Rev. Bras. Saúde Prod. An., v.6, n.1, p. 31-41 , 2005 **http://www.rbspa.ufba.br**
ISSN 1519 9940

Cytotoxic effects of an extract containing alkaloids obtained from *Prosopis juliflora* Sw. D.C. (Algaroba) pods on glioblastoma cells

Citotoxicidade do extrato alcaloidal de vagens de Prosopis juliflora Sw. D.C. (Algaroba) em células gliais

HUGHES, J.B.[1]; SOUSA, J.S.[1]; BARRETO, R.A.[1]; SILVA, A.R.[1]; SOUZA, C.S.[1]; SILVA, V.D.A.[1]; SILVA, B.M.P.[1]; FREITAS, S.R.V.B.[1]; COSTA, M.F.D.[1]; EL-BACHÁ, R.S.[1]; BATATINHA, M.J.M.[2]; TARDY, M[3]; VELOZO, E.S.[4] AND COSTA, S.L.[1*]

1.Laboratório de Neuroquímica e Biologia Celular, Departamento de Biofunção, Instituto de Ciências da Saúde, Universidade Federal da Bahia (UFBA), Salvador, BA, Brazil.
2.Laboratório de Toxicologia, Departamento de Patologia e Clínica, Escola de Medicina Veterinária, UFBA, Salvador, BA, Brazil.
3.INSERM U-421, Faculté de Médicine, Université Paris XII, Val-de-Marme, Créteil, France.
4.Laboratório de Pesquisa em Matéria Médica, Faculdade de Farmácia, UFBA; , Salvador, BA, Brazil.
*Corresponding Author: Prof. Dr. Silvia Lima Costa, Laboratório de Neuroquímica e Biologia Celular, Departamento de Biofunção, Instituto de Ciências da Saúde, Universidade Federal da Bahia, Av. Reitor Miguel Calmon s/nº, Salvador, BA, 40.110-100, Brazil, Tel: (55) 71 3245 86 02, Fax: (55) 71 3245 89 17. costasl@ufba.br

RESUMO

Estudos têm revelado que o consumo predominante de vagens de *Prosopis juliflora* desenvolve doença de caráter neurotóxico em animais de produção. No sistema nervoso central, as células gliais desempenham funções essenciais que têm impacto na fisiologia e integridade neuronal. Neste estudo, investigamos os efeitos de citotoxicidade, modificações fenotípicas e expressão de GFAP, proteína marcadora de reatividade astrocitária, do extrato alcaloidal (EA), obtido das vagens de *P. juliflora,* sobre células gliais da linhagem GL-15. Concentrações entre 0,03-30µg/ml do EA foram avaliadas por 24-72 h. O teste do MTT, LDH e coloração com Azul de Tripan mostraram citotoxicidade dose-dependente do EA, atingindo efeito máximo a 30µg/ml em 24 h. Curvas de crescimento avaliadas pela coloracao com Azul de Tripan revelaram redução significativa do número de células viáveis ($p<0,05$) em 24 h de tratamento com 0,3µg/ml de EA. Análises por imunocitoquímica e western blotting das proteínas do citoesqueleto, vimentina e GFAP revelaram que, na mais baixa concentração do EA (0,03µg/ml), as células GL-15 modificaram sua morfologia com aumento da proporção de células GFAP positivas, entretanto, a expressão de vimentina permaneceu estável. Em contrapartida, as células expostas às mais altas concentrações do extrato (3-30µg/ml) apresentaram muito pouca expressão dessas proteínas. Esses resultados sugerem que alcalóides presentes nas vagens de *P. juliflora* estejam envolvidos nas modificações fenotípicas observadas em células gliais testadas e também na expressão das proteínas do citoesqueleto, vimentina e GFAP, assim como na redução do número de células viáveis.

Palavras-chave: *Prosopis juliflora*, alcalóides, glia, citotoxicidade

SUMMARY

Prosopis juliflora is largely used for feeding dairy and beef cattle. However, this plant has been found to cause neurotoxic disease when it is the sole sustenance for the animal. In the central nervous system (CNS), the glial cells are essential in maintaining neuronal cell health and integrity. In this study, an extract containing alkaloids (AE) from *P. juliflora* pods was obtained and tested on glial cells of the lineage GL-15 to investigate: cell cytotoxicity, morphological changes and the expression of GFAP, a marker of astrocytic reactivity. Concentrations between 0.03-30µg/ml of AE were assayed for 24-72h. The MTT test, LDH activity, and Trypan blue assay revealed dose-dependant cytotoxicity for GL-15 cells. Growth curves designed using Trypan blue staining displayed significant reduction ($p<0.05$) of the number of viable GL-15

cells, which reached maximum effect at 30µg/ml of AE. Immunocytochemistry and western blotting assay performed for the cytoskeletal proteins, vimentin and GFAP, revealed expression of vimentin in GL-15 cells at the lowest concentration (0.03µg/ml), and a change on their morphology together with an increase of the number of the GFAP positive cells. These effects were not detected when the highest concentrations (3-30µg/ml) were tested. The results suggest that the extract containing alkaloids may be involved in the phenotypical modifications detected on the lineage studied, in the expression of the cytoskeletal proteins, and in the reduction of the viable cell number.

Key words: *Prosopis juliflora*, alkaloids, glia, cytotoxicity

INTRODUCTION

Prosopis juliflora is a shrub, which was introduced in the northeast Brazil in the 1940's (SPA, 1989). Due to their palatability and nutritional value, the pods of *P. juliflora* or its bran are largely used for feeding dairy and beef cattle with good nutritional and economic results (SILVA, 1981). However, this plant causes disease when it is the sole sustenance for the animal, specially when it is offered during extreme drought conditions. Moreover, intoxication with *P. juliflora* has been reported in the US (DOLLAHITE et al., 1957), Peru (BACA, 1967), and in Brazil. In the latter, the illness called "cara torta" has been demonstrated periodically since 1981 (FIGUEIREDO et al., 1995; DANTAS, 1996). According to Figueiredo et al. (1995) the disease is characterised by neuro-muscular alterations like emaciation, muscular atrophy of the masseter, spongiosis and gliosis. Similar alterations were observed in goats fed with rations containing 60-90% of *P. juliflora* pods, specially after cronic exposure (>210 days) (TABOSA et al. 2000). Histologic lesions were also characterised by fine vacuolation of the perikaryon of neurons from trigeminal motor nuclei, and occasionally damages on neurons of the oculomotor nuclei and wallerian degereration in mandibular and trigeminal nerves were observed. The authors suggested that the clinical sings, from feeding *P. juliflora* pods in this more susceptible specie, were caused by a selective toxicity to neurons of some cranial nerve nuclei (TABOSA et al., 2000).

In the past, pharmacological properties *in vitro* such as antibacterial (SHANKARMURTHY e SIDDIQUI, 1948; AHMAD et al., 1986;AQEEL et al., 1989; KANTHASAMY et al., 1989a), antifungal (AHMAD et al., 1989; KANTHASAMY et al., 1989) and anti-inflammatory (AHMAD et al., 1989) were attributed to piperidine alkaloids present in the extracts of *P. juliflora* seeds (AHMAD et al.,1978; OTT-LONGONI et al., 1980; DAETWYLER et al., 1981; BATATINHA, 1997). Additionally, cytotoxic and antitumoral activity against human epithelial tumour cells (HeLa), human hepatic tumour (HepG2), and two fibroblast lineage, F26 and F57, (BATATINHA, 1997) was verified.

Glial cells, mainly astrocytes, are essential to the development, homeostasis and detoxification in the central nervous system (CNS) (LETOURNEL-BOULLAND et al., 1994). Additionally, these cells are known for their role of energetic support and immune response in the CNS against chemical, infectious or traumatic challenges (ASCHNER, 1998). They actively control synaptogenesis, synapse function, and synaptic plasticity (BARRES, 2000), in addition to mopping up transmitters and maintaining extracellular ion levels. These are important functions, which impact neuronal cell health and integrity (COYLE e SCHWARCZ, 2000). Astrogliosis is usually detected prior to any toxic effect on neurons and is, therefore, regarded as an early marker of neurotoxicity (O'CALLAGHAN, 1991; MONNET-TSCHUDI et al. 1995). When the

extracellular enviroment is damaged, the astrocytes react increasing the expression of the glial fibrillary acidic protein (GFAP), the major protein of their intermediate filaments and a specific marker for those cells. Increasing in GFAP production is a sign of astrogliosis, reactive injury, and even neurodegeneration (TARDY et al., 1991; COYLE e SCHWARCZ, 2000; COSTA et al. 2002; Tardy, 2002**).**

Estabilished and characterised glial cell lines constitute reliable and useful models to study cell biology (PFEIFFER et al., 1978, LAL et al., 1996). The cell line GL-15, the in vitro model chosen in this study, was characterised by Bocchini et al. (1993). These cells express GFAP, which is regulated when subjected to different stimuli like synthetic retinoic acid (COSTA et al., 2001), and the association with the proinflammatory cytokine tumour necrose factor-α (TNFα) (CHAMBAUT-GUÉRIN et al., 2000), altering their shape, enhancing the GFAP levels, and reducing the number of the viable cells.

Taking the advantage of the reliable glial cell model *in vitro* and the known pharmacological properties of the alkaloids present in the extract of *P. juliflora* pods, we investigated the cytotoxic effects of the extract containing alkaloids on the highly proliferative glioblastoma cell line GL-15. We emphasize the effects of this extract on cell viability, cytotoxicity and morphological changes.

MATERIALS AND METHODS

Extraction and isolation of alkaloids

The extract containing alkaloids (AE) from *Prosopis juliflora* pods was obtained by acid/basic extraction method previously described by Ott-Longoni et al. (1980). In brief, to eliminate the fats, the air dried plant material was submerged in hexane (1.5 l/kg) for 3 weeks at room temperature with occasional shake. The hexanic extract was filtered and the pods were flooded with methanol (1.5 l/kg) for 3 weeks using the above process. The methanol extract was concentrated under low pressure, stirred with 0.2 N HCl and filtered after 16 h. The product was shaken with chloroform to remove the non-basic material. The aqueous phase solution was basified with ammonium hydroxide until it reached pH 11 and was extracted with chloroform. The resulting solution, an extract containing alkaloids, was evaporated to dryness and confirmed for their positivity using the Dragendorf reagent identification test (WAGNER et al., 1983).

Cell line and treatments

The GL-15 cell line, derived from glioblastoma multiform (BOCCHINI et al., 1993), was cultivated as previously described (CHAMBAUT-GUÉRIN et al., 2000; COSTA et al., 2001). The cells were grown in polystyrene dishes (TPP, Switzerland) in Dulbecco's Modified Eagle's Medium (DMEM, Cultilab, SP, Brazil), supplemented with 100 UI/ml penicillin G, 100 µg/ml streptomycin, 7 mM glucose, 2 mM L-glutamine, 0.011 g/L pyruvate, and 10% fetal calf serum (Gibco, Grand Island, NY) in a humidified atmosphere with 5% CO_2, at 37 ºC. The dried AE was dissolved in dimethylsulfoxide (DMSO, Sigma, St Louis, MO) at a concentration of 3 mg/ml in a DMSO volume not exceeding 0.5%, and it was stored in the dark at –20 ºC. Extemporaneously, for all experiments, the extract was diluted in the medium at exponential final concentrations between 0.03 µg/ml to 30 µg/ml, for 24-72 h. The control group was treated with DMSO diluted in the culture medium at equivalent volume used by the treated group.

Cytotoxicity and cell membrane integrity assays

The AE was tested for cytotoxicity on confluent GL-15 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT test after 24 h and 72 h. The experiment was performed into 96 well plates (1 x 10^4 cells/well). The cell viability was quantified by the conversion of yellow MTT by mitocondrial dehydrogenases of living cells to purple MTT formazan (HANSEN et al., 1989). Three hours before the end of the time of exposure, the medium was removed and replaced by MTT containing medium (100µl/well of DMEM without supplementation) (Sigma, St Louis, MO) at a final concentration of 1 mg/ml. Thereafter, 100 µl/well of a buffer containing 20% sodium duodecil sulphate (SDS), 50% N, N-dimethyl formamide (DMF), pH 4.7 was added, keeping the plates for 12 h at 37º C for complete dissolution of formazan crystals. The optic absorbency of each sample was measured at a wave length of 560 nm using a BIO-RAD 550PLUS spectrophotometer. Four replicate wells were used for each analysis for each concentration tested in three independent experiments. The results were shown as the percentages of the control group.

Alternatively, to assess the membrane integrity of GL-15 cells, lactate dehydrogenase (LDH, E.C. 1.1.1.27) release into the culture media was assayed based on the oxidation of lactate to pyruvate and the reduction of NAD^+ to NADH, resulting in a change in absorbency at 500 nm. This experiment was performed in 340 mm ∅ plates (TPP Switzerland, 1.6 x 10^6 cells/plate). Supernatant for LDH determination was removed from each treatment group (24 – 72 h) on ice and it was immediately assayed. Relative LDH levels (IU/L) were determined by the enzymatic method (ANALISA kit, LABTEST Diagnóstica SA, MG, Brazil) adapted to microplates, according to the manufacturer's instructions. The results were expressed as LDH (IU/L) activity relative to that of non-treated cells.

In order to assay membrane integrity growth curves were obtained using Trypan Blue staining method. Both floating and adherent cells, cultured 340 mm ∅ plates (TPP Switzerland, 1.6 x 10^6 cells/plate), were harvested after trypsinization (trypsin 0.025%, EDTA 0.50%) and centrifuged for 10 minutes at 1000 rpm. The cells were suspended in 200 µl DMEM without supplementation and stained with Trypan blue at a final concentration of 0.1% (v/v). Five replicate plates were used for each analysis. The viable and non viable-blue cells/µl was determined after 24 – 72 h exposure to the AE by counting the number of cells in 10 µl of cell suspension for each experiment in the Neubauer chamber (Boehringer Mannheim). The results obtained were expressed as the mean ± standard deviation (S.D).

Phenotypic analysis

Morphological changes were studied analysing the cells by phase microscopy and by immunocytochemistry for the cytoskeletal proteins vimentin and GFAP. Increasing of the levels of GFAP was mainly detected by immunocytochemistry and western blotting. These experiments were performed in 340 mm ∅ plates (TPP Switzerland, 1.6 x 10^6 cells/plate).

Immunocytochemistry

All cells, control and treated, were rinsed three times with PBS and fixed with cold methanol at –20º C for 10 minutes. Cells were incubated with rabbit polyclonal anti-GFAP (1/500, DAKO, Denmark) or mouse monoclonal anti-vimentin (1/500, clone V9, Boehringer, Mannheim), and tetramethylahodamine isothiocyanate conjugated goat anti-rabbit (1/250, Biomakor, Israel), or sheep anti-mouse (1/250, Biomakor, Israel) antibodies respectively, for 30 min at room temperature, both under slow agitation. Chromatin

integrity or nuclear fragmentation/condensation was observed co-staining nuclear chromatin of fixed cells with the fluorescent dye Hoechst33258 (Sigma, St Louis, MO), at a final concentration of 5 µg/ml in PBS, for 10 minutes at room temperature in a dark chamber. Thereafter, the cells were analysed by fluorescent microscopy (Olympus AX70).

Protein assay and Western blotting

After time of exposure, cells were rinsed twice with PBS and harvested in a lysis buffer 2% (w/v) SDS, 62.5 mM Tris-HCl pH 6.8, 2 mM EGTA, 4 M Urea, 0.5% (v/v) Triton X-100, supplemented with 1µl/ml of a cocktail of proteinase inhibitors (Sigma, St Louis, MO). Protein content was determined using DC Protein Assay (Bio-Rad, Hercules, CA, USA). For GFAP and vimentin analysis, 18 µg of total protein was loaded onto a discontinuous 8% SDS polyacrilamide gel electrophoresis (SDS-PAGE), at 200 V for 45 minutes. Proteins were transferred onto a polyvinylidene fluoride membrane (PVDF, Bio-Rad, Hercules, CA, USA) for 1 h at 100 V. Subsequently, membranes were blocked for 1 h at room temperature in 20 mM Tris-buffered saline, pH 7.5, containing 0.05% Tween 20 (TBS-T) and 1% BSA and immediately incubated with mouse anti-GFAP (1:10,000, Boehringer, Mannheim) or mouse anti-vimentin (1:10,000, clone V9, Boehringer, Mannheim) monoclonal antibodies for 1 h. After three washes for 10 minutes each in TBS-T, conjugated alkaline phosphatase anti-mouse IgG secondary antibody (1:5,000, Promega Corporation, WI, USA) was added for 30 minutes, followed by three washes in TBS-T for 10 minutes and in TBS. Immunoreactive bands were visualised using Protoblot II AP System Kit (Promega Corporation, WI, USA), according to the manufacturer's instructions.

Statistical analysis

The SigmaStat for Windows (version 1.0) was adopted to analyse the results by Student-Newmann-Keuls test in order to determine the significance of the means. Values of $p < 0.05$ were considered as statistically significant.

RESULTS

Cytotoxic effects of the extract containing alkaloids on GL-15 cell line as confirmed by the MTT test, LDH activity and Trypan blue staining.The cytotoxic effects of AE from *P. juliflora* pods upon the viability were confirmed by MTT test, LDH activity and Trypan blue staining. The figure 1A summarize the MTT assay evidencing clearly a cytotoxic effect on GL-15 cells at 3 – 30 µg/ml ($p<0.05$; $p<0.0001$, respectively) after 24 h treatment. After 72 h exposure to the AE all concentrations tested exhibited toxicicity (0.03 – 0.3µg/ml: $p<0.05$; 3 – 30µg/ml: $p<0.0001$). Accordingly with MTT results, we proved the cytotoxicity of AE by measuring the LDH activity in culture supernatants as displayed on figure 1B. Three out to four concentrations tested provided high amount of LDH release inferring a cell membrane damage after 24 h exposure ($p<0.05$). Interestingly, the 0.3µg/ml of AE did not exhibit toxicity suggesting that the cells are able to recover after an injury. Additionally, growth curves approached by Trypan blue staining (figure 1C) detail that the number of viable cells/µl decreased in a dose-related fashion: since 24 h exposure to 0.3 µg/ml AE a marked reduction of the number of GL-15 cells ($p<0.05$) was observed, acquiring the maximum effect at 30 µg/ml after 72 h exposure.

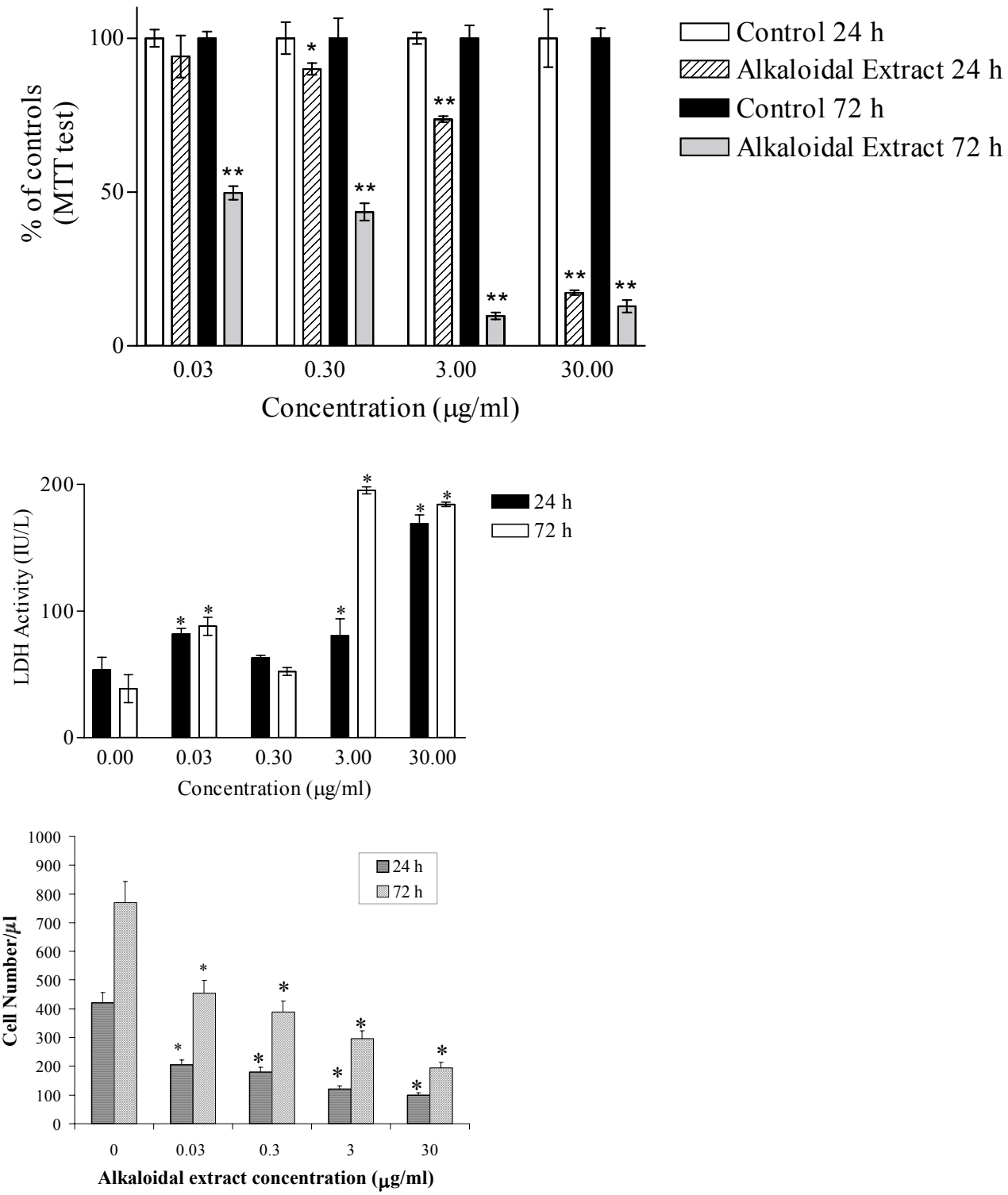

Figure 1 – Cytotoxicity assays. A: MTT test on GL-15 cells treated with AE at various concentrations (0.03 – 30 μg/ml) and evaluated at 24 and 72 h posttreatment. Values for untreated control samples were set at % of controls; B: LDH activity (IU/L) determined by enzymatic method at 500 nm; *$p<0.05$, **$p<0.0001$; C: Growth curve inhibition assessed by Trypan blue. Data are representative and expressed as means ± standard deviation of the mean values.

Modulation of GL-15 cells morphology

Immunolabelling of vimentin (figure 2A) and GFAP (figure 2B) illustrated that confluent control GL-15 cells had a bipolar shape with high vimentin and heterogeneous GFAP expression (figure 2Aa and 2Ba, respectively). Incubation of GL-15 cell line for 24 h with 0.03 – 3 µg/ml AE significantly provided morphological changes. At the lowest concentration tested (0.03 µg/ml, figure 2Ab and 2Bb) the AE induced differentiation on glioblastoma cells, illustrated by condensed cell body, emission of many thin filaments and an increase in GFAP immunolabelling. The latter was evident in the majority of the cells. At 0.3 µg/ml AE (figure 2Bc) only some zones of the culture dishes depicted differentiation, presenting cells with a bipolar multiprocessed phenotype and high levels of GFAP expression. The expression of vimentin was uniform for the concentrations between 0.03 – 0.3 µg/ml AE. On the other hand, at the highest concentration (3 µg/ml AE) it was clearly cytotoxic: the cells presented a contracted the cell body together with the low levels of vimentin and GFAP expression (figure 2AdBd) in the majority of the cells. As the concentration of 30 µg/ml was very cytotoxic, and cells detached, this concentration was not analysed by immunocytochemistry. Conversely, the vimentin and GFAP expressions in GL-15 cells were also detected by western blotting. The figure 3 illustrated vimentin and GFAP expressions in all control and treated cells when concentrations between 0.03 – 0.3 µg/ml AE were adopted for 24 – 72 h, and no apparent changes in their levels were observed. In contrast, after 72 h exposure to 3 µg/ml AE the vimentin and GFAP proteins were not detected. Any cells were viable after being treated with 30 µg/ml AE, conferring, therefore, any expression of those proteins since 24 h exposure.

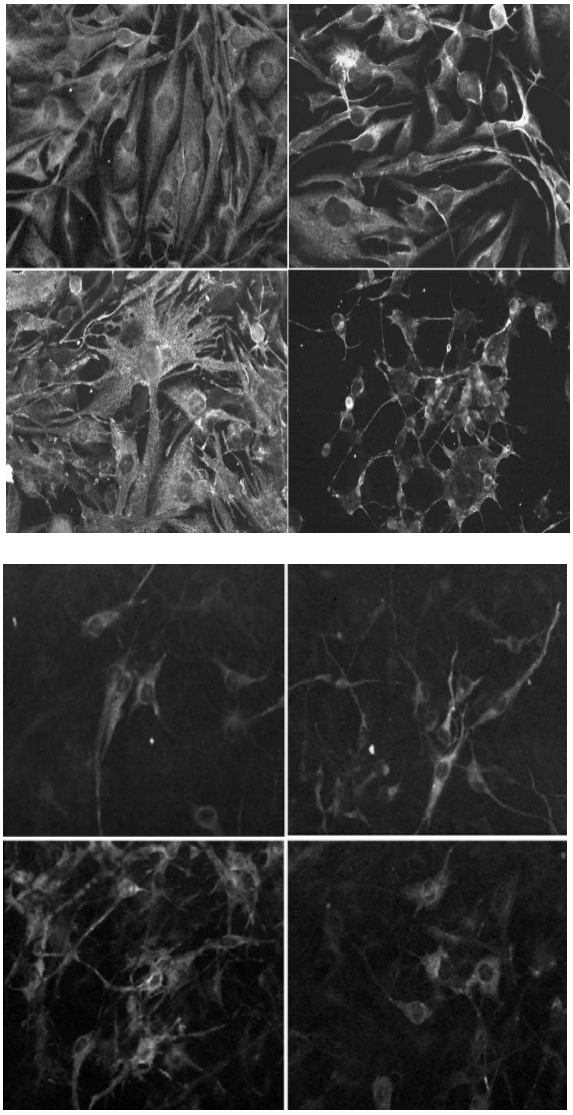

Figure 2. Immunofluorescence labelling of vimentin and GFAP in GL-15 cells treated with AE obtained from *P. juliflora* pods for 72 h; A: vimentin staining of GL-15 cells grown in control conditions (a) or with 0.03 – 3 µg/ml AE (b-d, respectively); B: GFAP staining of GL-15 cells grown in control conditions (a) or with 0.03 – 3 µg/ml AE (b-d, respectively). Obj.x20.

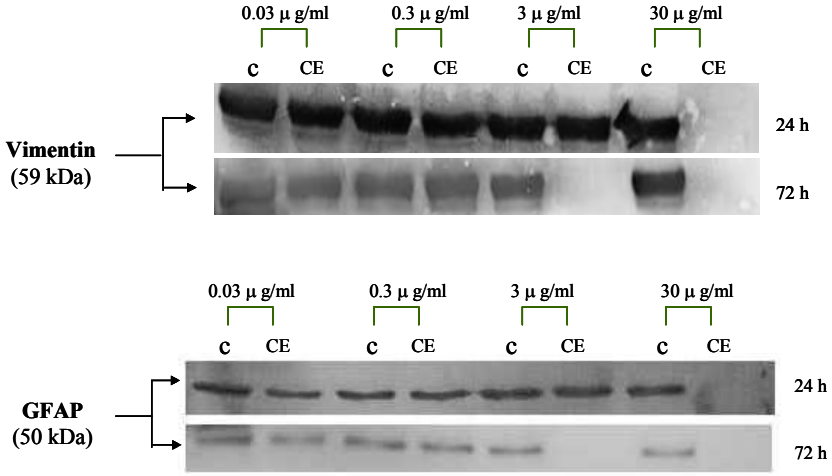

Figure 3. Western blot analysis of vimentin (A) and GFAP (B) protein expression from GL-15 cell in control conditions (lane 1, 3, 5, and 7) or treated with 0.03 – 30 µg/ml (lane 2, 4, 6, and 8) for 24 and 72 h. Samples containing 18 µg of protein were electrophoretically separated through 8% polyacrylamide gels containing 0.1% SDS in running buffer. These results are representative of three independent experiments.

DISCUSSION AND CONCLUSION

Animal studies conducted by Figueiredo et al. (1995) and Tabosa et al. (2000) revealed that intoxication with *P. juliflora* pods caused selective toxicity to neurons and gliosis. As the pharmacological properties of *P. juliflora* seeds were attributed to piperidine alkaloids (AHMAD et al.,1978; EL-MERZABANI et al., 1979), we investigated the cytotoxic effects of the extract containing alkaloids on the highly proliferative glioblastoma cell line GL-15. We think that some of the neurotoxic phenomena described by Figueiredo et al. (1995) and Tabosa et al. (2000) are due to the biological activity of those alkaloids. Hereby, we emphasize the effects of one extract containing alkaloids extratec from pods of *P. juliflora* on the cell viability, cytotoxicity and morphological changes on glioblastoma cell line GL-15. It is clear that depending on the concentration adopted and time of exposure, the GL-15 cell line can change their shape and become non-viable.

Two useful parameters was acquired to investigate cell toxicity *in vitro:* firstly, the measure of mithocondrial function, determined by the efficiency of cleavage of tetrazoline ring in MTT test, and the measure of the LDH activity on the culture medium. Treatment for 24 h with the AE at the lowest concentration (0.03 µg/ml) neither changed the mitocondrial functions nor altered the number of viable cells, even when an increase of the LDH activity on the culture medium was detected. At this concentration, a cytotoxic effect of the AE was obtained only after 72 h exposure. However, the MTT test, the LDH release, and the Trypan blue exclusion staining confirmed clearly that the AE is cytotoxic for GL-15 glial cells when at concentrations between 0.3 – 30µg/ml were

tested. This effect was observed as soon as 24 h treatment with the AE, suggesting a high sensitivity for the compound for the GL-15 glial cells. After 48 h treatment some condensed chromatin nuclei was detected and 72 h exposure to 30 µg/ml AE was necessary to reach 90% of cell death (data not shown). Most cell death might be attributed to necrosis, since cell membranes appeared damaged after 24 h of culture in the presence of the higher dosis of the AE (30 µg/ml). Such an effect was shown previously by Kanthasamy et al. (1989b) on erythrocyte subjected to alkaloids from *P. juliflora;* his former work suggested significant hemolysis due to membrane injury. These authors evidencied a dose-dependant effect which reached 90% in the presence of 150 µg/ml of the compound in both rat and human erythrocytes. Fibroblast (F26 and F57), hepthelial (HeLa) and hepatic (HepG2) cell lines exhibited cytotoxicity as verified by Batatinha (1997). These results validate our hypothesis that the GL-15 cell line are more sensitive to the effects of the AE than the other cell lines studied.

As it is known, glial cell functions are important cells to the brain. They are intimate partners of regulative functions, especially in the control of neuronal outgrowth and neuronal immune functions. Futhermore, astrocytes are the glial cells that build the blood–brain barrier and play a decisive role in biotransformation of xenobiotics like alkaloids, which can reach the central nervous system. Astrocytes react to external insults, undergo activation and change the pattern of expression of their most important cytoesqueletal protein, GFAP. The immunocytochemistry for the proteins vimentin and GFAP proved that the GL-15 cells changed their morphology when cultivated in the presence of the 0.03 – 0.3µg/ml AE. The vimentin and GFAP proteins appeared rather reorganised than overexpressed since no apparent changes in their levels could be observed by Western blotting. Moreover, growth curves observed by Trypan blue staining revealed that at those AE concentrations, the number of viable cells reduced, suggesting a growth inhibition, which may conduct these cells towards a differentiation pathway. On the other hand side, when the AE was adopted at the highest concentrations (3 – 30 µg/ml), the cells lost the expression of the major cytoesqueletal proteins, vimentin and GFAP. This finding corroborate with the decrease of the mithocondrial function and the lost of membrane selectivity, and support the hypothesis that alkaloids from *P. juliflora* acts directly on glial cells and are toxic. Decreases in GFAP expression by reactive astrocytes are associated to defects on neurotransmission (RAJKOWSKA et al., 2002; MOISES et al., 2002); our study infer that the neurological damages observed in intoxicated animals are related with the reduction in the GFAP levels in GL-15 cells after AE exposure.

The alkaloids from *P. juliflora* are toxic and this study confirmed that they induce growth inhibition and morphological changes in GL-15 glial cells. The further examination of the effect of these molecules on glial cells, and the possibility of a selective pattern of neuronal degeneration requires additional consideration.

ACKNOWLEDGEMENTS

We gratefully acknowledge the research support provided by Mestrado em Medicina Veterinária Tropical/UFBA, Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and by Conselho Nacional de Desenvolvimento Científico e Técnológico (CNPq); J. S. Souza, C.S. Souza and V.D.A Silva are recipient of fellowships from Programa de Iniciação Científica (PIBIC)-UFBA. Thanks to Mr. Carlos Alberto Rodrigues for technical support, and Elli Lewis and Michael Drew Bradford for corrections of the manuscript.

REFERENCES

AHMAD V.U.; BASHA, A.; HAQUE W.Z. New alkaloids from Prosopis juliflora. **Naturforsch Ser.** v.33, p.347-8,1978.

AHMAD, V.U.; QAZI, S. Studies on the structure of julifloricine. **Journal Chemistry Society of Pakistan,**. v.7,n.4, p.347-50,1985.

AHMAD, A.; KHURSHEED, A.K.; SABIHA, QUE. ;VIQARUDDIN, A. Antifungial activity of some hydrosoluble Prosopis juliflora alkaloids. **Fitoterapi**a; v.60,n.1, p.86-9, 1989.

AQEEL, A.; KHURSHEED, A.K.; VIQARUDDIN, A.; SABIHA, Q. Antimicrobial activity of julifloricine isolated from Prosopis juliflora. Arzneim. - Forsch./ **Drug Research**., v. 39,n.6,p.652-5. 1989.

ASCHNER, M. Astrocytes as Mediators of Immune and Inflammatory Responses in the CNS. **Neurotoxicology**, v.19, n.2, p.269-82. 1998.

BACA, S.F.; VALLENAS, A. Novoa C. estudio experimental de la "Coquera" en caprinos. **Revta Fac Med Vet.** v**.**18/20, p.131-59,1967.

BARRES, B.A.; BARDES, Y.A. Neuronal and glial cell biology. **Current Opinion in Neurobiology.** v.10, p.642-8, 2000.

BATATINHA, M.J.M. **Investigations on toxic influences of Prosopis juliflora D.C. (Algarobeira) on cell cultures as well as on ruminal fermentation in cattle (in vitro).**1997. 189p. Dissertação (mestrado).- Veterinary Medicine University, Hannover.

BOCCHINI, V.; BECCARI, T.; ARCURI, C.; BRUYERE, L.; FAGES, C.; TARDY, M. Glial fibrillary acidic protein and its encoding mRNA exhibit mosaic expression in a glioblastoma multiforme cell line of clonal origin. **Int. J. Devl. Neuroscience,** v.11, n.4, p.485-92, 1993.

CHAMBAUT-GUÉRIN, A.M.; COSTA, S.L.; LEFRANÇOIS, T.; FAGES, C.; GAUTHEREAU, X.; TARDY, M. Effects of retinoic acid and tumor necrosis factor alpha on GL-15 globlastoma cells. **Neuro Report** v**.**11, n.2, p.389-93, 2000.

COSTA, S.L.; PAILLAUD, E.; FAGES, C.; ROCHETTE-EGLY, C.; PLASSAT, J.L.; JOUAULT, H. et al. Effects of a novel synthetic retinoid on malignant glioma in vitro: inhibition of cell proliferation, induction of apoptosis and differentiation. **European Journal of Cancer**, v. 37, p.520-30, 2001.

COSTA, S.; PLANCHENAULT, T.; CHARRIERE-BERTRAND, C.; MOUCHEL, Y.; FAGES, C.; JULIANO, S.; LEFRANCOIS, T.; BARLOVATZ-MEIMON, G.; TARDY M. Astroglial permissivity for neuritic outgrowth in neuron-astrocyte cocultures depends on regulation of laminin bioavailability. **Glia**.,v.7, n.2, p.105-13, 2002.

COYLE, J.T.; SCHWARCZ R. Mind glue: implications of glial cell biology for psychiatry. **Arch Gen Psychiatry**, v.57, n.1, p.90-3, 2000.

DAETWYLER, P.; OTT-LONGONI, R.; SCHÖPP, E.; HESSE, M. Over juliprosine, a further alkaloid from Prosopis juliflora A. DC.]. **Helvetica Chimica Acta,** v.64, n.188, p.1959-63,1981.

DANTAS, J.R.F. Cara torta, uma doença que atinge bovinos da Paraíba, Pernambuco e Rio Grande do Norte, Brasil**. Rev. Criad.** v.66, p.32, 1996.

DOLLAHITE, J.W. Anthony WV. Malnutrition in cattle on an unbalanced diet of mesquite beans. **Texas Agricultural Experiment Station.** v.11, p.209-12,1957.

FIGUEIREDO, L.J.C.; FERREIRA, M,M.; TÁVORA, J.P.F.; DANTAS, J.; SIMÕES; S.D. Estudo clínico e anátomopatológico da doença "cara torta" em bovinos no nordeste brasileiro. **Arquivos de Medicina Veterinária – UFBA,** v.18, n.1, p.175-83, 1995.

HANSEN, M.B.; NIELSEN, S.E.; BERG, K. Re-examination, and further development of a precise and rapid dye method for measuring cell growth/cell kill. **J Immunol Methods**. v.119, p.203-210, 1989.

KANTHASAMY, A.; SUBRAMANIAN, S. GOVINDASAMY, S. Bactericidal and fungicidal effects of prosopis juliflora alkaloidal fraction. **Indian Drugs,** v.26, n.8, p.390-394, 1989a.

KANTHASAMY, A.; WILLIAM, S.; GOVINDASAMY, S. Hemolytic effects of Prosopis juliflora alkaloids. **Current Science**; v.58, n.3, p.142-144, 1989b.

LAL, P.G.; GHIRNIKAR, R.S.; ENG, L.F. Astrocyte-Astrocytoma Cell Line Interactions in Culture. **Journal of Neuroscience Research**, v.44, p.216-22, 1996.

LETOURNEL-BOULLAND, M.L.; FAGES, C.; ROLLAND, B.; TARDY, M. Lipopolysaccharides (LPS) up-regulate the IL-1-mRNA and downregulate the glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS)-mRNAs in astroglial primary culture euro. **Cytokine Network,** v. 5, n. 1, p.51-6, 1994.

MOISES, H.W.; ZOEGA, T.; GOTTESMAN, II. The glial growth factors deficiency and synaptic destabilization hypothesis of schizophrenia. **BMC Psychiatry**. v.2, n.1, p.8, 2002.

MONNET-TSCHUDI, F.; ZURICH, M.G,; RIEDERER, B.M.; HONEGGER, P. Effects of trimethyltin (TMT) on glial and neuronal cells in aggregate cultures dependence on the developmental stage, **Neurotoxicology**, v.16, p.97–104, 1995.

O'CALLAGHAN, J.P. Assessment of neurotoxicity use of glial fibrillary acidic protein as a biomarker, **Biomed Environ Sci**. v. 4, p.197–206,1991.

OTT-LONGONI, R.; VISWANATHAN, N.; HESSE, M. The structure of the alkaloid juliprosopin from Prosopis juliflora A. DC. **Helvetica Chimica Acta,** v.63, n.222, p.2119-29, 1980.

PFEIFFER, S.E.; BETSCHART, B. Glial cell lines. In: FEDEROFF, S.; HERTZ, L. **Cell, tissue and organ cultures in neurobiology**. New York: Academic Press, 1978. p.110-200.

RAJKOWSKA, G.; MIGUEL-HIDALGO, J.J.; MAKKOS, Z.; MELTZER, H.; OVERHOLSER, J.; STOCKMEIER, C. Layer-specific reductions in GFAP-reactive astroglia in the dorsolateral prefrontal cortex in schizophrenia. **Schizophr Res**. v.57, n.2-3, p.127-38, 2002.

SHANKARMURTHY, P.; SIDDIQUI S. Antibiotic activity of leaf extracts of Prosopis juliflora. **Journal of Scientific and Industrial Research,** v.7b. n.11, p.188,1948.

SILVA DS. **Substituição progressiva do farelo de trigo pela vagem da algaroba na alimentação de bovinos em engorda.** Paraíba. 1981.p.86 Dissertação(graduação) - Universidade Federal da Paraíba.

SPA.SECRETARIA DE PRODUÇÃO ANIMAL. A algarobeira (Prosopis juliflora Sw, D.C.) no nordeste do Brasil. Brasília, 1989. 74p

TABOSA, I.M,.; SOUZA. J.C.; GRACA, D.L.; BARBOSA-FILHO, J.M., ALMEIDA, R.N.; RIET-CORREA, F. Neuronal vacuolation of the trigeminal nuclei in goats caused by ingestion of Prosopis juliflora pods (mesquita beans). **Vet Hum Toxicol**. v.42, n.3, p.155-158, 2000.

TARDY, M. Astrocyte et homeostasie. **Médecine Science** v.8, n.7, p. 799-804,1991.

TARDY, M. Role of laminin bioavailability in the astroglial permissivity for neuritic outgrowth. **An Acad Bras Cienc**.v.74, n.4, p.683-90, 2002.

WAGNER, H.; BLADT, S.; ZGAINSKI, E.M. Drogen Analyse. In: VERLAG SPRINGER, (ed.) **Dünnschichtchromatographie Anlyse von Arzneidrogen**. Berlin: Heidelberg New York Publishers, 1983. p.249-250.