et al., 2013). The results of the acute toxicity of the decoction of *D. integrifolia* showed that the lethal dose 50 of the plant is higher than 5000 mg/kg thus *D. integrifolia* is safe for human consumption (OECD, 2011).

Conclusion

At the end of this study, we can conclude that the decoction of *D. integrifolia* counteracts MSG-induced hyper excitation, alteration in behavior and oxidative stress. These results can at least explain the wide use of this herb in traditional medicine in the treatment of central nervous disorders. Nevertheless, to understand the exact mechanism by which this plant exerts neuroprotection, other specific animal models of central nervous system disease are needed.

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Conflict of Interest Disclosure: The authors declare no conflict of interests.

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