Neuroprotective impact of *Ximenia americana* aqueous bark extract on Diazepam-induced memory impairment in mice via its antioxidant potential

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**Abstract**

In traditional medicine, *Ximenia americana* (XA) is used to treat mental disorders, and headaches. The current study aimed to show the preventive and biochemical impacts of XA aqueous extract on diazepam-induced amnesia. Mice were randomized as follows: distilled water (10 mL/kg); diazepam (3 mg/kg); piracetam (PIR) (150 mg/kg); and XA experimental groups (25, 50 and 75 mg/kg). Mice were then treated in groups, 14 straight days. Radial arm maze (RAM) and T-maze were employed to assess different behaviours 30 min after each treatment. After the test was completed, the brains were isolated for histological and biochemical examinations. The results obtained showed that XA extract seriously (p < 0.001) reversed mistakes in working remembrance in the radial arm maze test contrasted to the normal control factions. In the T-maze test, pretreatment of mice with XA extract seriously (p < 0.001) expanded the time spent in the preferred arm when contrasted to the DZP-only treated faction. The XA-treated DZP groups showed subsequent (p < 0.001) improvement in catalase (CAT) and reduced glutathione (GHS). A diminish in malondialdehyde (MDA) level was observed in brain homogenates of mice treated with the extract contrasted with the DZP group. These few results regarding the neuroprotective and antioxidant effects of XA extract at least partially demonstrate its empirical use in the treatment of certain pathologies.

**Keywords:** amnesia; antioxidant; diazepam; neuroprotective; *Ximenia americana*

**Introduction**

Amnesia is defined as the pathological inability to integrate new information or to remember previously acquired information. Depending on the degree of impairment, it can be a symptom of brain dysfunction and is classified into categories ranging from moderate to more severe and permanent dementia (Healey and Kahana, 2016). Worldwide, approximately 46.4% of men and 53.6% of women suffer from amnesia, and it affects 40% of people over the age of 65 (Bartsch and Butler, 2013). According to Thakur *et al.* (2016),...
behavioral and/or neurological disorders account for 12.3% of the total disease burden worldwide. Although the causes of amnesia are not known, many drugs can interfere with cognitive functioning and especially memory function. Those that act on the brain can be particularly deleterious. Among the drugs, benzodiazepines are the most widely prescribed of the hypnotic, sedative, and anxiolytic group of drugs for the treatment of behavioral disorders (Cloos, 2016). In addition, they are one of the drugs with the most reported memory-related adverse effects (Tan et al., 2011). Some research results revealed that benzodiazepines induced anterograde memory loss in rodents through hyperpolarization of neurons and diminished excitability by lipid peroxidation and oxidative stress of neurons (Darinka et al., 2015). Indeed, diazepam at 2 and 2.5 mg/kg has been shown to impair learning and reminiscence (Beppe et al., 2020). Behavioral therapy and interpersonal therapy are increasingly used in depressed people, but 80% of patients are prone to relapse. In addition, the therapy of moderate and severe depression requires a combination of antidepressants, particularly benzodiazepines, with psychotherapy, which is effective only for mild forms of depression (Keck, 2010). Therapy of cognitive disorders is increasingly based on acetylcholinesterase inhibitors and N-Methyl-D-aspartate (NMDA) receptor antagonization (Upadhyaya et al., 2010). However, these treatments are only symptomatically and side impacts such as motor and psychological compilations, diarrhea, vomiting, insomnia, muscle cramps, and loss of appetite remain major problems for the population (Bae et al., 2019). Moreover, current treatments, promote neuronal regeneration (Wang et al., 2016).

Hence the interest in turning to herbal medicine, which is readily available to people and may have fewer side effects than synthetic drugs. Ximenia americana L. (Olacaceae) is a shrub whose roots are used in treatments such as headaches, and mental illnesses (Kefelegn and Desta, 2021). Bakrim et al. (2022) showed its anti-inflammatory. Furthermore, phytochemical studies of XA have shown that it has a pharmacological interest through the presence of compounds such as sterols, polyphenols, and flavonoids, molecules widely known for their antioxidant and biochemical effects on amnesia. Thus, the interest of this study focused on the preventive and antioxidant impacts of the water extract of XA stem bark on diazepam-induced amnesia in mice.

**Materials and Methods**

**Chemicals**

Diazepam, acetylthiocholine, 5,5-dithiobis (2-nitro-benzoic acid) (DTNB), 2-thiobarbituric acid (TBA), and piracetam were all ordered from Sigma Aldrich, USA.

**Plant material and extract preparation**

The stem bark of XA was harvested in 2018. The plant was then authenticated at the School of Fauna, where a reference number HEFG N°1918 was deposited. The bark was pulverized. One hundred and fifty (150 g) of bark powder was then mixed with 3 L of distilled water and boiled for 15 min. The resulting extract was filtered through a No. 4 filter paper and evaporated in an oven for 24 h at 45 °C.

**Phytochemical screening of the water of the root bark of Ximenia americana**

The determination of total polyphenols, total flavonoid, tannins, and saponins contents was carried out according to methods described by Vermerris and Nicholson (2006), Mimica-Duckic (1999), Bainbridge et al. (1996), Alilou et al. (2014) respectively. Reference compounds used were gallic acid, quercetin, catechin, and diosgenin respectively for total polyphenol, total flavonoid, tannins, and saponins.

**Evaluation of the in vitro antioxidant activity of the aqueous extract of Ximenia americana bark**

The iron-reducing antioxidant capacity (FRAP) and the anti-free radical activity with DPPH (2,2-Diphenyl-2-Picrylhydrazyl) were used to evaluate the in vitro antioxidant activity of the methanolic extract of...
**Ximenia americana.** The reducing power of iron (Fe$^{3+}$) was determined according to the method described by Soural *et al.* (2022) while the effect of the extract on DPPH was measured by the procedure described by Sanchez-Moreno (2002).

*Treatment of animals*

Male mice (25-30 g) aged 8-10 weeks old were used in this study. The animals were acclimated to the laboratory for 14 days, before the start of treatments. Mice were housed in polyacrylic cages under a natural light/dark cycle and were provided with water *ad libitum*. The treatment and care of the animals were carried out by the guidelines of the Bioethics Committee of Cameroon (Reg N° FWA-IR00001954) and following the NHI-Care and use of Lab animal manual (8th Edition). Each animal was subjected to a single behavioral test and efforts were made to reduce animals suffer. The mice were then separated into groups (n=6). The normal group received distilled water (DW, 10 mL/kg p.o), the negative category was treated with diazepam (DZP, 3 mg/kg p.o.), and the positive faction received piracetam (PIR 150 mg/kg, p.o.). The experimental factions received water extract of XA at doses 25, 50, and 75 mg/kg body weight, p.o., respectively, for 14 consecutive days.

**Behavioral tasks**

*Radial arm maze*

The labyrinth used had eight arms, numbered from 1 to 8 and the dimensions were (48 cm × 12 cm). Its arms extended radially from a middle region. The device was located on a Lab bench above the floor. Some of the arms had 50 mg of food at the end. Each mouse was maintained at 85% of its total body weight and was simultaneously placed in the central area of the maze. It was authorized to examine the environment for 5 minutes and to take food freely. To prevent the animal from seeing where it had previously searched, the food pellets were hidden behind small raised walls. The animals were accustomed for 7 days to visit the arms. The entry into an arm containing no bait was considered as a reference and while the entry into an arm containing bait but previously visited was considered as a working memory mistake (Beppe *et al.*, 2014). The time used to ingest all the foods was also noted.

*The T-maze test*

This labyrinth is a T-shaped device made of boards. It is made of a starting compartment and two others (30 cm × 10 cm × 25 cm). There are openings at the exit and entrance of each passage. This tool is widely used to assess the cognitive abilities of rodents (Deacon and Rawlins, 2006). Mice were placed one after the other in the box facing the central platform of the T-maze, and were required to explore the maze for 5 minutes. The procedure for assessing spatial memory consolidation was divided into three phases: habituation, acquisition, and retention phases, assessed daily, each, and a single free-choice trial. During habituation, the preferred and discriminated arms of each mouse were scored. 24 hours after habituation, mice were subjected to a forced-choice test, blocking the discriminated arm. An animal released in the start branch was allowed to explore the maze by entering the preferred branch and had to return to the start branch. During this time, all of the gates in the guillotine were open. When mice were released to explore all arms, time spent in one arm as well as the number of returns to the starting arms were recorded. To avoid place preference and olfactory stimuli, the inside of the maze was carefully cleaned after each mouse passed through it with 70% alcohol.

*Biochemical assays*

Hippocampi were extracted from brains, homogenized and centrifuged at 3000 rpm for 15 min. Malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GHS) and catalase (CAT) levels were analyzed in the collected samples. MDA concentrations were estimated according to the method related by Wills (1969). SOD activity was note according to Misra and Fridovich (1972). GHS was evaluated
using the essay by Fukuzawa and Tokumara (1976), and CAT activity was noted using the method estimated by Shina et al. (1972). Acetylcholinesterase quantities were measured in accordance with the protocol of Ellman et al. (1961). A portion of the supernatant was used to note dopamine levels (Ngatanko et al., 2019).

**Statistical analysis**

GraphPad Prism version 8.00 software for Windows was used to analyze the results. Results were expressed as mean ± standard error at the mean (SEM). Data were analyzed using one-way and two-way analysis of variance (ANOVA) followed by Tukey’s and Bonferroni’s post hoc tests, respectively. Results with p <0.05 were considered significant.

**Results**

**Phytochemical analysis Ximenia americana root bark methanolic extract**

The result of quantitative photochemical assay of the crude extract of *Ximenia americana* bark is reported in Table 1.

### Table 1. Content in mg/100 g DM of some compounds present in the aqueous extract of *Ximenia Americana* root bark

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polyphenols</td>
<td>54.52</td>
<td>mg eq Gallic acid / g DM</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>67.77</td>
<td>mg eq Quercetin / g DM</td>
</tr>
<tr>
<td>Saponins</td>
<td>45.68</td>
<td>mg eq galactose / g DM</td>
</tr>
<tr>
<td>Tannins</td>
<td>17.73</td>
<td>mg eq catechin / g DM</td>
</tr>
</tbody>
</table>

**Antioxidant activities of Ximenia americana aqueous extract**

DPPH and FRAP assays were used to measure the antioxidant potential of the extract. Results obtained, revealed that the extract has a DPPH inhibition percentage of 58.74% and a FRAP inhibition percentage of 51.32% compared to the positive control (BHT) which has an inhibition percentage of 61.04%.

**Impact of Ximenia americana on memory**

**Impact of Ximenia americana on short-term reminiscence in the radial arm maze**

Figure 1 depicts the influence of the aqueous extract of *X A* bark on working remembrance in the radial arm maze (RAM) essay. These data show that from day one to seven, the working memory errors were high in negative control group compare to normal. Pretreating animals at all doses seriously (p < 0.001) diminished memories mistakes compare with DZP-non-treated group. On day 1, the 25 mg/kg and 75 mg/kg test factions made subsequently (p < 0.001) fewer repeat visits to the baited arms than the normal group. This number of errors decreased from day 5 of the experiment until the end of the experiment (day 7). PIR on days 3 and 7 significantly (p < 0.01; p < 0.01) reversed the number of working memory errors compared to normal animals (1.2 and 1) respectively. (Figure 1A).

The impact of the aqueous extract of *X A* on the RAM from day 1 to day 7 showed significant diminish in reference remembrances mistakes in all treated factions. These reference memory mistakes diminished from an average of 2.4 ± 0.2 in the 25 mg/kg (p < 0.001, day 2) extract-treated group, 3.2 ± 0.2 in the 50 mg/kg (p < 0.01, day 4) extract-treated group, and 3.0 ± 0.2 in the 75 mg/kg (p < 0.001, day 7) extract treated group compared to negative group 4.6 ± 0.1, 3.6± 0.2 and 4 ± 0.2 respectively on seems days. On days 2, 6 and 7, the PIR-treated group reversed seriously (p < 0.01) reference memory mistakes contrasted to the normal group.
PIR-pretreated and all dose groups of XA aqueous extract subsequently (p < 0.001) reversed the effect of DZP on day 7 (Figure 1B).

Pretreatment of the animals subsequently expanded the time taken to ingest all the baits on days 2, 4, 5 and 7 at all the doses tested contrasted with animals given DZP and not treated with the extract. In the positive control faction, the PIR reference product significantly reversed time to bait consumption contrasted to negative control faction. (Figure 1C).

Figure 1. Effect of aqueous extract of *Ximenia americana* on working memory errors (A), reference memory error (B) and the time taken to consume baits (C) in the radial arm maze. Each point represents the mean ± S.E.M, (n= 6), ***p < 0.01 when compared to normal control, *p < 0.5, **p < 0.01; ***p < 0.001 when compared to diazepam treated group. DZP: Diazepam, PIR: Piracetam, XA 25, 50, 75: *Ximenia americana* (25, 50, 75 mg/kg).
Impact of Ximenia americana on long-term remembrance in the T-maze test

Induction of pathology with DZP to the negative control category seriously ($p < 0.05$) expanded the time spent in the discriminated arm (Figure 2A) when contrasted to the normal control group, with respective mean values of $59.6 \pm 1.59$ s and $44 \pm 3.02$ s. Going in the same direction, treatment of animals with aqueous $XA$ extract at all doses significantly ($p < 0.001$) (25 mg/kg; $p < 0.001$ for 50 and 75 mg/kg) reversed the time of visit in the discriminated arm with respective means of $36 \pm 4.07$ s; $15 \pm 2.08$ and $35 \pm 3.40$ s. The reference substance PIR, at a single dose of 150 mg/kg also seriously ($p < 0.001$) reduced the visit time in the discriminated arm with a mean of $29.40 \pm 4.28$ s. In Figure 2B, it was observed that; the DZP treated factions exhibited serious ($p < 0.05$) diminish time spent in preferred arm contrasted with normal factions. Premedication with 50 and 75 mg/kg of the extract noted a substantial time ($p < 0.001$ for 50 mg/kg; $p < 0.05$ for 75 mg/kg) spent in the preferred arm, with mean values of $138.4 \pm 6.7$ s and $96.8 \pm 6.95$ s, respectively, compared to the DZP-treated group, whose time was $33.6 \pm 5.81$ s.

![Figure 2. Impact of aqueous extract of Ximenia americana on long-term memory in the T-maze test. (A): Discriminated arm, (B): Preferred arm](image_url)

Each diagram represents the mean ± S.E.M. of 6 animals, *$p < 0.05$ when compared to normal control, **$p < 0.01$; ***$p < 0.001$ when compared to diazepam treated group. DZP: Diazepam, PIR: Piracetam, $XA$ 25, 50, 75: Ximenia americana (25, 50, 75 mg/kg)
Impact of aqueous extract of Ximenia americana on the latency and the number of returns to the starting arm

Figure 3A shows the latency in the T-maze. The latency to choose an arm was significantly \( p < 0.01 \) expanded in the DZP-treated factions contrasted to the normal group. The mean time was 6.8 ± 2.51 s in the normal group compared to DZP treated group with 44 ± 5.05 s. In contrast, the groups treated with aqueous XA extract at all doses subsequently \( p < 0.01 \) reduced the time to select an arm contrasted to the DZP group. The mean times for these respective doses were 6.4 ± 0.87 s, 4.6 ± 1.5 s, and 7.2 ± 2.05 s, compared to 44 ± 5.05 s in the DZP group.

In the number of returns to the starting arm (Figure 3B), the DZP-treated animals made more returns to the starting arm with a mean value of 6 ± 0.44 s contrasted to the normal group (3 ± 0.31 s) whose number of returns to the starting branch was subsequently \( p < 0.05 \) reduced. In addition, the animals treated with PIR had a seriously \( p < 0.001 \) reduced number of returns to the starting arm compared to the group treated with DZP with a mean of 1.8 ± 0.37 s contrasted to 6 ± 0.44 s. As observed above, animals treated with the extract noted a meaningfully diminishes number of returns \( p < 0.05 \) for 25 mg/kg; \( p < 0.001 \) for 50 and 75 mg/kg) compared with the group treated with DZP.

**Figure 3.** Effect of aqueous extract of *Ximenia americana* on the latency (A) and the number of returns to the starting arm (B) in the T-maze test

Each diagram represents the mean ± S.E.M of 6 animals. \( * p < 0.05 \); \( ** p < 0.001 \) when compared to normal control, \( * p < 0.5 \); \( ** p < 0.01 \); \( *** p < 0.001 \) when compared to diazepam treated group. DZP: Diazepam, PIR: Piracetam, XA 25, 50, 75: *Ximenia americana* (25, 50, 75 mg/kg)
Biochemical effect of water extract of Ximenia americana on amnesia in mice

Impact of aqueous extract of Ximenia americana antioxidant markers

The impact of water extract XA on SOD activity in the hippocampus of mice is shown in Figure 4A. Contrasted to the normal faction, these results revealed a subsequently ($p < 0.05$) diminish in SOD activity in the group treated with the inducer which is DZP. Pre-treatment of the animals with all doses of the extract seriously elevated SOD activity contrasted to the DZP-treated group. Regarding catalase, treatment with DZP, subsequently ($p < 0.001$) decreased CAT activity contrasted to the normal faction (Figure 4B). Pretreatment with extract resulted in a subsequent ($p < 0.001$) expand in CAT activity compared with the DZP-treated category. Figure 4C shows the impact of the aqueous extract of XA root back on the concentration of GSH. A serious diminish ($p < 0.001$) in GSH was observed in the DZP treated group ($0.01 \pm 0.01$) contrasted to the normal control ($0.03 \pm 0.00$). In contrast, the GSH concentration at all doses of the extract treatment was subsequently expanded ($p < 0.05; p < 0.01$) contrasted to the DZP-treated group.

The histogram in Figure 4D shows the impact of water extract of XA root on MDA concentration. A subsequent reduction ($p < 0.01$) in MDA concentration was noted in the DZP-treated category contrasted with the normal control. In contrast, the MDA concentration at all doses of the extract treatment was meaningfully reversed ($p < 0.05; p < 0.01$) contrasted to the DZP-treated faction.
Impact of water extract of Ximenia americana on the microarchitecture of the hippocampus

The microarchitecture of the hippocampus (Figure 5) showed in normal-group animals, a normal structure with intact-looking neurons in the different layers, dentate gyrus, CA1, CA2, and CA3. Contrasted with the normal category, the DZP-treated faction showed several histopathological changes in the hippocampus, marked by a diminish in the number of neuronal cells in the CA1, CA2, and CA3 layers and vacuolation of the dentate gyrus cells. Contrasted to the DZP-treated faction, treatments with the extract at all doses, as well as in the PIR-treated group, resulted in a restructuring of all hippocampal structures.

Figure 4. Impact of aqueous extract of *Ximenia americana* on superoxide dismutase (SOD) (A), catalase (CAT) (B) activities, reduced glutathione (GSH) activity (C), and malondialdehyde (MDA) content (D) in the hippocampus of mice

Each diagram represents the mean ± S.E.M of 6 animals. $p < 0.05$, **$p < 0.01$; ***$p < 0.001$ when compared to normal control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ when contrasted to diazepam treated faction. DZP: Diazepam, PIR: Piracetam, XA 25, 50, 75: *Ximenia americana* (25, 50, 75 mg/kg).
Discussion

This work aimed to investigate the anti-amnestic and antioxidant activities of *Ximenia americana* against diazepam-induced memory impairment in mice. The assessment of memory impairment was based on the radial arm maze (RAM), and T-maze tests. DZP is a drug used to treat epileptic seizures, anxiety, and panic.
attacks (Cao et al., 2018). At higher doses, this molecule causes anterograde amnesia. As a benzodiazepine receptor agonist, it causes amnesia and blocks long-term potentiation (LTP) in slices of the hippocampus (Prabhakar et al., 2011). One aspect that amplifies neurological complications is inflammation. However, previous work has shown anti-inflammatory effects of XA in mice (Gaichu et al., 2017), and based on this fact, the anti-amnesic and antioxidant activity of XA aqueous extract was evaluated. RAM is widely used for the study of spatial working memory disorders (Egashir et al., 2018). This tool is useful for assessing the effects of medication, stress, and various other environmental influences on learning and remembrance (Beppe et al., 2015). In this test, the number of working remembrance mistakes and the time required to ingest a bait were both significantly higher in DZP-treated mice than in normal mice. Treatment of mice with PIR and all doses of XA significantly reduced the number of working reminiscence mistakes and time to ingest bait, suggesting an anti-amnestic impact of the plant extract. Remembrance loss may result in an expand in the number of reference reminiscence mistakes (Foyet et al., 2019). It has been known that benzodiazepines, such as DZP, act primarily on gamma-aminobutyric acid type A (GABA_{A}) receptors located at synapses (Cao et al., 2018). The T-maze test is the tool of choice for assessing working and reference memory in rodents (Shoji et al., 2012). In this test, DZP-treated mice spent significantly longer time in the discrimination arm than normal mice. According to Beppe et al. (2020), mice treated with DZP-induced memory impairment spent more time in the discrimination arm. In addition, mice with good memory remembered their first choice in the T-maze test (Lodiot, 2009). The inability of mice to recall their first choice may be the cause of the amnesia. However, the time spent in the discrimination group decreased significantly after treatment with PIR and all the XA doses, but increased in the preferred group, reflecting the anti-amnestic effects of the extract and the reference drug. Brain tissue has low endogenous antioxidant capacity and is susceptible to oxidative stress (Barai et al., 2019; Tanwar et al., 2014). SOD, GSH and CAT are enzymatic and non-enzymatic antioxidants that can protect the cell from damage caused by reactive oxygen species (ROS) (Foyet et al., 2019). The enzymatic activity of CAT, which was significantly reduced after treatment with DZP, was increased when mice were treated with the extract, as reported by Prabhakar et al. (2011).

SOD and CAT play a role in the detoxification of superoxide anions that damage cell membrane macromolecules (Che et al., 2016). This reduction in enzymatic activity thus reflects the vulnerability of cells in the brain. GSH is the body's antioxidant defense mechanism that works by scavenging free radicals in brain tissue (Barai et al., 2019) and its level was significantly reduced following DZP treatment. Given that GSH is an effective scavenger of free radicals and that disruption of GSH homeostasis can lead to oxidative damage in neurons, the reduction in GSH levels in the hippocampus caused by DZP administration could be at least partially involve in the memory deficit observed in mice (Rodrigues et al., 2013). The vulnerability of CA1 neurons to metabolic stress plays a central role in the pathophysiological cascade leading to hippocampal damage (Spiegel et al., 2017). Nevertheless, treatment with aqueous XA extract at all doses as well as with PIR has significantly increased CAT activity, while the GSH level was significantly elevated only with doses of the extract. In fact, phytochemical studies of this plant revealed the presence of alkaloids, flavonoids, steroids, saponins, cardiac glycosides, phenolics, and terpenoids (Gaichu et al., 2017). These results prove the antioxidant activity of the aqueous extract of XA. Malondialdehyde (MDA) is used as an important biomarker involved in lipid peroxidation, and is the most abundant individual aldehyde that arises as a result of lipid peroxidation (Singh et al., 2014). DZP administration significantly increased the concentration of MDA in comparison to the normal group. An elevated MDA level indicates the severity of tissue attack by free radicals (Rebe et al., 2021; Ionita et al., 2017), administration of DZP at a dose of 2.5 mg/kg resulted in a similar effect (Beppe et al., 2020). However, this effect was significantly reserved by PIR and all doses of XA. Overall, these results indicate a beneficial effect of aqueous XA extract through its antioxidant effect on hippocampal cells.

The hippocampus is the major brain structure involved in the remembrance process (Moriiasi et al., 2020). The GABA_{A} receptor, which is highly expressed in the hippocampus, especially in the CA1 and CA3
regions, plays an important role in spatial remembrance performance (Auta et al., 2008), hence the importance of observing its main cells in behavioral studies. DZP treatment in mice resulted in a diminish in the number of neuronal cells in the CA1, CA2, and CA3 layers and vacuolation of the dentate gyrus cells. The overproduction of ROS and the imbalance of detoxification systems produce severe oxidative stress conditions in hippocampal neurons (Cai et al., 2017). The observed effects could be related to a disorder of individual hippocampal cells. Furthermore, the target of BZDs is the GABA_A receptor, which is a ligand-dependent chloride channel activated by GABA. GABA exerts inhibitory neurotransmission in the central nervous system, thereby reducing the excitability of neurons. However, administration of the plant extract at all doses, as well as the PIR, resulted in the preservation of all hippocampal structures.

Conclusions

This study demonstrates the beneficial effects of an aqueous extract of *Ximenia americana* on cognitive impairment induced by diazepam. Our findings suggest that aqueous extracts of the bark have neuroprotective and antioxidant effects. Further neurochemical studies are important to elucidate the influence of this aqueous extract on acetylcholine and acetylcholinesterase, which are critically involved in the memory process.

Authors’ Contributions

GM-IM and RNR have investigated the traditional healers, in order to choose the plant, provided extract and proposed the methodology. BGJ and BMB have validated the methodology and wrote the manuscript. JGB analyzed data, GNA-D and AIF revised the English version of the manuscript. BAD has corrected the protocol and brought an expertise to the whole manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Animals were handled according to the guidelines of the Cameroon Bioethics Committee (reg. no. FWA-IRB00001954). The protocol was approved by the ethics committee of the Faculty of Sciences of the University of Maroua (ref. no. 14/0261/Uma/D/FS/VD-RC).

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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