# Cognitive Disorders and Oxidative Stress Status Attenuated by Chrysophyllum Perpulchrum Extract in Alzheimer- like Rat Model of Intracerebroventricular $A\beta_{1-40}$ injection

## Pacôme Kouadio N'Go[1,2]*, Lazare Tehoua[1], Omar Touhami Ahmed Ahami[2], Fatima-Zahra Azzaoui[2] and Youssef Aboussaleh[2]

[1]Peleforo Gon COULIBALY University,Training and Research Unit of Biological Sciences, P.O.Box 1328, Korhogo, Ivory Coast.
[2]Clinical and Cognitive Neurosciences Group, Biology and Health Lab, Ibn Tofail University, P.O.Box 133, Kenitra, Morocco.
*Corresponding Author E-mail: pacngo.neuro@gmail.com

**Chrysophyllum perpulchrum is an endemic medicinal plant used in ivorian tranditional pharmacopeiaeas antipyretic to heal malaria fever. Since three flavonoid compounds have been isolated, catechin and two procyanid in dimers, we are proposed to testthe neuroprotective effectiveness effects using a rat model of Alzheimer Disease (AD). Adult Wistar rats were used as model.Sham-operated rats as controlwere injected by intracerebroventricular route (i.c.v) with1% ammonia(Group1), Aß rats were microinjected with 10μg/side (i.c.v route, (Group 2)).From 14th day post-surgery required for neuro inflammation and oxidative stress induction,some Aß-injected rats were daily treated with the extract (300 mg/kg bw, oral route, (Group 3)) for 21 days,sham-operated rats were treated only with plant extract (300 mg/kg bw, oral route, (Group 4)). Rats were then submitted to memory tests with Y maze, object recognition test and Morris water maze. Some oxidative stress markershave been assessed.AD-like rats exhibited significant recognition memory as well as learning and spatial memory deficits.The treatment of AD like-rats with methanolic extract of Chrysophyllum perpulchrum alleviated cognitive disorders by improving the memory recognition index and spatial learning strategy to find the hidden platform. Furthermore,Chrysophyllum perpulchrum extract prevented significantly Aß-induced lipid proxidation through a decrease of malondialdehyde (MDA) level in the hippocampus and the prefrontal cortex, and also helped to increase the non protein-thiol (NP-SH) antioxidant level.These findings suggest the neuroprotective actions of Chrysophyllum perpulchrum extract on AD-like rats. However,further pharmacological studies are needed to test ability of isolated compounds from Chrysophyllum perpulchrum to counteract full Aß physiopathology mechanisms before promising to be a drug candidate for AD treatment.**

**Keywords:** Alzheimer disease; brain; Chrysophyllum perpulchrum; oxidative stress; recognition memory; spatial learning.

Alzheimer Disease (AD) is the common form of dementia disorders which is mostly prevalent among ageing population through world. AD is a complex multifactorial neurodegenerative disease characterized by extracellular â-amyloid (Aâ) senile plaques deposition and intracellular neurofibrillary tangles of hyperphosphorylated Tau protein in selective brain areas as hippocampus

and cerebral cortex,[1] causing memory loss, progressive cognitive and mood disorders. According to one review of worldwide data, AD represents more than 60% of all dementia in developing countries including those of Sub-Saharan Africa (SSA),[2] and the global dementia in people over 60 years is approximately 1.6% in Africa compared to 5.9% and 6.4% in western Europe and north America respectively.[3] However, SSA countries show some rapid demographic and economic transitions, and that is accompanied by an improvement of lifestyle quality. It could project to an increase of the life expectancy and the incidence of age-related neurodegenerative diseases as AD during upcoming years. Although entire mechanisms of the Aâ neurotoxicity are still unclear, it has been considerably demonstrated that the oxidative stress status plays a critical role in the physiopathogenesis of AD leading to neuronal death.[4] Thus, a big challenge is to find out drugs with free radicals scavenging activity for helping to prevent cognitive and behavioral deficits related to oxidative stress. Today, the most drugs classes approved for transiently slow down AD progression are from synthetic chemicals,[5] with possible existing of side effects on health. However, medicines from natural products seem to be an alternative safety therapy approaches for AD treatment. From this perspective, several studies using cell culture or animal's model have been conducted with promise to find news drugs from medicinal plants targeting Aâ peptide and its subsequent pathophysiological effects.[6] Almost of all valuable researches testing pharmacological properties of medicinal plants extract against AD have used species originated from Asia. Paradoxically, African forests with a abundant and diversity of traditional use plants, scarce studies have so far focused the potential effects of their natural products as anti-AD. In this setting, we plan to promote the effectiveness of african tropical area endemic specie as *Chrysophyllum perpulchrum* (Sapotaceae) by using of an AD-like rat model. It is used as antipyretic for cure malaria fever in Ivory coast traditional pharmacopeia, and a provider of antioxidant source.[7]

Here, we tested whether methanolic bark extract of *Chrysophyllum perpulchrum* could have neuroprotective effects on memory deficits due to oxidative stress in a rat model of intracerebroventricular (i.c.v) Aâ $_{1-40}$ injection-induced AD.

## MATERIALS AND METHODS

### Animals

The experiment was carried out using adult Wistar rats locally obtained at the animals breeding house of Ibn Tofail University (Kenitra, Morocco). Animals were maintained for acclimation under controlled reference room at 22-25°C with a good relative humidity (50-60%), submitted to a 12 H light/ dark cycle and have free access to standard food (ALF SAHEL Company of Casablanca) and tap water. All experimental protocols were carried out according to NIH guide for the care and use of laboratory animals and approved by local Ibn Tofail university local ethic committee.

### Experimental Design
### Chemicals of plant material

Bioactive compounds from *Chrysophyllum perpulchrum* termed Catechin (P1) and two dimeric procyanidins (P2 and P3) (Fig.1) were chromatographically isolated and tested for free radicals scavenging activity using 2,2-diphényl-1-picrylhydrazyl, their activity is similar to quercetin.[7,8] The processes of methanolic, total phenolic contents or isolation compounds extraction have been described elsewhere.[7] Acute toxicity study estimated lethal dose 50 of *Chrysophyllum perpulchrum* to 1250 mg/kg of body weight (bw).[8]

### Aâ preparation and treatments

Synthetic Aâ $_{1-40}$ peptide (Sigma Aldrich, St. Louis, USA) were prepared as stock solution at concentration 1mg/ml in 1% ammonia, and aliquots were stored at - 20°C. Before use, Aâ $_{1-40}$ solution was aggregated by incubation at 37°C for 4 days.[9]

For the stereotactic surgery, animals were prior anesthetized with chloral (300 mg/Kg, i.p, in 7% solution) and were bilaterally i.c.v injected with Aâ at the lateral ventricle level ( coordinates Antero-poosterior = -0.8, Lateral = ± 1.4, Dorso-ventral = -3.4) with a 10-µl Hamilton microsyringe.

Animals were randomly divided into four experimental groups of 6-7 animals each: **(1)** Sham group (10µl of 1% ammonia by i.c.v route), **(2)** Aâ group (10µg/side of Aâ $_{1-40}$, i.cv route), **(3)** Aâ + CP rats (10 µg/side i.c.v route and treated with 300

mg/kg of methanolic bark extract of *Chrysophyllum perpulchrum*), **(4)** Sham + CP (Sham operated rat and treated 300 mg/kg of methanolic bark extract of *Chrysophyllum perpulchrum*).

The treatments with plant extract was done from 14th day post-surgery, a required period for Aâ to cause neuroinflammation and oxidative stress.[10, 11]

**Cognitive Testing**

**Y-maze**

It was used to evaluate spatial working memory in rodent,[12,13] after all the treatments. The apparatus was made with in fine-wood with three arms (A, B and C) measuring 40 in length, 10cm in width and 13 cm in height) and painted in different colour patterns. A central platform is formed by tri-angles of 120° between each arm. The procedure consists to give 8 min-session to each rat for exploring freely the maze. The sequence of arms entries was monitored with a camera video. An entry was validated when the four paws are within the arms. Alternation is defined as a triad of successive entry in different arms (*i.e.* ABCACBAACB= 5 alternations). Spontaneous alternation behaviour was calculated with following equation: % alternation = 100 x (number of alternation/ total arm entries – 2).

**Recognition Memory Test (NORT)**

The NORT is suitable to assess AD-related cognitive impairment.[14] The object recognition test procedure was conducted as describe by Ennaceur and Delacour.[15] The apparatus is an Open box with floor measurements 50 cm in length, 50 cm in width, and 40 cm in height walls. In the trial (familiarization session), rats were allowed 5 min to explore freely the box with two identical objects and return in their home cage. After 2 h delay, to evaluate short term recognition Memory (STM), rats were return in open field in which one object was switched by another one different for colour, sharpe and size, and experiment was repeated during 5 min with one novel object and one identical previously explored. To evaluate long -term memory (LTM) 24h later from the familiarization phase, rats were submitted to explore again two objects for 5 min, one identical and another novel one. Objects and box were cleaned with ethanol 70% during intertrial period. The exploration time of each object was recorded the video tracking, and the exploration feature is defined as the directing the nose at a distance less than 1 cm from the object. The ratio of preference of the novel object of each animal was calculated from the exploration frequency of novel object divided the total frequency spent for exploring both objects.

**Morris Water Maze (MWM)**

The MWM was performed to study spatial learning and memory. The apparatus was slightly modified of Morris Water maze test,[16] with circular open lightly grey pool (120 cm in diameter and 40 Cm in depth) filled with tepid water (22°C) at 30 Cm of width. It is divided into four virtual quadrants (North, South, West, and East) each including a visual cue outside the surface of water on the wall of the maze. In the habituation phase, animals were allowed 60 s to swim freely in the tank in order to define the best position of the platform which remained fixed during the all test sessions. Then, in the training phase, rats were left in the tank facing the wall and then allowed to swim to find the hidden platform submerged to 1 cm to the surface of water. If the animal did not find the platform after 60 s elapsed, it was gently guided to it and stay for 15s before returning in home cage. The testing was completed in five consecutive days including four trials each. The behaviour of rat was recorded by a video camera placed on the ceiling above the maze.

We considered the escape latency parameter as the time spent to find the platform.

**Oxidative stress level assay**

After behavioural testing, rats were anesthetized with choral with (300mg/kg) and killed by decapitation. Brain regions correspondent to Hippocampus and Prefrontal cortex areas were quickly removed and homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.4). The homogenate was then centrifuged at 3000 rpm for 30 min at 4°C to obtain a supernatant that was used.

**Analysis of Non-protein Thiols (NP-SH) level**

NP-SH level was assessed according to the method described by Ellman et *al.*[17] The supernatant was treated with 10% of trichloroacetic acid to precipitate proteins, and the sample was centrifuged at 2.000 rpm for 10 min. To the supernatant, was added 1 M of potassium buffer (pH 7.4) and 1mM of Ellman's reagent

(5,5-dithiobis-2-nitrobenzoic acid). The NP-SHs levels were determined at 412 nm and expressed as µmol/g of tissue.

**Determination of Lipid peroxidation level**

The assay Malondialdehyde (MDA) level, an important index of lipid peroxidation, was described in the method of Satoh *et al.*[18] Briefly, 500 µl of supernatant correspondent to sample of hippocampus or CPF was mixed with 1.5 ml of trichloroacetic acid (10%), vortexed and incubated at room temperature for 10 min. Then it was added to the mixture 1,5 ml of thiobarbituric acid (0.67%), and heated in boiling bath water for 15 min. After a cooling, 1.5 ml of n-butanol was mixed to the solution and strictly vortexed. The sample was centrifuged at 800 rpm for 5 min, and the supernatant was collected. The absorbance was determined spectrophotometrically at 532 nm. The results were expressed as MDA level nmol/g of tissue.

**Statistical analysis**

The experimental results data were expressed as means ± S.E.M (Standard Error of Mean). Statistical analysis were done using one-way analyses of variance followed by Tukey post-hoc test for multiple comparison, with value of $p < 0.05$ considered statistically significant.

**RESULTS**

Effects of Chrysophyllum perpulchrum extract on cognitive abilities in AD-like rats model induced by i.c.v $A\hat{a}_{1-40}$ injection

**Spatial working memory in Y-maze**

The i.c.v injection of $A\hat{a}_{1-40}$ caused an alteration of spatial working memory through a significant reduction of the percentage of spontaneous alternation behaviour ($p < 0.05$), when compared to the performance of sham-operated rats and treated with 300mg/kg of *Chrysophyllum perpulchrum* extract. However, even if, the treatment with 300 mg/kg of *Chrysophyllum perpulchrum* extract improved $A\hat{a}_{1-40}$-induced working spatial memory impairment that was not significant (Fig.2).

**Recognition memory in NORT**

As indicated on the Fig.3A, i.c.v injection of $A\hat{a}_{1-40}$ impaired significantly STM recognition performance in Aâ rats when compared to vehicle rats ($p < 0.01$) and sham operated rats treated with 300 mg/kg of crude extract ($p < 0.05$). The treatment of AD-like rats during 3 weeks with 300 mg/kg of crude extract of *Chrysophyllum perpulchrum* improved the short-term recognition capacity as reflected by an increase of the index.

Regarding to the LTM recognition, the treatment with *Chrysophyllum perpulchrum* extract prevented the impairments induced by Aâ i.c.v injection through a significant increase of recognition index above the threshold of 50% ($p < 0.05$) (Fig.3 B).

**Spatial learning and memory in MWM**

We used MWM to assess spatial learning and memory which are hippocampal functions dependents. Our results showed that the experiment groups performed differently to locate the hidden platform in the maze (Fig.4). The i.c.v Aâ injected rats learnt with difficulty to find the platform during training day sessions, remarkably at day 1 ($p < 0.001$), day 3 ($p < 0.01$), day 4 ($p < 0.001$), day 5 ($p < 0.001$) compared to others groups. On the other hand, the treatment of i.c.v Aâ rats with 300 mg/kg of plant extract over 3 weeks relieved significantly spatial learning and memory deficit. Interestingly, the latency time to find the platform was shortened by the training day's sessions in the rats treated only with 300mg/kg of *Chrysophyllum perpulchrum* crude extract.

Effects of Chrysophyllum perpulchrum extract on oxidative stress status in AD-like rats model induced by i.c.v $A\hat{a}_{1-40}$ injection

**Analysis of NP-SHs level**

In prefrontal cortex, a significant decrease of NP-SH level was found in the rats of Aâ group ($p < 0.001$) when compared to others ones. We noted that the Aâ rats treated with 300 mg/kg of crude extract of *Chrysophyllum perpulchrum* did not showed significant increase of NP-SH level. However, the highest concentration of the NP-SH level was found in sham-operated rats treated with 300 mg/kg of plant extract (Fig.5). The dosage of NP-SH in hippocampal area revealed the lowest level in Aâ rats. However, the given treatment with *Chrysophyllum perpulchrum* extract to Aâ rats caused significant increase of the level of NP-SH ($p < 0.01$, Aâ + CP vs Aâ group). The same observation was done in the sham-operated rats treated with extract of *Chrysophyllum perpulchrum* (Fig. 5).
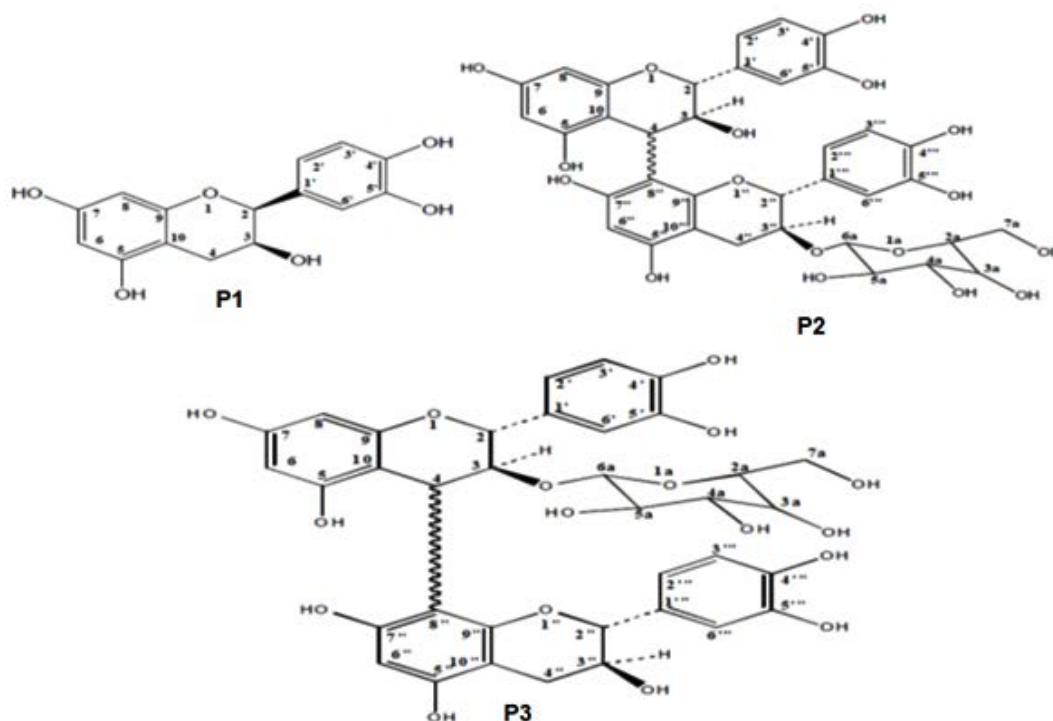
**Fig. 1.** Chemical structure of compounds isolated from *Chrysophyllum perpulchrum*
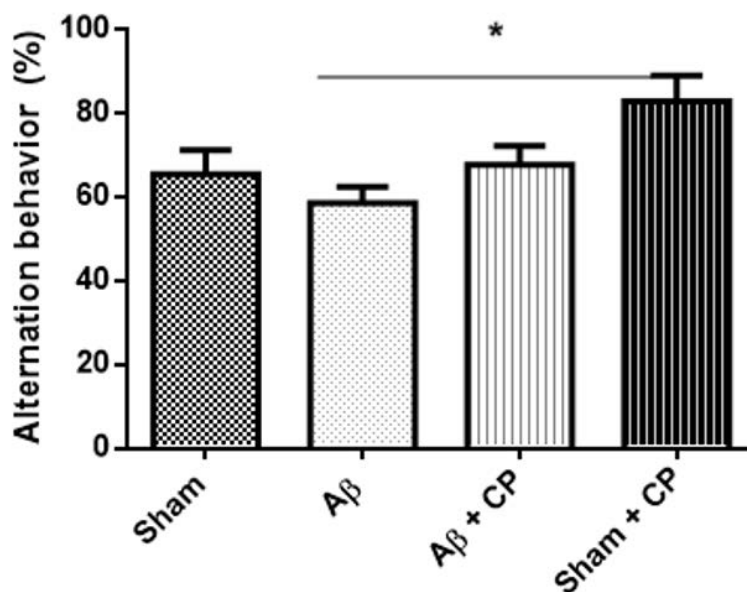


**Fig. 2.** *Chrysophyllum perpulchrum* extract effect on spatial working memory ability in an AD-like rat's model. Sham (control rats as vehicle, i.c.v injection with 10μl of 1% ammonia), Aâ (i.c.v injection Aâ 10μg by side ), Aâ+ CP (i.c.v injection of Aâ (10μg/side) and treated from 14th day post-surgery with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks), Sham + CP (sham-operated rats treated with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks). Data are presented as mean ± SEM (one-way ANOVA/post hoc Tukey test  analysis). *p<0.05 (Aâ vs Sham + CP)
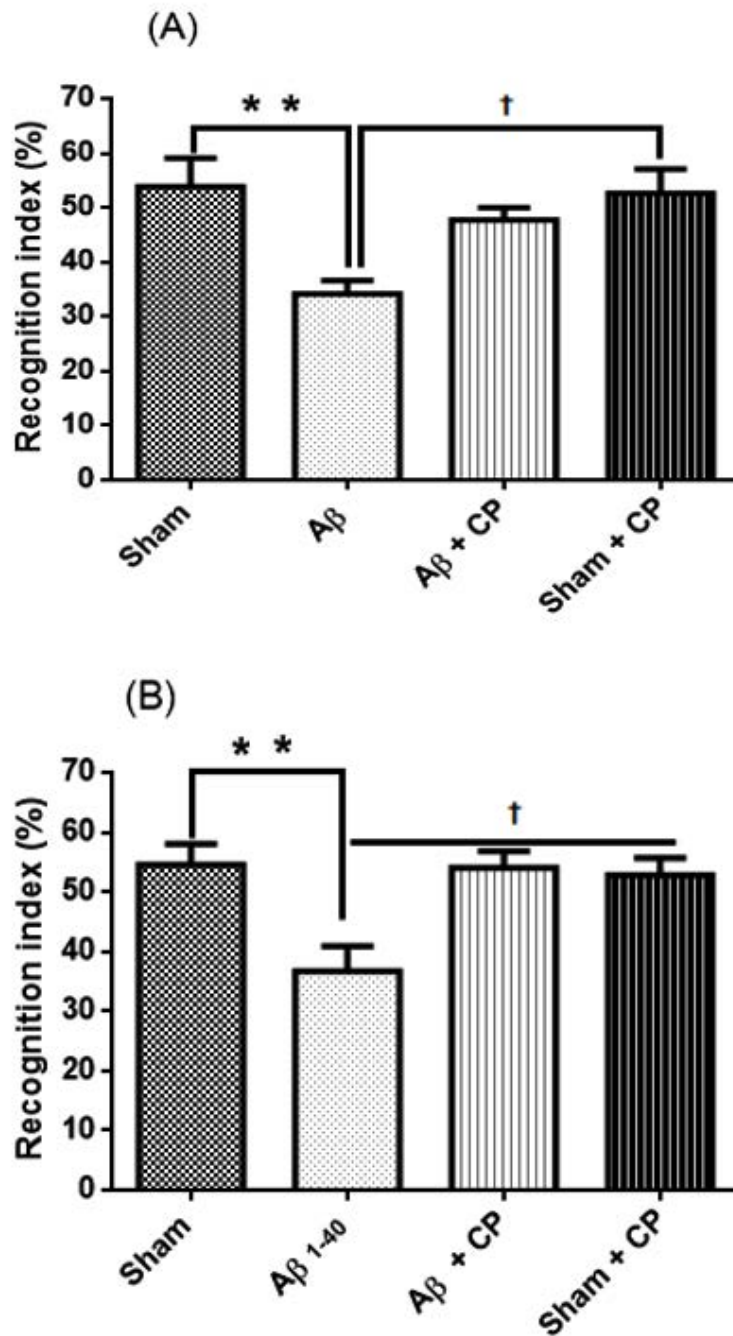
**Fig. 3.** *Chrysophyllum perpulchrum* extract effect on STM recognition **(A)** and LTM recognition **(B)** in an AD-like rat's model. Sham ( i.c.v  injection with 10μl of 1% ammonia), Aâ (i.c.v injection Aâ 10μg by side), Aâ + CP (i.c.v injection of Aâ (10μg/side) and treated from 14th day post-surgery with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks ), Sham + CP (sham-operated rats treated with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks ). Data are presented as mean ± SEM (one way ANOVA/post -hoc Tukey). **p<0.01 (sham vs Aâ ), [†] p<0.05 (Aâ vs Aâ + CP, and Sham + CP)

**Analysis of MDA level**

The i.c.v Aâ injection showed an increased level of lipid peroxidation which is reflected by high amount of MAD in both the prefrontal cortex and hippocampus (Fig.6). In the prefrontal cortex, there was very high significant increase of the MDA level in the Aâ rats group (p<0.001). The treatment with *Chrysophyllum perpulchrum* extract help to counteract the lipid peroxidation observed in Aâ rats. At the same manner, in hippocampus, there was high significant raising of MDA concentration in Aâ rats group (p<0.001), while the treatment with bark crude extract of *Chrysophyllum perpulchrum* avoided high level of lipid peroxidation. The sham-operated rats with plant extract treatment were not significantly affected compared to Aâ rats with plant treatment (p<0.05).

**DISCUSSION**

The increase of risk factors of AD sporadic form could rise the prevalence in SSA population during upcoming years. Thus, as none drug is yet efficient against AD, it becomes now worthwhile to conduct further experiments with goal to find drugs based on natural products from African's endemic medicinal plants. To our knowledge, there is no yet researches having studied pharmacologically and clinically the beneficial effects of any Ivoirian's medicinal plants in the neurodegenerative diseases context. The present study is a first one launched to evaluate whether methanolic extract of *Chrysophyllum perpulchrum* could relieve memory and cognitive deficits, and alleviate oxidative stress status in an AD-like rats induced by i.c.v Aâ$_{1-40}$ injection. We found as main findings significant deficits of short-term and long-term object recognition, and spatial learning and memory abilities in Aâ rats. However, the treatment of AD-like rats with crude extract of *Chrysophyllum perpulchrum* rescued cognitive impairments. Interestingly, AD-like rats treated with plant extract performed sometimes behavioral tests at the same manner as the rats of control or sham operated-treated with plant extract groups. Also, the treatment by *Chrysophyllum perpulchrum* extract prevents oxidative stress and lipid peroxidation in hippocampus or prefrontal
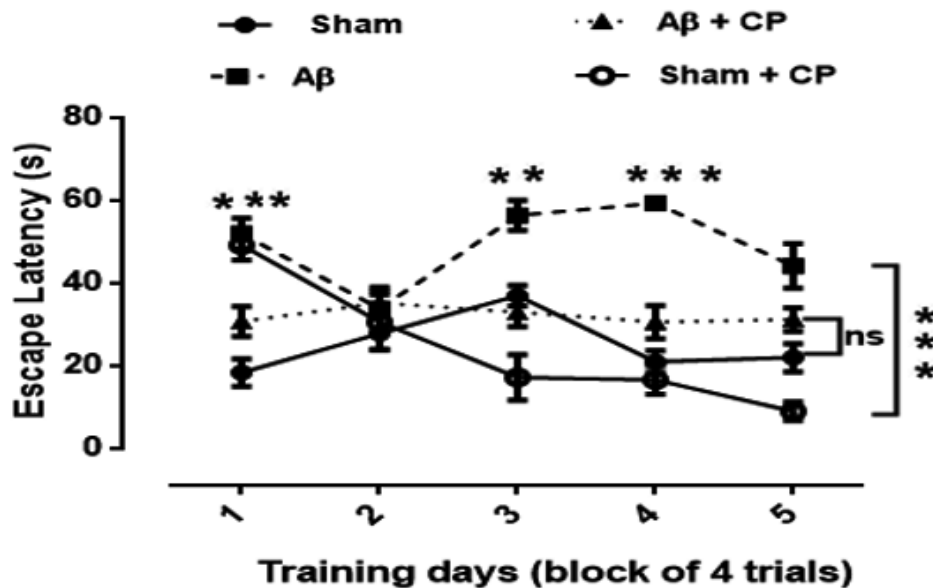


**Fig. 4.** *Chrysophyllum perpulchrum* extract effect on spatial learning and memory abilities in an AD-like rat's model. Sham (control vehicle, i.c.v with 10µl of 1% ammonia), Aâ (i.c.v injection of 10µg of Aâ by side), Aâ + CP (i.c.v injection of Aâ (10µg/side) and treated from 14$^{th}$ day post-surgery with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks), Sham + CP (sham-operated rats treated with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks). Data are presented as mean ± SEM (measure repeated ANOVA/post -hoc Tukey). ns, non-significant. **p<0.01 and ***p<0.001 (vs Aâ group)

cortex observed in AD-like rats. Here, we discuss our results with others possible actions supposed to be due to bioactive compounds from *Chrysophyllum perpulchrum* against AD.

It has been reported that object recognition task is a suitable behavioral test to evaluate AD-related cognitive impairments as well as

hippocampus-dependent spatial learning and memory assessed with MWM.[14] Poor performances of i.c.v Aâ rats compared to others in behavioral testing arise the question to know the chronology events occurred in the physiopathology mechanisms according to our experimental procedure. Firstly, we suggest that the target of one bioactive
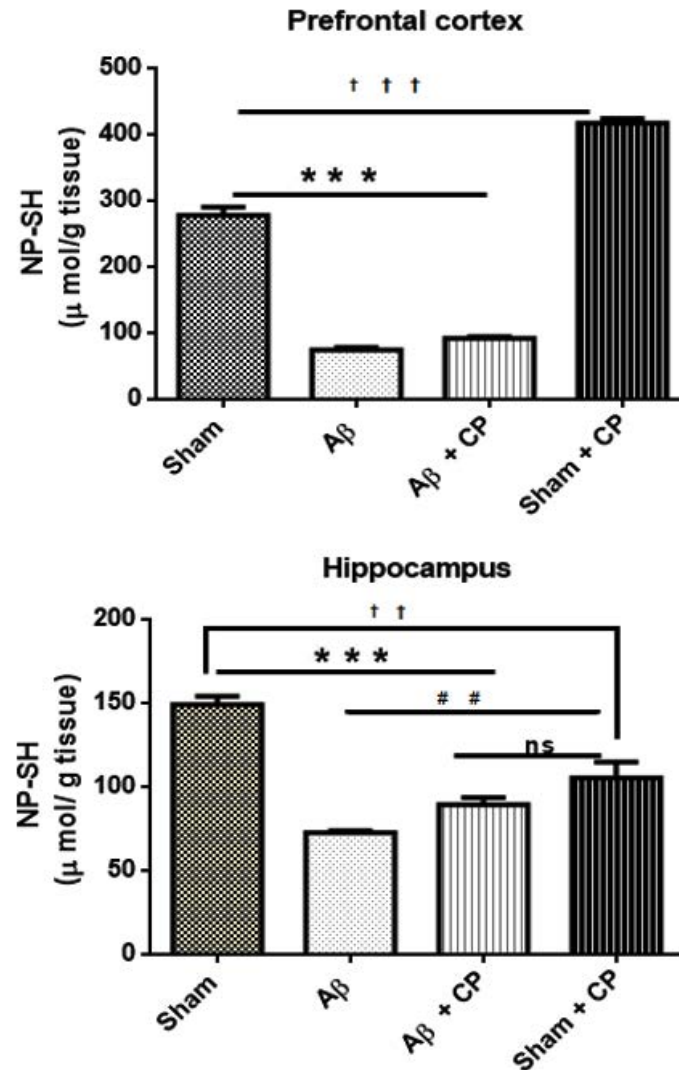


**Fig. 5.** *Chrysophyllum perpulchrum* extract effect on NP-SH level in the prefrontal cortex and hippocampus of an AD-like rat's model. Sham (control vehicle, i.c.v with 10µl of 1% ammonia), Aâ (i.c.v injection of 10µg of Aâ by side), Aâ + CP ( i.c.v injection of Aâ (10µg/side) and treated from 14th day post-surgery with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks), Sham + CP (sham-operated rats treated with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks). Data are presented as mean ± SEM (measure repeated ANOVA/post-hoc Tukey). ***p<0.001 (sham vs Aâ, and Aâ + C); †† p<0.01 and †††p<0.001 (sham vs Sham + CP); ## p<0.01 (Aâ vs Aâ + CP, and Sham + CP); ns, non-significant

component of *Chrysophyllum perpulchrum* namely catechin is AChE inhibitor. In fact, AChE is largely recognised to promote acceleration of Aâ sheet polymerization, fibrillization and its aggregation in amyloid plaque.[19,20]

A previous study mentioned that polyphenolic compounds are well known to inhibit AChE which is a key enzyme in AD.[21] More specifically, Olasehende et *al* [22] when testing the anti-amyloidogenic effects of Catechin and derived compounds isolated from seaweeds, have *in vitro* highlighted a significant reduction of aggregated Aâ$_{1-42}$ level. Another experiment using *in vivo* mice's model of AD confirmed that
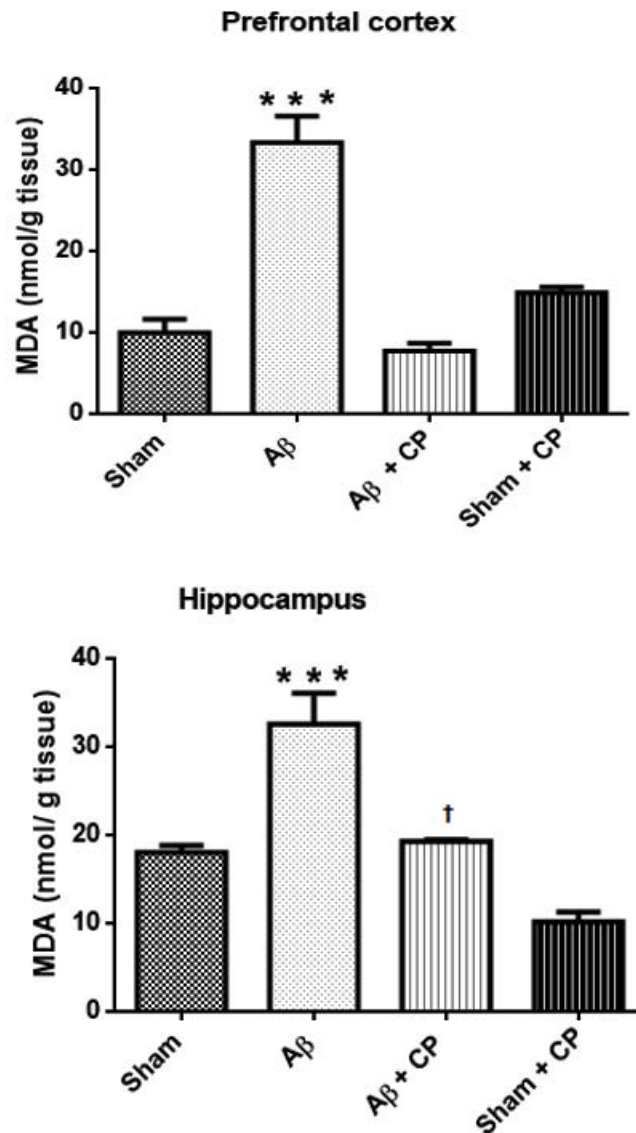


**Fig. 6.** *Chrysophyllum perpulchrum* extract effect on lipid peroxidation in the prefrontal cortex and hippocampus of an AD-like rat's model. Sham (control vehicle, i.c.v with 10µl of 1% ammonia), Aâ (i.c.v injection of 10µg of Aâ by side), Aâ + CP (i.c.v injection of Aâ (10µg/side) and treated from 14th day post-surgery with 300 mg/kg of extract of *Chrysophyllum perpulchrum* for 3 weeks), Sham + CP (sham-operated rats treated with 300 mg/kg of *Chrysophyllum perpulchrum* extract for 3 weeks). Data are presented as mean ± SEM (measure repeated ANOVA/post-hoc Tukey). ***$p<0.001$ (vs Aâ); † $p<0.05$ (Aâ + CP vs sham + CP)

catechin from *Rhizophora mucronata* enhanced cognitive functions by inhibiting AChE and others cholinesterases.[23] The neuroprotective effects of *Chrysophyllum perpulchrum* extract on memory performances suggest partly to be related to its effect on Aâ clearance. It is clearly established that Aâ mediates oxidative stress by different processes leading to production of ROS like superoxide anion radical, hydrogen peroxide or hydroxyl radical among others.[24] Oxidative stress occurred when the level of free radicals generated by Aâ exceeds the cells natural antioxidants including glutathione and antioxidant enzymes as superoxide dismutase, catalase and glutathione peroxidase. Oxidative stress in brain is a major factor that underlies neurotoxicity in the AD, with consequence of cells damage or death by apoptosis.[25] An approach of supplementation with exogenous antioxidants from natural substance may be effective to prevent Aâ-induced toxicity or halt AD progression.[26] In our study, there was an increased of NP-SH level (90% represented by gluthation) in hippocampus of Aâ rats after treatement with *Chrysophyllum perpulchrum* extract, when compared to Aâ rats without treatment. However, we found that this difference was less observed in the prefrontal cortex. Our results are in agreement with previous study that showed significant increase in glutathione in a rat's model of AD treated with natural antioxidant plant *Cantella asiatica*.[27] In fact, it has been demonstrated that Aâ is able to cause high-depleted level of glutathione in brain and reduce synaptic density.[28]

It plays neuroprotective role from oxidative stress by reacting with prooxidant hydroxyl peroxide to give rise to the oxidized form of glutathione. We can suggest that active compounds of *Chrysophyllum perpulchrum* extract help to combate Aâ$_{1-40}$-induced oxidative stress by enhancing NP-SH level. In another way, high concentration of NP-SH in hippocampus is thought to be linking to its great vulnerability to oxidative stress,[29] it is the primary brain site affected in AD condition before extending toward the cerebral cortex for advanced cognitive disorders. Healthy brain contains much macromolecules which are susceptible to oxidative stress. One of subsequent effects of Aâ-facilitated extensive oxidative stress is peroxidation of membrane lipids, leading to neuronal damage and death by apoptosis.[25] It has

been clearly found a positive correlation beetwen Aâ plaques and lipid peroxidation in AD brain.[30] Our experiment highlighted high level of MDA in hippocampus and prefrontal cortex in Aâ rats, certainly due to high lipid peroxidation of neuronal membranes. However, the treatment with *Chrysophyllum perpulchrum* extract attenuated Aâ$_{1-40}$-induced membrane lipid peroxidation. A significant lessening of MDA level was observed in brain of Aâ rats treated with plant crude extract. Similar results were reported by Schimidt et al [31] which sustain that catechin from *Camellia sinensis* avoids lipid peroxidation in AD rat model, and relieves oxidative stress and memory deficits. Another key toxicity action of Aâ is based on its high affinity toward redox-active metal copper or iron which releases hydrogen peroxide and hydroxyl and responsible for membrane lipid peroxidation.[32]

The supplementation of catechin contained in *Chrysophyllum perpulchrum* extract could be considered as potent redox metal-chelator for the prevention membrane peroxidation and cognitive disorders related to AD. Otherwise, oxidative stress condition promotes progressively the neuronal loss mainly by apoptotic pathway.[33] In this sense, a previous study investigating the beneficial effects of Catechin-rich from methanolic extract of *Rhuzophora mucronata* demonstrated an inhibition of hippocampal neurons apoptosis by down–regulating caspase 3 in a mice model of i.c.v administration of aggregated Aâ.[23]

We remind that *Chrysophyllum perpulchrum* extract contains two dimeric procyanidin compounds.[7] An important factor suggested to prevent Aâ-induced cognitive deficits is probably the neuroprotective effects of procyanidin. For this purpose, procyanidin B1 from a medicinal plant (*Uncaria hook*) suppressed actively Aâ oligomeric-induced neurotoxicity, their neuroprotective action consists so to attenuate activation of Caspase 3 by inhibiting the pro-caspase 8 or 9.[34] All results regarding *Chrysophyllum perpulchrum* or others plant species mentioned rich in catechin and procyanidin suggest that this should be proposed as phytotherapeutic drug for AD because of their antioxidante, anti-apoptosis, and likewise their anti-AChE properties.

Although we found some neuroprotection effects of crude extract of *Chrysophyllum*

*perpulchrum sp* on memory and cognitive performances in i.c.v injected Aâ rats, we cannot exclude the possibles mechanisms of Aâ clearance from lateral ventricle in order to validate our experimental model. The processes of Aâ influx or efflux beetwen brain parachyma and blood-cerebrospinal fluid via brain barriers remains poorly understood as well as the threshold amount of Aâ level to provoke AD. It has been experimented in mice's model of AD that the efflux of Aâ from central nervous to plasma increasing the blood baseline level 200 pg/mL to 5-10 ng/mL ((0.5-1).$10^{-2}$ μg/μl) within 24 h.[35] That suggest that the burden of i.c.v Aâ injected in our experiment (10μg of Aâ/side) could be sufficient to extend half-life avoiding so a faster Aâ clearance. However, some authors conclude their experiment *in vitro* by suggesting that choroid plexus of CSF reduces potentially Aâ from normal brain or AD brain because that area contains potent mechanism of Aâ efflux via lipoprotein receptor-related protein,[36] as also early demonstrated in vivo.[37] Another limit is the effectiveness of therapeutic concentration of bioactive compounds catechin and procyanidins contained in the dose of *Chrysophyllum perpulchrum* (300mg/kg for 21 days) used in our experiment. Even if we found positive cognitive and antioxidant outcomes with our medicinal plant, some pharmachokinetic properties including bioavailibilty and excretion must be take account. In fact, none study regarding catechin or procynadin from *Chrysophyllum perpulchrum* has been addressed in this sense, but a past report revealed for instance that by oral route catechin from green tea reached a blood peak 1-2 hours followed by an elimination by 5-6 hours.[38] Those authors suggested that an administration every 4 h should allow to maintain high blood level of catechin. It could be interesting to investigate using larger or lower concentration, and duration of treatment in order to further appreciate the dose and time-dependant effects.

## CONCLUSIONS

The present study is a first to promote neuroprotective actions of ivorian's traditional plant *Chrysophyllum perpulchrum sp* using AD-like rat model induced by i.c.v Aâ injection. We can predict that the bioactive compounds catechin and dimer procyanidins (catechin + Hexose) act to attenuate oxidative stress status and relieve memory deficits in the AD condition. However, the challenge remains to tackle its full pharmacological targets.

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