Histol Histopathol (2005) 20: 1147-1153

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Histological evaluation of brain damage caused by crude quinolizidine alkaloid extracts from lupines

J. Bañuelos Pineda[1], G. Nolasco Rodríguez[1], J.A. Monteon[1], P.M. García López[2], M.A. Ruiz Lopez[2] and J. García Estrada[3]

[1]Departament of Medicine Veterinary, [2]Departament of Botanic and Zoology, University of Guadalajara, Mexico and [3]Research Biomedical of Western Center, Mexican Institute of Social Security

Summary. The effects of the intracerebroventricular (ICV) administration of crude extracts of lupin quinolizidine alkaloids (LQAs) were studied in adult rat brain tissue. Mature *L. exaltatus* and *L. montanus* seeds were collected in western Mexico, and the LQAs from these seeds were extracted and analyzed by capillary gas chromatography. This LQA extract was administered to the right lateral ventricle of adult rats through a stainless steel cannula on five consecutive days. While control animals received 10 µl of sesame oil daily (vehicle), the experimental rats (10 per group) received 20 ng of LQA from either *L. exaltatus* or from *L. montanus*. All the animals were sacrificed 40 h after receiving the last dose of alkaloids, and their brains were removed, fixed and coronal paraffin sections were stained with haematoxylin and eosin. Immediately after the administration of LQA the animals began grooming and suffered tachycardia, tachypnea, piloerection, tail erection, muscular contractions, loss of equilibrium, excitation, and unsteady walk. In the brains of the animals treated with LQA damaged neurons were identified. The most frequent abnormalities observed in this brain tissue were "red neurons" with shrunken eosinophilic cytoplasm, strongly stained pyknotic nuclei, neuronal swelling, spongiform neuropil, "ghost cells" (hypochromasia), and abundant neuronophagic figures in numerous brain areas. While some alterations in neurons were observed in control tissues, unlike those found in the animals treated with LQA these were not significant. Thus, the histopathological changes observed can be principally attributed to the administration of sparteine and lupanine present in the alkaloid extracts.

Key words: Quinolizidine alkaloids, Wild lupines, Brain damage, Sparteine, Lupaine

Offprint requests to: Dr. Jacinto Bañuelos Pineda, Centro Universitario de Ciencias Biológicas y Agropecuarias de la Universidad de Guadalajara, Kilómetro 15.5, Carretera Guadalajara-Nogales, Zapopan, Jalisco, México CP 45110. e-mail: jpineda@cucba.udg.mx

Introduction

Quinolizidine alkaloids (QA) are toxic compounds synthesized by lupin plants and other species of the GENISTEAE family as a defense mechanism against predators, both herbivores and microorganisms (bacterium, fungi and viruses; Wink, 1992, 1993b). In mammals, QA intoxication is characterized by trembling, shaking, excitation and convulsions (Pothier et al., 1998). QA intoxication also causes a wide range of other responses including, a transient delay in the gain of body weight, a decrease in the total red blood cell count, abnormal prenatal development and acute intoxication that can be fatal (Keeler, 1976; Butler et al., 1996; Robbins et al., 1996; Ruiz and Sotelo, 2000). The alkaloids sparteine (*lupinidine*), lupanine, angustifoline and anagyrine are among those most frequently detected QAs in lupin extracts (Davis, 1982; Hatzold et al., 1983; Muzquiz et al., 1989). While the first two of these are considered to be the most toxic (Yovo et al., 1984; Pothier et al., 1998), at least another forty minor alkaloids are also present in crude lupin extracts.

While it is known the QAs cause neural damage, it is still unclear by what mechanism this might occur. Some researchers suggest that they inhibit ganglionic transmission in the sympathic nervous system (Schmitt, 1980; Yovo et al., 1984), while others have proposed that the QAs affect the acetylcholine receptors (nicotinic and muscarinic) as well as inhibiting Na^+ and K^+ channels (Kinghorn and Balandrin, 1984; Wink 1987, 1992, 1993ab; Schmeller et al., 1994; Korper et al., 1998). However, these studies have all been carried out in vitro and do not provide information about the effects of the QAs in the central nervous system, on peripheral nerves, or of the histopathological effects on the nervous tissue. Therefore, the aim of this study was to establish the neuropathological damage caused by the intracerebroventricular (ICV) administration of the QAs present in raw extracts from two wild lupin species collected in western Mexico, *L. exaltatus* and *L. albus*.

Materials and methods

Mature seeds of *L. exaltatus* were collected from an altitude of 1,560 metres at the end of spring, 2003 in the locality "El Fresnito" in Zapotlan el Grande, Jalisco, Mexico. Similarly, mature seeds of *L. montanus* were collected from an altitude of 3200 m during the autumn of 2003, at the peak of the "Nevado de Colima" mountain. The plants were identified by their morphologic features as set out in McVaugh, 1987, and by comparing them with herbarium specimens stored at the University of Guadalajara's Botanical Institute.

Preparation of Lupin alkaloid extracts

A crude extract of Lupin quinolizidine alkaloids (LQAs) was prepared from the flour of *L. exaltatus* or *L. montanus* seeds from which the fats had been removed, following the method described by Muzquiz et al. (1993). The lipid free flour (5 g) was homogenized for 1 min at room temperature with 5 ml of a 5% trichloroacetic acid solution. The mixture was then centrifuged at 700g for 5 min and the supernatant was recovered in a separating funnel. This procedure was repeated twice, and the final supernatant volume of 15 ml was alkalinated with 1 ml of 10 M sodium hydroxide. The alkaloids were extracted three times with 5 ml of methylene chloride, and the solvent was evaporated to dryness using a rota-vapour.

A pale-yellow oily mass containing the crude alkaloids was diluted in 5 ml of methyl- hydroxide and filtered (Millipore 0.46 µm). Then, 1 µl of the filtrate was injected into a gas chromatograph (Perkin Elmer™, type split-less) fitted with a capillary column SPB-1 (30x0.4 mm id; Teknokroma™) and a flame ionization detector. The temperature of the injector and detector was set at 240°C and 300°C, respectively. The oven temperature was programmed to rise from 150°C to 235°C at a rate of 20°C/min and the final temperature was maintained for 15 min. The carrier gas was helium (1.1 ml min^{-1} flow, at 5 psi). The alkaloids were identified and quantified by the appropriate elution times and using standard curves.

Alkaloid administration

Thirty male *Swiss-Wistar* rats (250-300 g) were housed in individual cages under controlled ambient conditions and fed with a standard diet (Ralston-rations, USA). An intracerebral stainless steel cannula (0.72 mm outer diameter, 0.37 mm inner diameter, 9.0 mm length) was placed in the right lateral ventricle of each rat using a stereotaxic apparatus (David-Kopf). The cannula was positioned at coordinates 1.4 mm medio-lateral, 8.2 mm before to lambda and -4.0 mm dorso-ventral (Paxinos and Watson, 1982), and it was prior adjusted with a fixed flat obstacle to reach the appropriate depth. Following insertion of the cannula, the animals were randomly assigned to the control or one of the two experimental groups (10 animals each). All animals received a daily intracerebroventricular (ICV) injection of sesame oil (vehicle) or lupin alkaloid extract between 9:00-10:00 h, on five consecutive days. The control group received only 10 µl of vehicle (Treatment I), while the experimental groups received 20 ng of either *L. exaltatus* or *L. montanus* crude alkaloid extract in 10 µl of vehicle, at a rate of 1 µl/30 sec (Treatments II and III, respectively). The total volume was divided into two 5 µl doses administered at an interval of 1 h between injections.

The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) 40 hours after receiving the last dose. They were then sacrificed by intracardiac perfusion with 100 ml of saline solution at 37°C for 3 min, followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. The brain of each animal was removed and post-fixed for 24 h in 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. The brains were then washed in a phosphate buffered saline solution, dehydrated in increasing concentrations of alcohol and embedded in paraffin.

Two sets of sections (10 µm thickness), in the coronal or sagittal plane, were obtained from each brain using a rotating microtome. One series was stained with Nissl stain (cresyl violet) and the other with haematoxylin and eosin. The stained tissues were dehydrated in alcohol solutions and mounted with Entellan resin (Merck). All brain sections from the hemisphere contralateral to the side of the cannula insertion were examined microscopically with a 40X objective. Regions with signs of neuronal degeneration such as eosinophilic cytoplasm, nuclear pyknosis, acidophilia, hypochromasia, cellular swelling and cytolysis were identified as described by Li et al. (1998).

In each one of the cerebral regions in which damage was detected, the proportion of degenerate neurons was recorded in 20 microscopic fields (0.78 mm^2/field) using a Leica microscope fitted with an image analysis program (QWIN-500). Only those neurons in which the nucleus (except for the "ghost neurons") and abnormal structures could be observed in a single plane of focus were considered. Cerebral structures were identified according to Paxinos and Watson terminology (Paxinos and Watson, 1982).

Statistical analysis

The Kruskall-Wallis test was used to evaluate the statistical significance of the differences for the distinct parameters evaluated between control and experimental groups. A P value <0.05 was considered to be statistically significant.

Results

The principal QAs that were found by gas chromatography in the two different seed types analyzed

here are represented in Table 1. While sparteine, lupanine, 3-OH lupanine and 13-OH lupanine were the principal QAs identified in *L. montanus*, in *L. exaltatus* only lupanine, 3-OH lupanine and sparteine were found.

Clinical symptoms

Immediately after the first ICV administration of the LQA from *L. exaltatus*, the animals became excited and piloerection, tail erection and a moderate loss of equilibrium was observed. The animals remained in this altered state for the next 4 min before they progressively recovered their normal physiological parameters. However, despite this rapid recovery, their consumption of food decreased for the following 12 hrs. Successive treatment with the crude alkaloid extract resulted in wasting, anorexia and auto-mutilation of the forelimb digits.

The animals that received the extract from *L. montanus* extract suffered convulsions of variable intensity for 1 min, followed by a period of depression that lasted less than five min. During this period, an increase in the cardiac and respiratory frequency was registered in the animals. Similarly, piloerection, tail erection, grooming, muscular contractions (principally of limbs), a loss of equilibrium, excitation, tachypnea and an unsteady gait was also observed in this period, followed by a decrease in food and water consumption. Subsequent administrations of the crude alkaloid extract produced more severe signs of depression and the animals required a longer time period to fully recover. By the end of the experimental period the animals were lethargic, showing variable postures and cachexia.

Table 1. Principal QA present in *L montanus* and *L. exaltatus* alkaloids extracts. Data represents the mean ± SD of 3 independent determinations.

ALKALOID	ALKALOID CONTENT (mg/g)	
	L. montanus	*L. exaltatus*
Sparteine	35.4±0.13	0.284±0.004
Lupanine	17.7±0.096	5.832±0.46
3-OH-Lupanine	3.8±0.47	1.526±0.19
13-OH-Lupanine	3.2±0.43	-

Microscopic analysis of cerebral damage

In the rats treated with sesame oil alone, a relatively small number of degenerating neurons were found along the borders of the cannula and in other regions of the coronal brain slices stained with haematoxylin and eosin (data not shown). These cells had a slightly shrunken eosinophilic cytoplasm and pyknotic nuclei. Furthermore, some neuronal swelling, mild gliosis and a few ghost cells could also be observed (Fig. 1). In contrast, more severe damage was readily observed in cerebral slices from both groups of experimental animals as a result of LQA administration. Numerous degenerative neurons and pathological signs of variable intensities were observed. The most frequent findings corresponded to the accumulation of abundant eosinophilic neurons (red neurons), particularly in the middle layers of the cerebral cortex. These neurons had a shrunken eosinophilic cytoplasm and strongly stained pyknotic nuclei. Other pathological signs included a varying degree of neuronal swelling, perineuronal

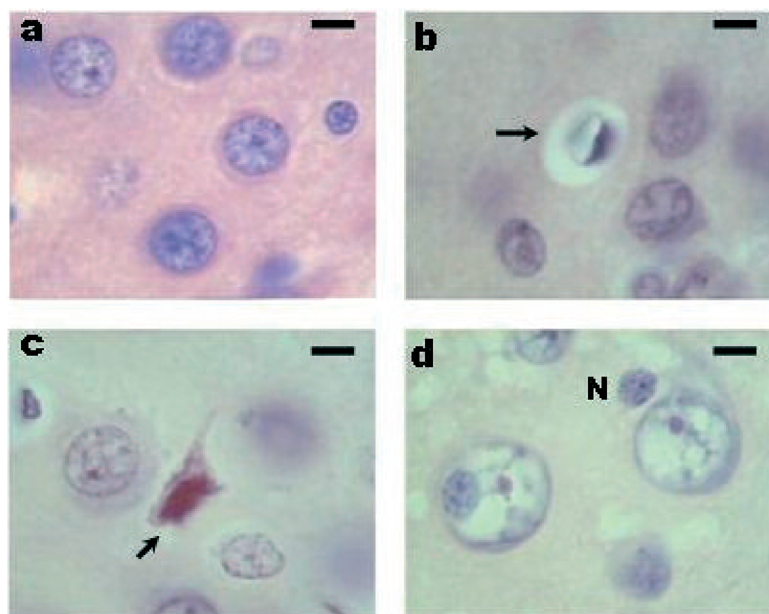

Fig. 1. Coronal slices of rat brain stained with H&E that show normal and affected neurons. **a.** Tissues from TI rats have a normal appearance, the parenchyma appears compact and the neurons have a spherical nucleus with scattered heterochromatin, **b.** the dark arrow points to a "ghost neuron", characterized by an oval empty space with some membranes remains inside (TIII group), **c.** an eosinophilic neuron is indicated (red cell), which appears with a dark nucleus color due to an altered pH (TII group), **d.** shows enlarged swollen neurons with a translucent cytoplasm containing dissolved organelles and eccentric nucleus. N indicates the nucleus of a microglia cell, the nervous parenchyma appearing to have a spongy appearance (TII group). Bar: 5 µm.

Table 2. Cerebral regions and specific structures where degenerating neurons were identified after ICV injection of LQA extracts (Treatment II or III).

TREATMENT II	
Amygdaloid body	Nucleus: anterior cortical, basomedial, intercalated, medial, posterolateral cortical, and posteromedial cortical. Areas: amygdalo-hippocampal and anterior amygdaloid area
Cerebellum	Interpositus nucleus, Purkinje cells in: lobules 1, 2, 3, 4, 5, 6, 7, crus 1 and crus 2 ansiform lobule, flocculus, paraflocculus and simple.
Cortex	Cortex-amygdale, frontal, lamina dissecans, retrosplenial cortex and striate cortex (area 18, 18a, 17)
Hippocampus	Nucleus: habenular, dorsal hippocampus comissure and septohippocampal Areas: fasciola cinereum, hilus, parasubiculum and presubiculum.
Hypothalamus	Nucleus: arcuate, lateral and medial mammillary, magnocellular (tuberal and caudal magnocellular), posterior and dorsomedial ventromedial, premammillary, periventricular, perifornical, paraventricular, preoptic suprachiasmatic, supraoptic, suprachiasmatic, suprammillary. Areas: anterior, dorsal, lateral, lateral preopotic, medial, and retrochiasmatic
Midbrain	Nucleus: accessory oculomotor, accessory optic tractus, Darkschewitsch, deep mesencephalic, interstitial of Cajal, interfascicular, interpeduncular, interstitial magnocellular of posterior commissure, intracommissural of the posterior commissure, medial terminal, mesencephalic tractus trigeminal nerve, olivary pretectal, optic tractus, parabrachial pigmented, paranigral, posterior pretectal, red and retrorrubral. Areas: central grey matter, inferior colliculus, prerubral, pretectal, retrorrubral, substantia nigra, superior colliculus, and ventral tegmental.
Pons and Medulla	Nucleus: cochlear, cuneiform, granule cell layer cochlear, Kolliker-Fuse, lateral cervical, lateral lemniscus, locus coeruleus, motor trigeminal, parabigeminal, parabrachial, pedunculopontine, pontine, pontine prepositus hypoglossal, reticular, raphe, raphe pontis, principal sensory trigeminal, reticular, reticulotegmental pons, retrorubral, spinal tractus trigeminal nerve, subcoeruleus, suprafacial, suprageniculate nuc pons, tegmental, ventral tegmental, trapezoid body and vestibular. Areas: inferior colliculus, superior colliculus and superior olive.
Subcortical Structures	Nucleus: anterior commissural, anterior olfatory, bed nuc accesory olfatory tract, bed nuc anterior commissure, bed nuc stria terminalis, entopeduncular, gemini, lateral, septofimbrial, septohypothalamic, fields of Forel and triangular septal. Areas: incerta zone, subfornical organ, Islands of Calleja, olfatory tract and taenia tecta.
Thalamus	Nucleus: anterodorsal, anteromedial, anteroventral, centrolateral, central medial, gelatinosus, dorsal lateral geniculate, intermediate geniculate, intermediodorsal, interanteromedial, lateral posterior, laterodorsal, medial geniculate, mediodorsal, paracentral, parafascicular, paratenial, paraventricular, posterior nuclear group, reuniens, rhomboid, submammillothalamic, subparafascicular, subthalamic, suprageniculate, ventrolateral and ventromedial.
TREATMENT III	
Amygadaloid body	Nucleus: anterior cortical, cortical, basomedial, intercalated, medial, posterolateral and posteromedial cortical. Areas: anterior amygdaloid and amygdalo-hippocampal.
Basal ganglia	Nucleus: septal, accumbens, subthalamic, vertical limb diagonal band and horizontal limb diagonal band. Areas: cell bridges between caudate putamen and olfatory tubercle, olfatory tubercle, substantia innominata and ventral pallidum.
Cerebellum	Purkinje cells in: lobules 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, crus 1 and crus 2 ansiform lobule, flocculus, paraflocculus, paramedian and simple.
Cortex	Cortex-amygdala (transitional zone), frontal, lamina dissecans, striate cortex (area 18, 18a, 17) and retrosplenial cortex.
Hippocampus	Nucleus: dorsal hippocampus comissure,habenular and septohippocampal. Areas: fasciola cinereum, hilus dentate gyrus, presubiculum and parasubiculum.
Hypothalamus	Nucleus: arcuate, dorsomedial, magnocellular, mammillary, median preoptic, paraventricular, perifornical, periventricular, posterior, premammillary, preoptic, suprachiasmatic, septohypothalamic, suprachiasmatic, supramammillary, supraoptic and ventromedial. Areas: anterior, dorsal, lateral, lateral preoptic, medial and retrochiasmatic.
Midbrain	Nucleus: Darkschewitsch, deep mesencephalic, interfascicular, interpeduncular, interstitial of Cajal, interstitial magnocellular of posterior commissure, intracommissural of the post commissure, medial terminal of accessory optic tractus, mesencephalic tract trigeminal nerve, olivary pretectal, optic tract, parabrachial pigmented, paranigral, posterior pretectal, red and retrorrubral. Areas: central grey matter, inferior colliculus, pretectal, prerubral, retrorrubral, substantia nigra, superior colliculus and ventral tegmental.
Pons and Medulla	Nucleus: cuneiform, facial, granule cell layer cochlear, K, Kolliker-Fuse, locus coeruleus, lateral lemniscus, motor trigeminal, parabigeminal, parabrachial (dorsal and ventral), pedunculopontine, pontine, pontine reticular, principal sensory trigeminal, raphe, raphe pontis, reticulotegmental pons, retrorubral, sagulum, subcoeruleus, trapezoid body, tegmental, ventral cochlear and ventral tegmental. Areas: inferior colliculus, superior colliculus and superior olive.
Subcortical Structures	Nucleus: anterior commissural, anterior olfatory, bed nucleus accesory olfatory tract, bed nuc anterior commissure, bed nuc stria terminalis, entopeduncular, fields of Forel, gemini, lateral olfatory tract, peripeduncular, septofimbrial, septohypothalamic and triangular septal. Areas: Islands of Calleja, subfornical organ, taenia tecta and zone incerta
Thalamus	Nucleus: anterodorsal, anteromedial, anteroventral, central medial, centrolateral, dorsal lateral geniculate, gelatinosus, interanteromedial, intermediate geniculate, intermediodorsal, laterodorsal, lateral posterior, medial geniculate, mediodorsal, paracentral, parafascicular, paratenial, paraventricular, posterior thalamic nuclear group, reuniens, rhomboid, submammillothalamic, subparafascicular, subthalamic, suprageniculate, ventrolateral and ventromedial.

vacuolation, ghost cells (hypochromasia), basophilia of the rough endoplasmic reticulum and numerous neuronophagy figures.

Significant differences were found in the neuronal populations in which eosinophilia, swelling and ghost cells were observed between control and experimental groups at P < 0.05 (Figs 2-4). Animals treated with sesame oil showed fewer degenerate cells (~10%), while animals treated with LQA showed a much higher number of neurons with degenerative alterations (30-40%). Only small differences were observed in the number of degenerate cells between animals subjected to treatments II and III (Figs. 2, 3).

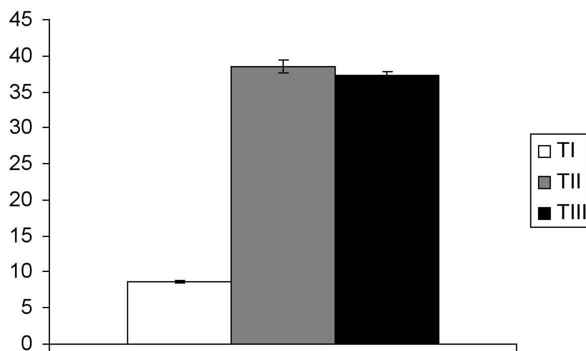

Fig. 2. Effect of ICV LQA administration on the number of eosinophilic neurons exhibiting signs of neurodegeneration in different cerebral areas. Animals received sesame oil alone (TI), or LQA (TII and TIII) and each bar represents the mean ± SEM of 20 determinations. *: indicates significant difference at P<0.05.

Discussion

The results of this study show that, with the exception of 13-OH-lupanine, the two lupine species studied contained the same principal QAs. The QA content in *L. exaltatus* was 6 to 13 times less than that detected in *L montanus*, nevertheless the relative proportions of the QAs was respected. As such, sparteine and lupanine were the main alkaloids in these extracts, followed by 13-OH-lupanine and 3-OH-lupanine. These alkaloids have also been found in *L. luteus*, *L. hispanicus*, *L. mutabilis* and other native species of lupines from Mexico (Wink et al., 1995). In addition, *L. mutabilis* was found to contain high concentrations of sparteine. Indeed, previous studies by our group found that sparteine and lupanine are the main alkaloids present in *L. montanus*, *L. exaltatus*, *L. resflexus* and *L. stipulatus* (García-Lopez et al., 1998).

We administered the alkaloids to the animals via the ICV route because of the limited quantities of the QA raw extracts isolated. In this way, free diffusion of these hydrophobic compounds throughout the cerebral parenchyma was permitted, without them having to pass through the blood-brain barrier. This method of administration also avoided any metabolic transformation of the active compounds before their contact with the cerebral tissue. However, the disadvantage of this procedure is that secondary alterations may appear in the cerebral cortex of the rats as a result of the presence of the cannula. These secondary alterations may be associated with the transitory increase in the intraventricular pressure caused by the volume of liquid injected, as observed in the rats that received the sesame oil vehicle alone (TI).

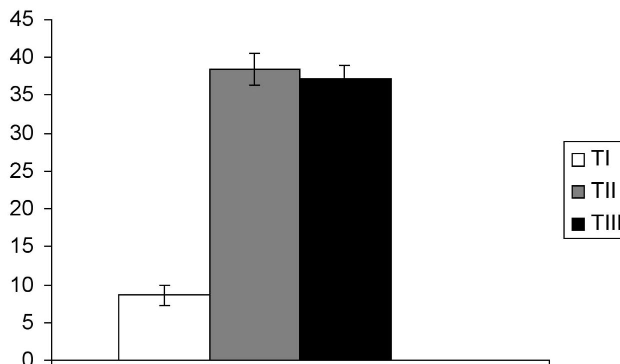

Fig. 3. The percentage of swollen neurons in brain tissue slices from animals treated with sesame oil alone (TI) or with LQA from L. montanus (TII) and L exaltatus (TIII). Each bar represents the mean ± SEM and *: indicates significant difference at P<0.05

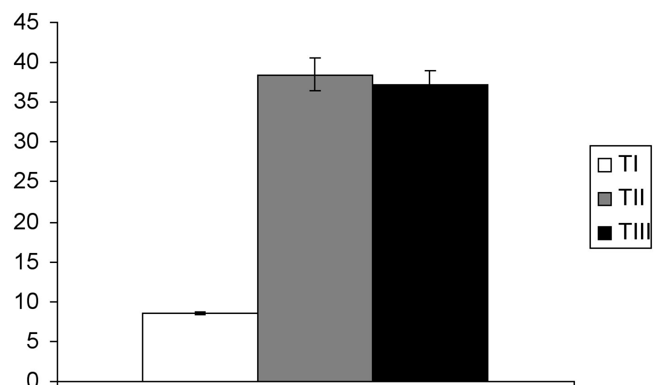

Fig. 4. Percentage of neurons with hypochromasia and no visible nuclei from cerebral areas in which features of degeneration were observed in animals treated with sesame oil alone (TI) or with LQA from *L. montanus* (TII) and *L exaltatus* (TIII). Each bar represents the mean ± SEM and *: indicates significant difference at P<0.05.

Therefore, the failure to observe significant alterations in the animals that received only the sesame oil indicated that the damage observed in the animals that received LQAs was not the result of changes in the intraventricular pressure.

Although it is well known that the alkaloids extracted from lupine seeds cause alterations of nervous behavior, to date there have been no studies regarding their possible damage to neurons. For this reason; the main objective of this study was to assess the clinical and neuronal effects caused by the ICV administration of crude QA extracts dissolved in sesame oil. Sesame oil was chosen as the carrier due to its frequent use in studies of neurotoxicity and its lipophilic properties, which assure its free diffusion in the cerebral tissue (Renner et al., 1986; Hammer 1988; Chen and Squier, 1990; Frye and Duncan 1996; Furuta et al., 2002; Li et al., 2002; Marriott and Korol, 2003). For this reason, the microscopic analysis and the analysis of the histopathological data was only performed in the contralateral unlesioned left hemisphere.

The QA extracts affected broad areas of the brain with varying intensity. A striking finding was that many of the cerebral structures that were damaged possess cholinergic neurons (septal nucleus, ventral pallidum, vertical and horizontal nucleus of the limb diagonal band, and tegmental nucleus), structures related to cholinergic paths (hippocampus, thalamic nucleus, different areas of the bark, bulb olfactory, amygdaloid nucleus), and structures of the basal ganglion that possess cholinergic receptors (muscarinic or nicotinic type). Since a number of the damaged areas corresponded to pathways that contain nicotinic and muscarinic receptors, these neurotoxic findings support the mode of action of QAs in the central nervous system proposed by Kinghorn and Balandrin (1984) and Schmeller et al. (1994).

The toxic degenerative effects of the QAs were also observed in neurons within cerebral structures and nuclei unrelated to the cholinergic pathways. In these areas, the mechanism of action is not understood. Additional studies to establish the affinity of purified QA for cholinergic receptors, the susceptibility of different neurons and the mechanism of neuronal death will help us to a better understanding the effects of QAs in the brain.

The neurons affected showed lesions typical of those caused by ischemia, hypoxia (eosinophilic neurons) and some neurotoxins. This type of damage is associated with an acute stage of cell death (Yamaoka et al., 1993; Jackson-Lewis et al., 1995; Kofke et al., 1996; Rosenblum, 1997; McD Anderson and Opeskin, 1998; Adogwa, 1999). Cellular swelling is associated with a failure in energy metabolism or a loss of selective membrane permeability (Majno and Joris, 1995). Indeed, our observations suggest that the QA might interfere with mitochondrial function. The presence of "ghost" cells is also associated with conditions in which hipochromasia prevails, and indicates an advanced neurodegenerative stage (García et al., 1995).

The main difference between the animals treated with *L. montanus* or *L. exaltatus* QA extracts was the severity of neuronal damage throughout all the areas examined. The QAs from *L. montanus* were more toxic than those of *L. exaltatus* extracts. These results are in agreement with the high content of sparteine found in QA extracts from L. montanus, which has been reported to be ten times more toxic than lupanine (Pothier et al., 1998). It is also possible that the *L. montanus* QA crude extracts contained other QAs with higher toxicity, or that synergistic neurotoxic effects occur among the alkaloids present.

In conclusion, the results of this study demonstrated that the ICV administration of crude LQA extracts causes neuronal damage in different cerebral regions, whose severity varied according to their lupanine and sparteine content.

Acknowledgements. This study was financed by grants from CONACYT (Mexico), Ref. 4047P-B.

References

Adogwa A.O., Alleyne T. and Mohammed A. (1999). Degenerative changes in the cerebral cortex in swayback disease lambs. Alzheimer's Disease Rev. 4, 19-22.

Butler W.H., Ford G.P. and Creasy D.M. (1996). A 90 day feeding study of lupin (Lupinus Angustifolius) flour spiked with lupin alkaloids in the rat. Food Chem. Toxicol. 34, 531-536.

Chen Y.P. and Squier C.A. (1990). Effect of nicotine on 7,12-dimethylbenz[a]anthracene carcinogenesis in hamster cheek pouch. J. Natl. Cancer Inst. 82, 861-864.

Davis A.M. (1982). The occurrence of anagyrine in a collection of western Americans lupines. J. Range Maneg. 35, 81-84.

Frye C.A. and Duncan J.E. (1996). Estradiol benzoate potentiates neuroactive steroids' effects on pain sensitivity. Pharmacol. Biochem. Behav. 53, 27-32.

Furuta M., Funabashi T. and Kimura F. (2002). Suppressive action of orexin A on pulsatile luteinizing hormone secretion is potentiated by a low dose of estrogen in ovariectomized rats. Neuroendocrinology 75, 151-157.

García J.H, Liu K.F. and Ho K.L. (1995). Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. Stroke 26, 636-643.

García-López P.M., Zamora-Natera J.F., Ruiz-Lopez M.A., Bañuelos-Pineda J., Ruiz-Moreno J.J. and Muzquiz-Elorrieta M. (1998). Determinación de alcaloides en tres especies de lupinus del estado de Jalisco (México). In: Proceedings of the VII Congreso Latinoamericano de Botánica y XIV Congreso Mexicano de Botánica, 18-22 Octubre, Cd. de México, México.

Hammer R.P. Jr (1988). Opiate receptor ontogeny in the rat medial preoptic area is androgen-dependent. Neuroendocrinology 48, 336-341.

Hatzold T., Elmaddfa I., Gross R. Wink M., Hartmann T. and Witte L. (1983). Quinolizidine alkaloids in seeds of Lupinus mutabilis. J. Agric. Food Chem. 31, 934-938.

Jackson-Lewis V., Jakowec M., Burke R.E. and Przedborski S. (1995).

Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neurodegeneration 4, 257-269.

Keeler R.F. (1976). Lupin alkaloids from teratogenic and nonteratogenic lupins. III. Identification of anagyrine as the probable teratogen by feeding trials. J. Toxicol. Environ. Health 1, 887-898.

Kinghorn A.D. and Balandrin M.F. (1984). Alkaloids. In: Chemical and biological perspectives. Pelletier S.W. (ed). John Wiley & Sons. New York. pp 105-148.

Kofke W.A., Garman R.H., Stiller R.L., Rose M.E. and Garman R. (1996). Opioid neurotoxicity: fentanyl dose-response effects in rats. Anesth Analg. 83, 1298-1306.

Korper S., Wink M. and Fink H.A. (1998). Differential effects of alkaloids on sodium currents of isolated single skeletal muscle fibers. FEBS Lett. 436, 251-255.

Li Y., Jiang N., Powers C. and Chopp M. (1998). Neuronal damage and plasticity identified by microtubule-associated protein 2, growth-associated protein 43, and cyclin D1 immunoreactivity after focal cerebral ischemia in rats. Stroke 29, 1972-1980.

Li Q.G., Mog S.R., Si Y.Z., Kyle D.E., Gettayacamin M. and Milhous W.K. (2002). Neurotoxicity and efficacy of arteether related to its exposure times and exposure levels in rodents. Am. J. Trop. Med. Hyg. 66, 516-525.

Marriott L.K. and Korol D.L. (1995). Short-term estrogen treatment in ovariectomized rats augments hippocampal acetylcholine release during place learning. Neurobiol. Learn Mem. 80, 315-322.

Majno G. and Joris I. (1995). Apoptosis, oncosis, and necrosis. An overview of cell death. Am. J. Pathol. 146, 3-15.

McD Anderson R. and Opeskin K. (1998). Timing of early changes in brain trauma. Am. J. Forensic Med. Pathol. 19, 1-9.

McVaught R. (1987). Leguminosae. A descriptive account of the vascular plant of Western México. In: Flora novogaliciana. Ann. Arbor (eds). The University of Michigan Press. USA. pp 567-569.

Muzquiz M., Burbano C., Gorospe M.J. and Rodenas I. (1989). A chemical study of Lupinus hispanicus seed. Toxic and antinutritional components. J. Sci. Feed Agric. 47, 205-213.

Muzquiz M., Burbano C., Cuadrado C. and De la Cuadra C. (1993). Determinación de factores antinutritivos termo-resistentes en leguminosas. I. Alcaloides. Inves. Agrar. Prod. Veg. Ed. V. A. impresores. Madrid, España. 8, pp 350-361.

Paxinos G. and Watson C. (1982). The rat brain in stereotaxic coordinates. Academic Press. pp 9-60.

Pothier J., Cheav L., Galand N. and Viel C. (1998). A comparative study of the effects of sparteine, lupanine and lupin extract on the central nervous system of the mouse. J. Pharm. Pharmacol. 50, 949-954.

Renner K.J., Allen D.L. and Luine V.N. (1986). Monoamine levels and turnover in brain: relationship to priming actions of estrogen. Brain Res Bull. 16, 469-475.

Robbins M.C., Petterson D.C. and Brantom P.G. (1996). A 90-day feeding study of alkaloids of lupinus angustifolius in the rat. Food Chem. Toxicol. 34, 679-686.

Rosenblum W.I. (1977). Histopathologic clues to the pathways of neuronal death following ischemia/hypoxia. J. Neurotrauma 14, 313-326.

Ruiz-López M.A. and Sotelo A. (2000). Chemical composition, nutritive value and toxicology evaluation of Mexican wild lupins. J. Agric. Food Chem. 49, 5339-5339.

Schmeller T., Sauerwein M., Sporer F. and Wink M. (1994). Binding of quinolizidine alkaloids to nicotinic and muscarinic acetylcholine receptors. J. Natural Product. 57, 1316-1319.

Schmitt H. (1980). Les neuroleptiques. In: Elements de pharcologie. 7th ed. Flammarion. Paris. p 356.

Wink M. (1987). Allelochemicals. Role in agriculture and forestry. No. 330 Waller G.R. (eds). ACS Syposium Series. Washington. D.C. pp 524-533.

Wink M. (1992). Insect plant interactions. Bernays E.A. (ed). CRC Press. Boca Raton. Florida. pp. 133-166.

Wink M. (1993a). Quinolizidine alkaloids. In: Methods in plant biochemistry. Waterman P. (ed). Academic Press. London. pp 197-239.

Wink M. (1993b). Allelochemical properties and the raison d'être of alkaloids. In: The alkaloids. Cordell G. (ed). Academic Press. San Diego. pp 1-188.

Wink M., Meibner C. and Witte L. (1995). Patterns of quinolizidine alkaloids in 56 species of genus lupinus. Phytochemistry 38, 139-153

Yamaoka Y., Shimohama S., Kimura J., Fukunaga R. and Taniguchi T. (1993). Neuronal damage in the rat hippocampus induced by in vivo hypoxia. Exp. Toxicol. Pathol. 45, 205-209.

Yovo K., Huguet F. Pothier J., Durand M., Breteau M. and Narcisse G. (1984). Comparative pharmacological study of sparteine and its ketonic derivative lupanine from seeds of lupinus albus. Planta Med. 50, 420-424.

Accepted June 27, 2005