

# Protective effect of ethanolic extract and raw juice of *Solanum betaceum* on aluminum induced oxidative stress and associated memory deficits in rats

Anne P.N. Kengne<sup>1,2</sup>, Rodrigue Tidjong<sup>1</sup>, Dieudonné Kuate<sup>1,2</sup>, Hermine T. Doungue<sup>1</sup>, Prosper N.Takam<sup>2</sup> and Inelle Makamwé<sup>2</sup>

## Abstract

**Background:** Oxidative stress is known to contribute to the mechanisms underpinning the pathogenesis of neurodegenerative diseases. Previous studies have identified the presence of flavonoids as the major constituents of *Solanum betaceum* (SB) with antioxidant activity. This work aims at investigating the protective effect of ethanolic extract and raw juice of *Solanum betaceum* (SB) on aluminum induced oxidative stress and associated memory deficits in *Wistar rats*.

**Methods:** Memory impairment was induced by aluminum chloride (4.2 mg/kg, i.p.) for 28 days. Memory function was assessed by Morris water maze test and the radial labyrinth tests.

SB raw juice (5 mL/kg, p.o.) and ethanolic extracts (200 and 400 mg/kg, p.o) were administered to rats for 28 days along with aluminum chloride. Biochemical parameters of oxidative stress (malondialdehyde) and antioxidants (total protein, catalase activity, glutathione) were estimated in brain homogenate after the treatment.

**Results:** The results of this study showed that aluminum altered memory function and oxidative stress. SB ethanolic extract and raw juice showed a significant ( $p < 0.05$ ) improvement in memory dysfunction and oxidative stress by decreasing the time spent in the baited arm and the latency, reducing the malondialdehyde levels ( $p < 0.05$ ) and enhancing the total protein ( $p < 0.05$ ), glutathione levels and catalase activity ( $p < 0.05$ ) compare to negative control group.

**Conclusion:** The present study clearly demonstrated the beneficial effects of SB ethanolic extract and raw juice which reduced oxidative stress and memory dysfunction in rats, indicating that it may be used in neurodegenerative disease prevention and management

**Keywords:** Antioxidants; *Solanum betaceum*; oxidative stress; neurotoxicity; rats; aluminium chloride.

\*Correspondence: Tel: +237 699 277 229; Email: [kengneannepas@yahoo.fr](mailto:kengneannepas@yahoo.fr) (Anne Pascale Nouemsi Kengne)

<sup>1</sup>Research unit of Biochemistry, Medicinal Plants, Food Sciences and Nutrition, Department of Biochemistry, University of Dschang, P.O. Box 67, Dschang, Cameroon; <sup>2</sup>Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, University of Yaounde I, P.O. Box 8418, Yaounde, Cameroon.

Citation on this article: Anne P.N. Kengne, Rodrigue Tidjong, Dieudonné Kuate, Hermine T. Doungue, Prosper N.Takam and Inelle Makamwé. Protective effect of ethanolic extract and raw juice of *Solanum betaceum* on aluminum induced oxidative stress and associated memory deficits in rats. *Investigational Medicinal Chemistry and Pharmacology* (2019) 2(1):27. Doi: <https://dx.doi.org/10.31183/imcp.2019.00027>.



## Background

Oxidative stress is induced by an imbalanced redox states, involving either excessive generation of reactive oxygen species (ROS) or dysfunction of the antioxidant system. The brain is one of organs especially vulnerable to the effects of ROS because of its high oxygen demand and its abundance of peroxidation-susceptible lipid cells. Previous studies have demonstrated that oxidative stress plays a central role in a common pathophysiology of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [1]. Aluminium (Al) contributes to neuronal oxidative stress, by generating reactive oxygen species (ROS), which cause damage to various proteins, DNA and membrane lipids [2]. Al is the most common neurotoxicant [3], and the evidences about its implication in developing Alzheimer's disease are getting increased [4]. It was also found that this trivalent cation can participate as a factor in the development of neural tube defects in human. Many studies showed that there were neuropathological, neurobehavioral, neurophysical and neurochemical changes after Aluminium exposure [5]. Aluminium (Al) is the most abundant metal present on the earth's crust. It is extensively used in daily life and was found in drinking water probably due to water purification procedures [6]. The new 20th century industrial products containing aluminium salts like antiperspirants are another source of exposure; vaccines adjuvants, phosphate binders, dialysis, total parenteral nutrition solutions and foods provide easy exposure of Al to human being [7].

To protect against the deleterious effects of radical oxygen species (ROS), the body has a complex set of antioxidant defenses. In fact, antioxidants represent all molecules that can directly inhibit production, limit the spread or destroy active oxygen species [8]. Natural products derived from diets are known to exhibit a variety of biological effects including antioxidant [9]. Several studies have been conducted to prove the existence of a relationship between the prevention of neurodegenerative diseases and some foods. In France, large study was conducted on 3777 men and women over 65 ages to show that an average consumption of flavonoids (antioxidants) of 14.4 mg/day (mainly from fruits and vegetables) leads to a significant reduction in the risk of dementia [10].

These observations prompt us to focus on the fruit *Solanum betaceum* commonly called tree tomato whose phenolic content and antioxidant activity of fruit extract have been show by Asih et al. [11]. The present study was designed to evaluate the protective effect of *Solanum betaceum* against aluminium chloride (AlCl<sub>3</sub>) induced neurochemical alterations and memory deficits in rats and associated oxidative biochemical markers.

## Methods

### Animals

3-months *Wistar rats* weighing between 200 and 230 g were obtained from the Department Animal Centre and allowed to be accustomed to the new environment for 1 week. They were maintained in accordance with the guidelines of the OECD [12]. The animals were individually housed under controlled temperature (25 °C) and lighting (12-h light/12-h dark cycle) and had free access to water and diet. They were fed *ad libitum* a staple diet as formulated by Telefo [13]. The animal housing and handling were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines. All experiments were carried out according to the regulations and ethical approval

of the Experimental Animal Welfare and Ethics Committee of the Institution (No. 2017/056)

### Aluminum chloride

Aluminum chloride was purchased from local chemical suppliers for the induction of the oxidative stress.

### Plant material

*Solanum betaceum* fruits were purchased from a local market in the west region of Cameroon identified with the national herbarium under the number 34728 / HNC and transported to the laboratory.

### Preparation of *Solanum betaceum* raw juice

The fruits were washed, opened with a knife and then crushed to obtain a paste before pouring into a colander in a container and further stirred to recover the raw juice.

### Preparation of fruit extracts

The freshly harvested fruit was washed, cut and allowed to dry in its entirety (including all parts) in an oven at 45°C with ventilation. When dry, it is finely ground with a grinder and powder obtained were used for extraction. The extraction with solvents (aqueous, ethanolic and hydroethanolic) was carried out according to the method described by Iqbal and Bhanger [14]. Aqueous, ethanolic, and hydroethanolic (20/80) extracts were obtained by soaking 20 grammes of *Solanum betaceum* fruits powder in 600 milliliter of corresponding solvent (water, ethanol, and hydroethanolic mixture) for 24 h with gentle stirring, after which the mixtures were filtered using a Whatman No. 4 filter paper. The resulting filtrates were dried at 45 °C using an air oven to obtain the respective extracts.

### Determination of total phenol and flavonoids content of fruits extract

Total phenol levels of the various extracts were determined by the spectrophotometric method using the Folin-Ciocalteu reagent as described by Dohou et al. [15]. The content of flavonoids was determined according to the method described by Bahorun et al. [16].

### Evaluation of in-vitro antioxidant activity

The antioxidant activity of the various extracts was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl), as described by [17].

The ability of the extracts tested to reduce ferric iron (Fe<sup>3+</sup>) present in the K<sub>3</sub>Fe (CN)<sub>6</sub> complex to ferrous iron (Fe<sup>2+</sup>) was determined as described [18, 19]. The ability to inhibit the hydroxyl radical was carried as described [20].

### Induction of neurotoxicity and experimental protocol

Albino *Wistar rats* were selected. Aluminum chloride was dissolved in water and injected to rats through intraperitoneal route at a dose of 4.2 mg/kg body weight. Aluminum chloride was given for 28 days along with ethanolic extract and raw juice of *Solanum Betaceum* fruit.

Six groups of six animals each were used. AlCl<sub>3</sub> (4.2 mg/kg/ip) was administered daily to all groups except the normal animal group. The dose of AlCl<sub>3</sub> was selected on the basis of the literature reports [21]. *Solanum betaceum* ethanolic extract (200, and 400 mg/kg

body weight ) and raw juice (5ml /kg body weight ) was administered daily through oral route to different groups of rats for 28 consecutive days. Otherwise, positive control group received 200 mg vitamin C per Kg body weight and negative control group received no treatment during the administration of aluminum chloride.

#### Assessment of cognitive performance

The memory was evaluated using radial eight-arm maze (RAM) [22, 23] and the Morris water maze (MWM) . From the first to fourth day after 30 minutes of administration of AlCl<sub>3</sub> the rats were exposed to pre-training session using RAM and the Morris water maze (MWM). From the 6th to 10th day after 30 minutes of administration of Al, the rats were exposed to training session using RAM and the Morris water maze (MWM). On the 28 st day, retention of the learned task (memory) was recorded.

#### Biochemical assessment

After 28 days of treatment, animals were sacrificed under anesthesia using steam chloroform. Immediately after brain were removed carefully and homogenized in ice-cold phosphate buffer pH 7. The brain homogenate were centrifuged at 800xg for 5 min at 4 °C to remove the nuclear debris. The supernatant was used for estimation of malondialdehyde (MDA), total protein (TP), glutathion level GSH) and catalase activity (CAT).

#### Statistical analysis

Statistical analysis was performed using SPSS program version 21. *In vitro* experiments were performed in triplicate. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Bonferroni test used for statistical analysis of the mean difference among groups. The post hoc Tukey helped highlighting the significant differences between the threshold averages. Differences were considered significant at P < 0.05 (at 95% confidence interval

## Results and Discussion

#### Phenolic content of *Solanum betaceum* extracts

The ethanol extract had the highest phenol value (96.81 ± 3.84 mg gallic acid equivalent / g extract), and flavonoid value (15.67 ± 1.37 mg catechin equivalent / g extract) (Table 1)

#### *In vitro* antioxidant activities

*In-vitro* antioxidant analysis using DPPH method revealed that free radical scavenging was observed with all extracts but the significantly highest results were obtained using ethanolic extract (Figure 1). Figure 2 shows the evolution of the reducing power of different extracts of *Solanum betaceum* at different concentrations compared to that of vitamin C. The reducing power of ethanolic extract was greater than those of the hydroethanolic and aqueous extracts at 56.8 µg/ml. The ability to trap the hydroxyl radical of all extracts of *Solanum betaceum* is relatively low and does not differ significantly from one extract to another (Figure 3). The antioxidant activity of *Solanum betaceum* by scavenging free radical , hydroxyl radical and ion reducing power *in vitro*. The reducing

power of *Solanum betaceum* is link to hydroxyl group in phenolic compounds such as flavonoids . The increase in the amount of ferrous ion would reflect an increase in the reducing power of the extract. Therefore, antioxidants are considered oxidative reducers and inactivators [24]. It is important to maintain a balance between antioxidants and oxidants in living organisms, and increased intake of dietary antioxidants may help in maintaining an adequate antioxidant status.

#### Effect of *Solanum betaceum* ethanolic fruits extract and raw juice on aluminum spatial memory deficits

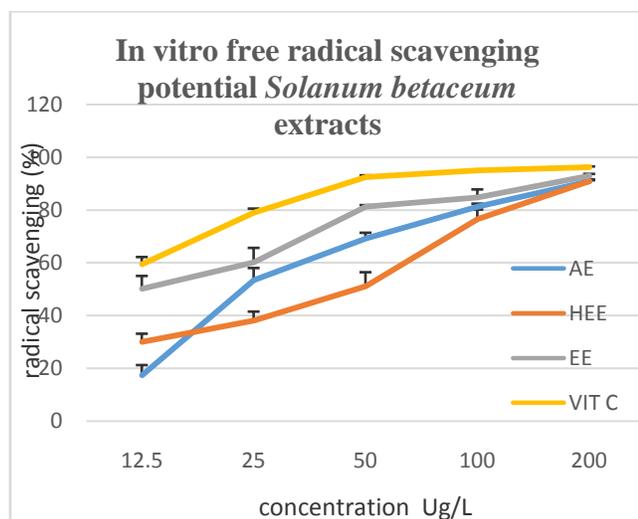
In the present study, administration of aluminum chloride to the control group resulted in a significant decrease in the average time spent in the correct (baited) arms during acquisition trials as compared to the normal group. This result shows a significant lack in the learning ability and memory development in the AlCl<sub>3</sub> treated group as compared to the normal group. Aluminium is well known to accumulate in brain tissue. Hippocampus, which is the site of memory and learning, is majorly affected, resulting in learning impairment [25]. Administration of *Solanum betaceum* ethanolic fruits extracts at doses of 200 and 400 mg/kg and raw juice resulted in a significant increase in the average time spent in the correct (baited) arms during acquisition trials as compared to the AlCl<sub>3</sub> treated group (Figure 4) indicating improvement in the learning ability and memory development under the influence of fruit extracts and raw juice. The best average time was obtained with raw juice followed by 200 mg/Kg extract means that raw juice consumption is the best way to prevent memory deficit.

Aluminium chloride treated animals showed an increase in escape latency during the training for spatial navigation task. From the results obtained, Figure 5 explains that there was a significant difference in the mean escape latency period of aluminium treated group when compared to the control group. The performances are analyzed in terms of average of the 4 tests. The analysis of the behavioral data confirms the existence of a reference memory deficit in rats treated with AlCl<sub>3</sub> compared to untreated. The AlCl<sub>3</sub> treatment generates a disturbance of the acquisition and the rats treated with *Solanum betaceum* fruit extracts and raw juice improved the memory capacities that can be noted by a reduction of the latency

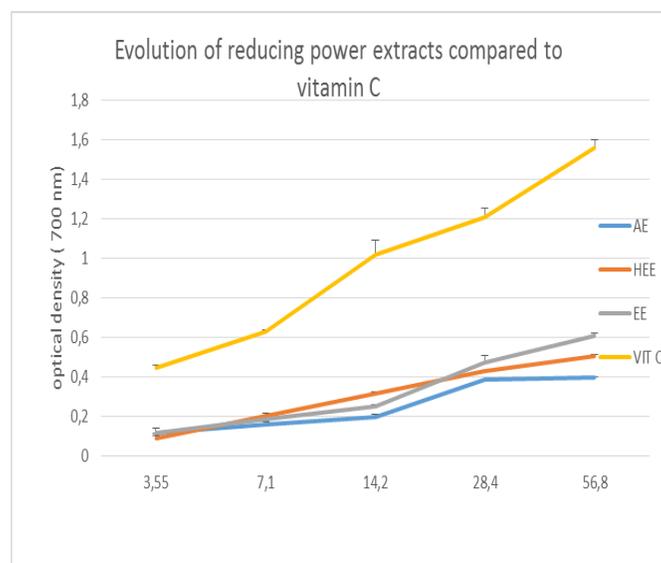
The significant decrease in the time spent in the baited arm clearly indicate impairment of learning ability. This findings are in concordance with previously documented effects of Al on neuroinflammation and associated spatial memory impairment [26] . In Morris water maze, aluminum exposure was associated with decrease spatial memory and this was evidenced by the results. Oxidative stress caused by the formation of reactive oxygen species, such as hydrogen peroxide, superoxide radical (O<sup>2-</sup>). The free radical production caused cell damage by damaging the DNA, cytosolic and membrane bound macromolecules. Free radicals have been linked to neurodegeneration and cognitive decline [27]. Accumulation of ROS in the brain has been reported to severely attenuate the neuronal functions forming a valid reason for cognitive impairment [28] . Moreover, oxidative stress severely overburdens the antioxidant defense system of the brain [29] , which further induces neurodegeneration and ultimately, memory impairment.

#### Effect of *Solanum betaceum* fruit extract and raw juice on aluminum chloride-induced changes in brain tissue biochemical levels

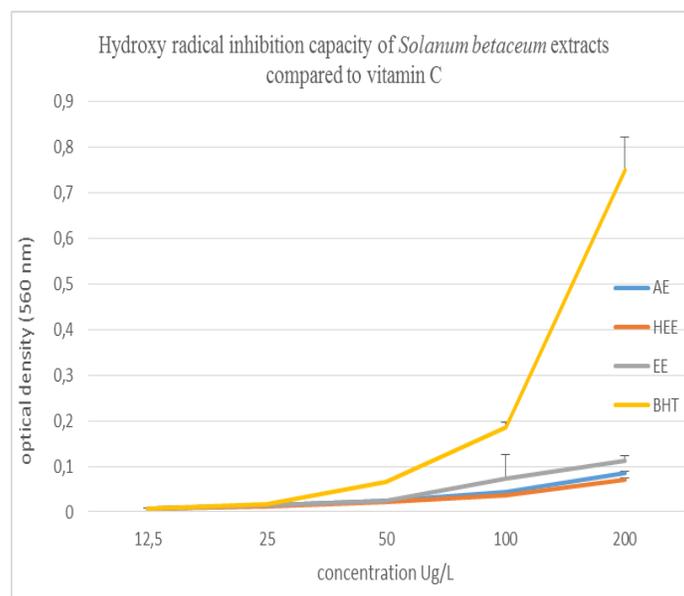
Al is considered as an etiological factor in a range of neurodegenerative disorders [30] there was a significant increase in the brain tissue malondialdehydes levels in the negative control group as compared to the normal group indicating a significant increase in the generation of free radicals in the brain tissue in the  $AlCl_3$  treated group. Administration of ethanolic extract of *Solanum betaceum* fruit and raw juice at doses of 200 and 400 mg/kg resulted in a significant decrease in brain homogenate with the best activity by raw juice (Figure 6). According to total protein we found that stress induction resulted in a significant ( $P < 0.05$ ) decrease in brain protein level of negative control group compare to ethanolic and raw juice treated rats (Figure 7). Stress induction resulted in a significant ( $P < 0.05$ ) decrease in brain catalase activity. Administration of vitamin C, different doses of extract and raw juice resulted in a significant ( $P < 0.05$ ) increase in brain homogenate catalase activity in all groups compared to negative control group (Figure 8). *Solanum betaceum* is an excellent source of minerals like calcium, cobalt, iron, phosphorus, potassium, sulfur, zinc, antioxidant vitamins such as A, B, C, E and antioxidant. Brain tissue glutathione levels increased significantly ( $P < 0.05$ ) in vitamin C treated group, and ethanolic extracts compared to negative control group (Figure 9). The phenolic compounds present in *Solanum betaceum* helps rid of free radicals thus improving memory. Ethanolic extracts and raw juice containing antioxidant properties is anticipated to exert neuroprotective effects via the regulation of cellular homeostasis and increase the self-defense to oxidative stress [31].



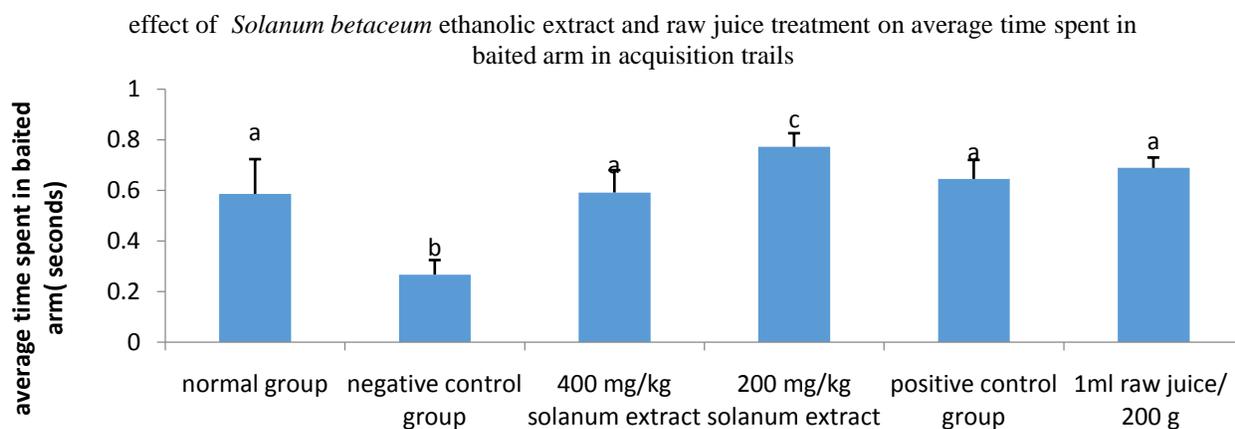
**Figure 1.** Evolution of the antiradical activity of *Solanum betaceum* extracts at different concentrations compared to vitamin C. AE: aqueous extract; HEE: hydroethanolic extract; EE: ethanolic extract; VIT C: vitamin C



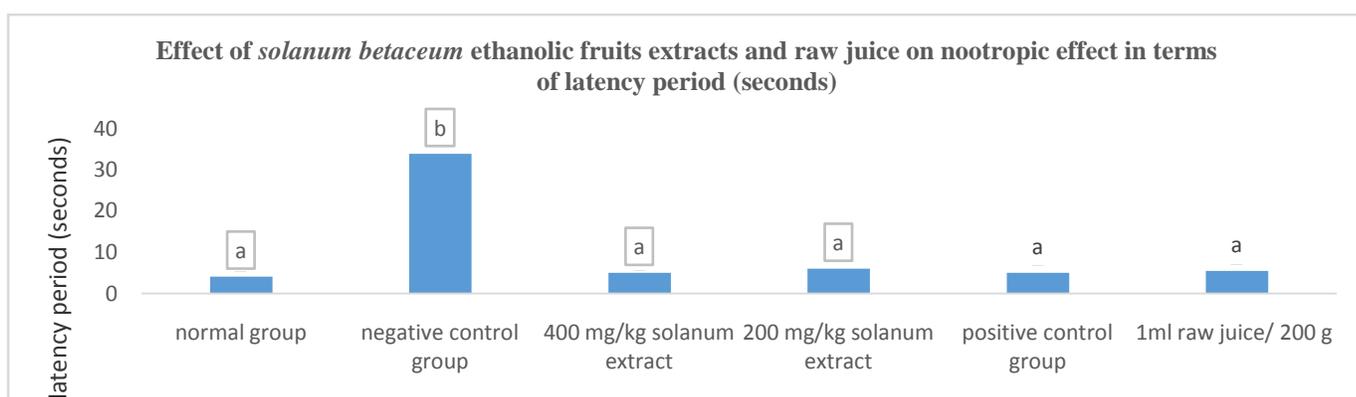
**Figure 2.** Evolution of reducing power of *Solanum betaceum* extracts compared to vitamin C. AE: aqueous extract; HEE: hydroethanolic extract; EE: ethanolic extract; VIT C: vitamin C.



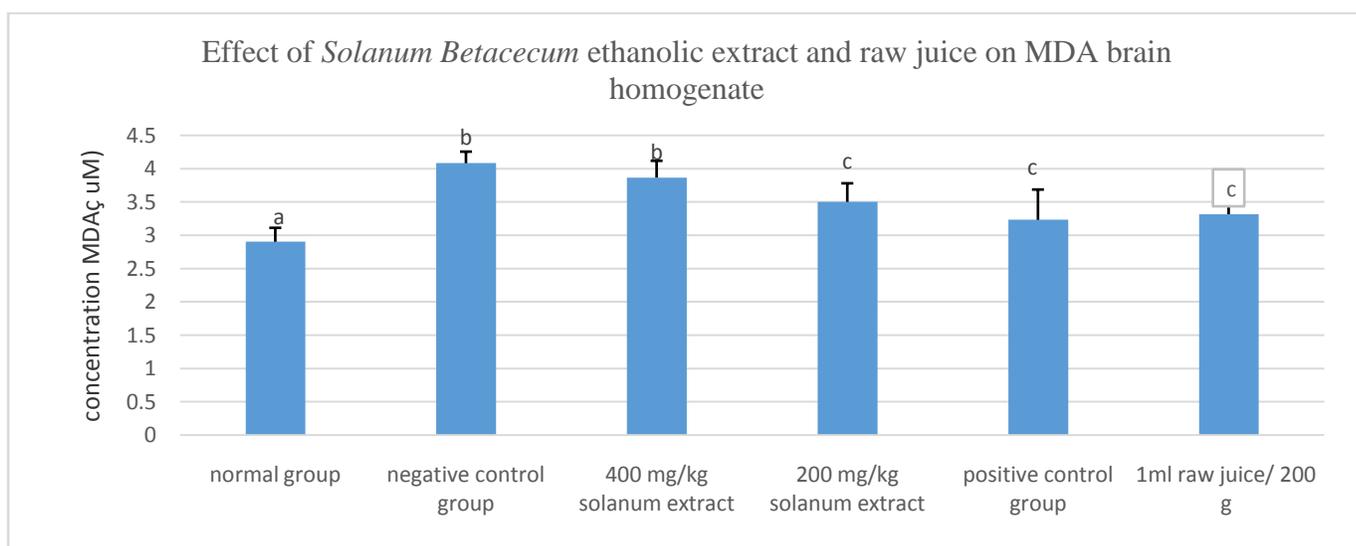
**Figure 3.** Evolution of hydroxyl radical inhibition capacity of *Solanum betaceum* extracts at different concentrations compared to vitamin C. AE: aqueous extract; HEE: hydroethanolic extract; EE: ethanolic extract; VIT C: vitamin C



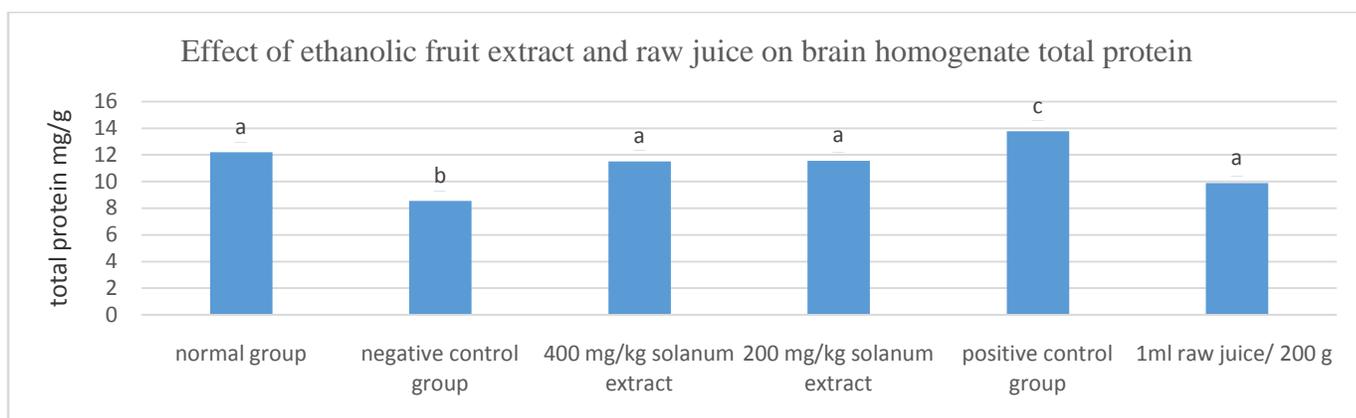
**Figure 4.** Changes in average time spent in baited arm (seconds) during acquisition trials of the normal, AlCl<sub>3</sub> extract and raw juice treated groups. Values with different letters differ significantly at P < 0.05.



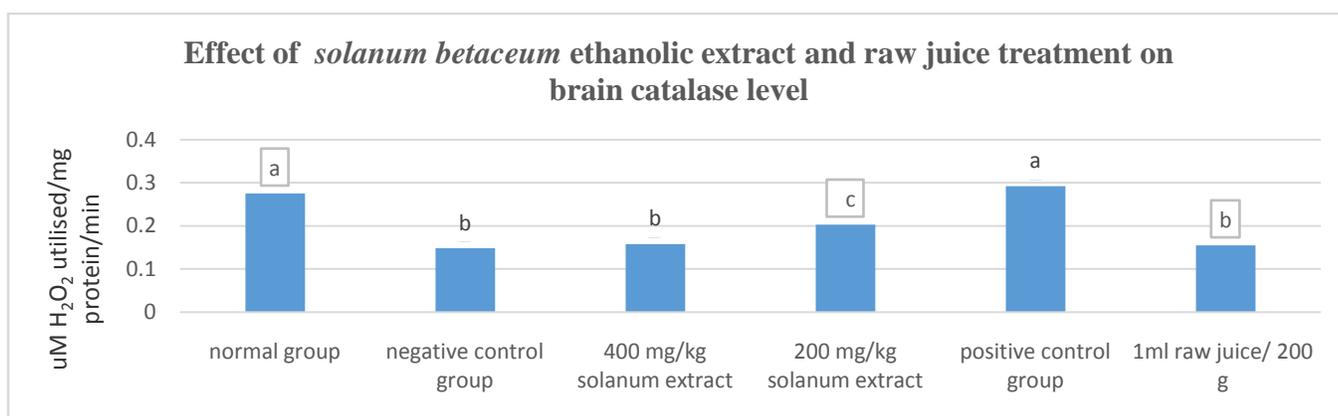
**Figure 5.** Effect of *Solanum betaceum* fruit extracts and raw juice on nootropic effect in terms of latency period (seconds) Values with different letters differ significantly at P < 0.05.



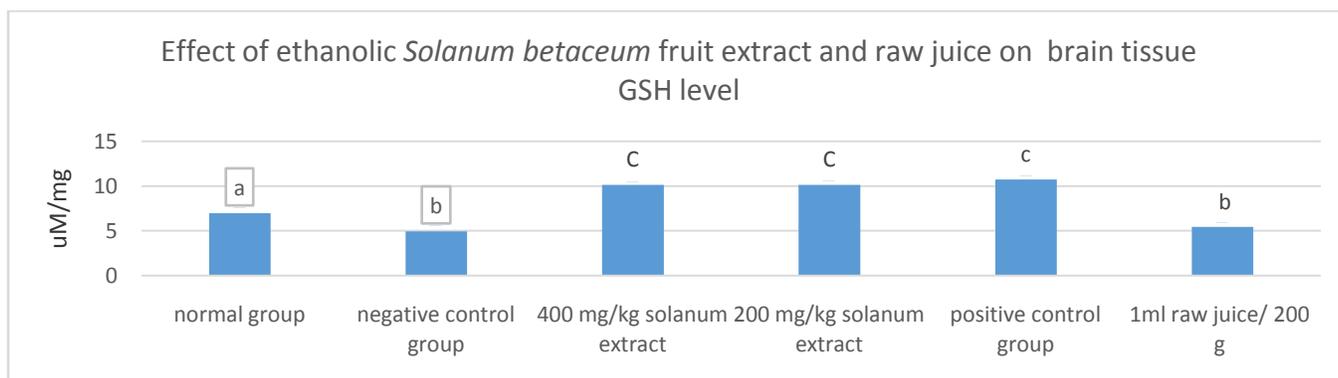
**Figure 6.** Effect of *Solanum betaceum* fruit extracts and raw juice on MDA brain homogenate. Values with different letters differ significantly at P < 0.05



**Figure 7.** Effect of *Solanum betaceum* fruit extracts and raw juice on brain homogenate total protein. Values with different letters differ significantly at  $P < 0.05$



**Figure 8.** Changes in brain homogenate catalase levels of the normal, AICI<sub>3</sub>, extract and raw juice treated groups. Values with different letters differ significantly at  $P < 0.05$ .



**Figure 9.** Changes in brain homogenate GSH levels of the normal, AICI<sub>3</sub> extract and raw juice treated-groups. Values with different letters differ significantly at  $P < 0.05$

**Table 1.** Total phenolic and flavonoids content of *Solanum betaceum* extracts

Extracts	Total phenolic (mg GAE/g of extract) content	flavonoïd (mg CATE/g of extract) content
Hydroethanolic (HEE)	32.27± 4.36 <sup>a</sup>	3.76± 1.32 <sup>a</sup>
Aqueous (AE)	40.45±1.9 <sup>b</sup>	7.52 ±2.65 <sup>c</sup>
Ethanolic (EE)	96.81±3.84 <sup>c</sup>	15.67± 1.37 <sup>b</sup>

GAE: gallic acid equivalent CATE: Catechin equivalent; Values with different letters differ significantly at P <0.05

## Conclusions

In conclusion, our study demonstrated that aluminum exposure was associated with impaired memory and cognitive functions in *Wistar rats*. Such situation was reversed by ethanolic *Solanum betaceum* fruit extract and raw juice. The results suggested that *Solanum betaceum* may have preventive and/or therapeutic potential in the management of aluminium induced oxidative stress. Raw juice of *Solanum betaceum* can be use as functional food for preventive aluminum induced oxidative stress and flavonoids from *Solanum betaceum* should be tested on aluminum induced oxidative stress

## Abbreviations

SB: *Solanum betaceum*  
 Al:aluminum  
 RAM : radial eight arm maize  
 MWM: morris water maze  
 MAD: malondialdehydes  
 TP: total protein  
 GSH: glutathione  
 CAT :catalase activity  
 ROS : reactive oxygen species

## Authors' Contribution

APNK defined intellectual content, drafted and review the manuscript; RT carried out the study; DK made the experimental design; HTN performed data analysis and statistical analysis; PTN did the data acquisition; IM made the literature search.

## Acknowledgments

The authors thank gratefully Professor Kuate Jules Roger and Professor Zambou Ngoufack Francois of the Department of Biochemistry, University of Dschang.

## Conflict of interest

The authors declare that there is no conflict of interest.

### Article history:

Received: 7 April 2019

Received in revised form: 26 April 2019

Accepted: 26 April 2019

Available online: 26 April 2019

## References

1. Geon Ha Kim, Jieun E. Kim, Sandy Jeong Rhie, and Sujung Yoon. 2015. The Role of Oxidative Stress in Neurodegenerative Diseases *Exp Neurobiol.* 24(4): 325–340
2. Shaw CA, Tomljenovic L. 2013. Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants and autoimmunity. *Immunol Res*;56(2–3): 304-16.
3. Kawahara M, Kato-Negishi M. 2011. Link between aluminum and the pathogenesis of Alzheimer's disease: the integration of the aluminum and amyloid cascade hypotheses. *Int J Alzheimers Dis.* 2011: 276393.
4. Walton JR. 2013. Aluminum involvement in the progression of Alzheimer's disease. *J Alzheimers Dis* 2013;35(1): 7-43
5. Walton JR. 2012. Evidence that ingested aluminum additives contained in processed foods and alum-treated drinkingwater are a major risk factor for Alzheimer's disease. *Curr Inorg Chem* 2012;2: 19-39.
6. Verstraeten SV, Nogueira LV, Schreier S, Oteiza PI. 1997 Effect of trivalent metal ions on phase separation and membrane lipid packing: role in lipid peroxidation. *Arch Biochem Biophys.* 338(1):121–7
7. Al-Kahtani MA. 2010. Renal damage mediated by oxidative stress in mice treated with aluminium chloride: protective effect of taurine. *J Biol Sci.* 10: 584-9
8. Ramírez-Altamirano MDJ, Fenton-Navarro P, Sivert-Chiñas E, Harp-Iturribarria FDM, Martínez-Cruz R, Cruz PH, et al. 2012. The relationship of aluminium and silver to neural tube defects; a case control. *Iran J Pediatr.* 22(3): 369-74.
9. Esposito E, Rotilio D, Di Matteo V, Di Giulio C, Cacchio M, Algeri S. 2012. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiol Aging.* 23:719–35.
10. Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues J. 2000. Intake of flavonoids and risk of dementia. *Eur J Epidemiol.* 16:357–63.
11. Asih IARA, Puspawati NM, Rita WS. 2017. *In vitro* evaluation of antioxidant activity of flavonoid compounds from *Terong belanda* (*Solanum Betaceum*, cav.) *Journal of Health Sciences and Medicine.* 1(2): 23-26.
12. OECD. 2008. Guidelines for chemicals trials: repeated dose oral toxicity studies for 28 days in rodents: OECD; p. 1–29. <https://ntp.niehs.nih.gov/iccvm/suppdocs/feddocs/oecd/oecd407-2008.pdf>
13. Telefo P. 1998. Contribution à l'étude des plantes médicinales du Cameroun :Influence de l'extrait aqueux du mélange des feuilles d'Aloe buettneri A. Berger .
14. Iqbal S, Bhangar M. I 2007. Stabilization of Sunflower Oil by Garlic Extract Under Accelerated Storage. *Food Chemistry* 100 (1): 246-254.
15. Dohou N., Yamni K., Tahrouch S., Idrissi-Hassani L., Badoc A., Gmira N. 2003 Screening phytochimique d'une endémique ibéro-marocaine, *Thymelaea lythroides*. *Bull. Soc. Pharm. Bordeaux* 142 : 61-78
16. Bahorun T., Gressier B., Trotin F., Brunete C., Dine T., Vasseur J., Gazin J., Boizot N., Charpentier J. 2006 Méthode

- rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre foustier. Le cahier des Techniques de l'Inra, Pp 79-82. *Bulletin de la société pharmacologique de Bordeaux* 142: 61-78.
17. Mensor L., Menezes F., Leitao G., Reis A., Dos Santos T., Coube C., Leitao S 2001 Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research* 15: 127-130
  18. Oyaizu M. 1986 Studies on products of browning reaction prepared from glucoseamine products derived from bees. *Journal of Pharmaceutical and Biomedical Analysis*.41: 1220-1234
  19. Hubert A. 2006 Caractérisation biochimique et propriétés biologiques des micronutriments du germe de soja. Étude des voies de sa valorisation en nutrition et santé humaine, Thèse de doctorat de l'institut national polytechnique de Toulouse, école doctorale des Sciences Écologiques, Vétérinaires, Agronomiques et Bioingénieries. p174
  20. Nagulendran K., Velavan S., Mahesh R., Haseena B. 2007. In Vitro Antioxidant Activity and Total Polyphenolic Content of *Cyperus rotundus* Rhizomes. *Journal of Chemistry* 4(3): 440-449.
  21. Rao DB, Kiran CR, Madhavi Y, Rao PK, Rao TR 2009 Evaluation of antioxidant potential of *Clitoria ternate* L. and *Eclipta prostrata* L. *Ind J Biochem Biophys*. 46:247-52.
  22. Srikumar BN, Raju TR, Rao BS. 2006. The involvement of cholinergic and noradrenergic systems in behavioural recovery following oxotremorine treatment to chronically stressed rats. *Neuroscience* 143:679-88.
  23. Titus ADJ, Rao BS, Harsha HN, Ramkumar K, Srikumar BN, Singh S, et al 2007. Hypobaric hypoxia-induced dendritic atrophy of hippocampal neurons is associated with cognitive impairment in adult rats. *Neuroscience* 145:265-78.
  24. Siddhuraju P., Becker K., 2007 The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp) seed extracts. *Food Chemistry* . 101(1):10-19.
  25. Veera Raghavulu Bitra, Deepthi Rapaka, Nalini Mathala, Annapurna Akula. 2014 Effect of wheat grass powder on aluminum induced Alzheimer's disease in Wistar rats *Asian Pac J Trop Med* . 7(Suppl 1): S278-S281.
  26. Rao SB, Chetana M, Uma, Devi P. 2005 Centella asiatica treatment during postnatal period enhances learning and memory in mice. *Physiol Behav* 86:449-57.
  27. Al-Amin MM, Reza HM, Saadi HM, Mahmud W, Ibrahim AA, Alam MM, et al. 2016 Astaxanthin ameliorates aluminum chloride-induced spatial memory impairment and neuronal oxidative stress in mice. *Euro J Pharmacol*. 777:60-9.
  28. Serrano F, Klann E 2004 Reactive oxygen species and synaptic plasticity in the aging hippocampus. *Ageing Res Rev*. 3(4):431-43.
  29. Massaad CA, Klann E 2011. Reactive oxygen species in the regulation of synaptic plasticity and memory. *Ant Red Sig* . 14(10):2013-54.
  30. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, et al. 2005. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* . 64(7):1152-6.
  31. Savory J, Herman MM, Ghribi O 2006 Mechanisms of aluminum induced neurodegeneration in animals: implications for Alzheimer's disease. *J Alzheimers Dis*. 10:135-44.
  32. Lucca G, Comim CM, Valvassori SS, Reus GZ, Vuolo F, Petronilho F, et al. 2009 Effects of chronic mild stress on the oxidative parameters in the rat brain. *Neurochem Int*. 54(5-6):358-62.