

Diagrams

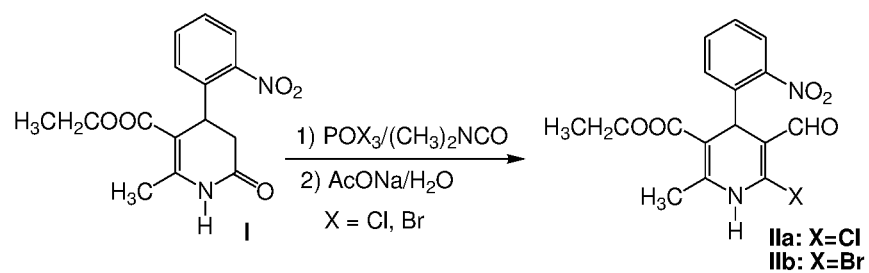


Figure 1. Obtaining **IIa** and **IIb** Diagram

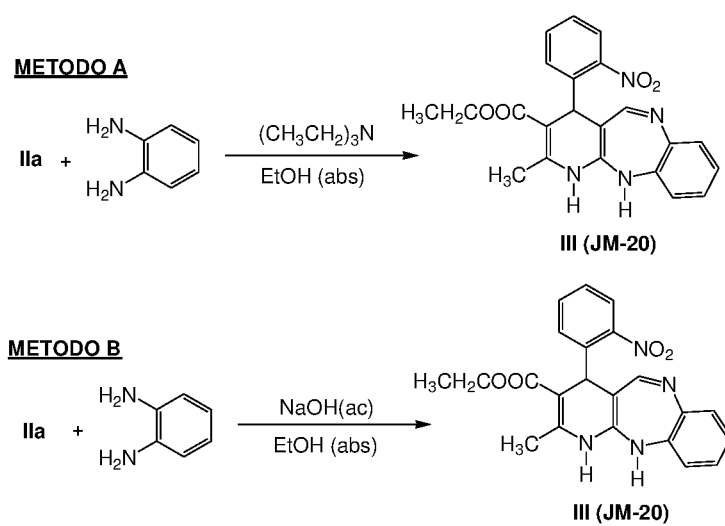


Figure 2.

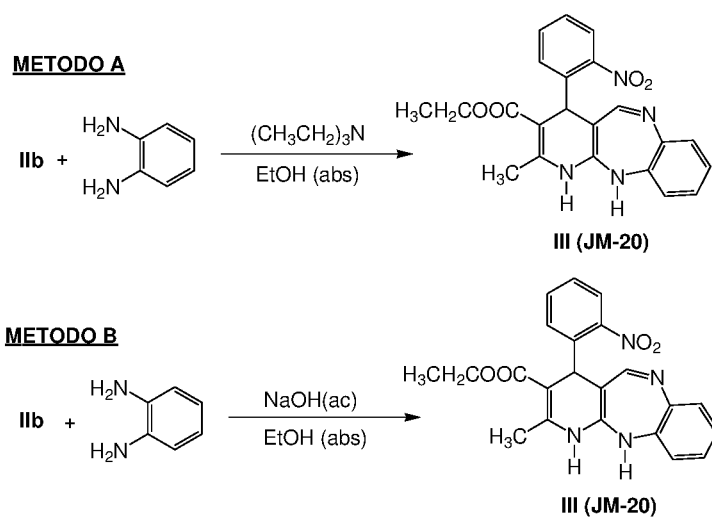


Figure 3.

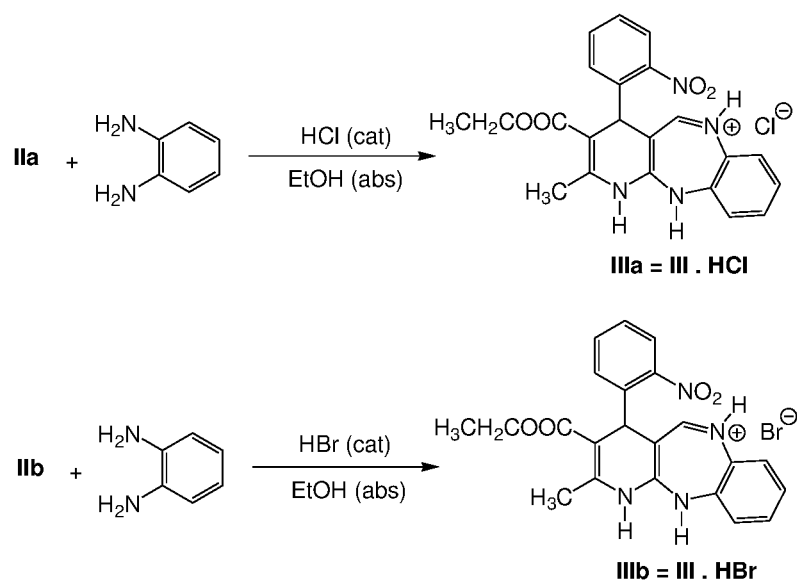


Figure 5. Obtaining Compound **III** and its Halohydrates Diagram

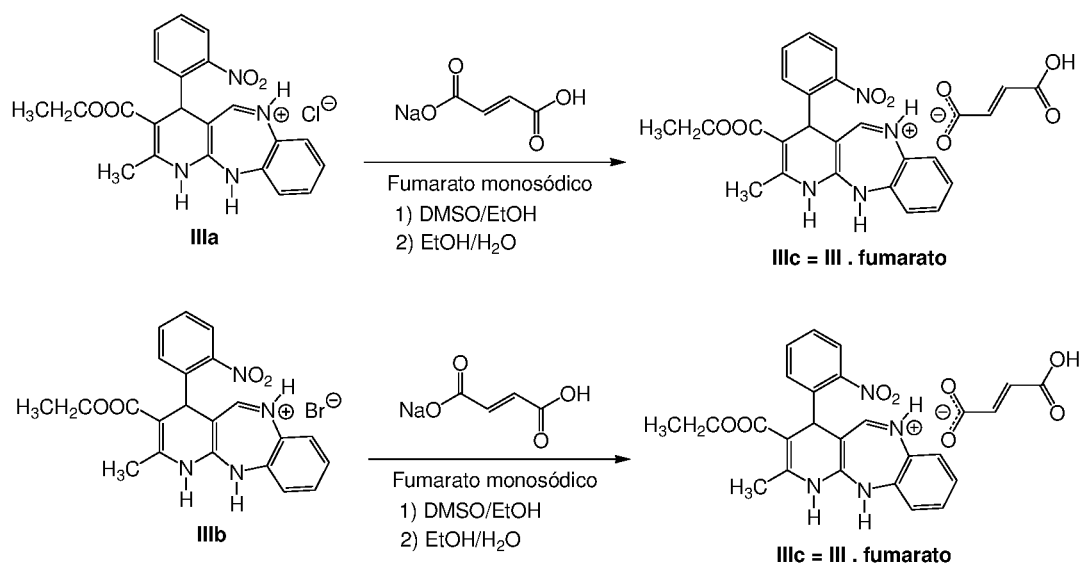


Figure 6. Obtaining fumarate salt from JM-20 (**IIIc**).

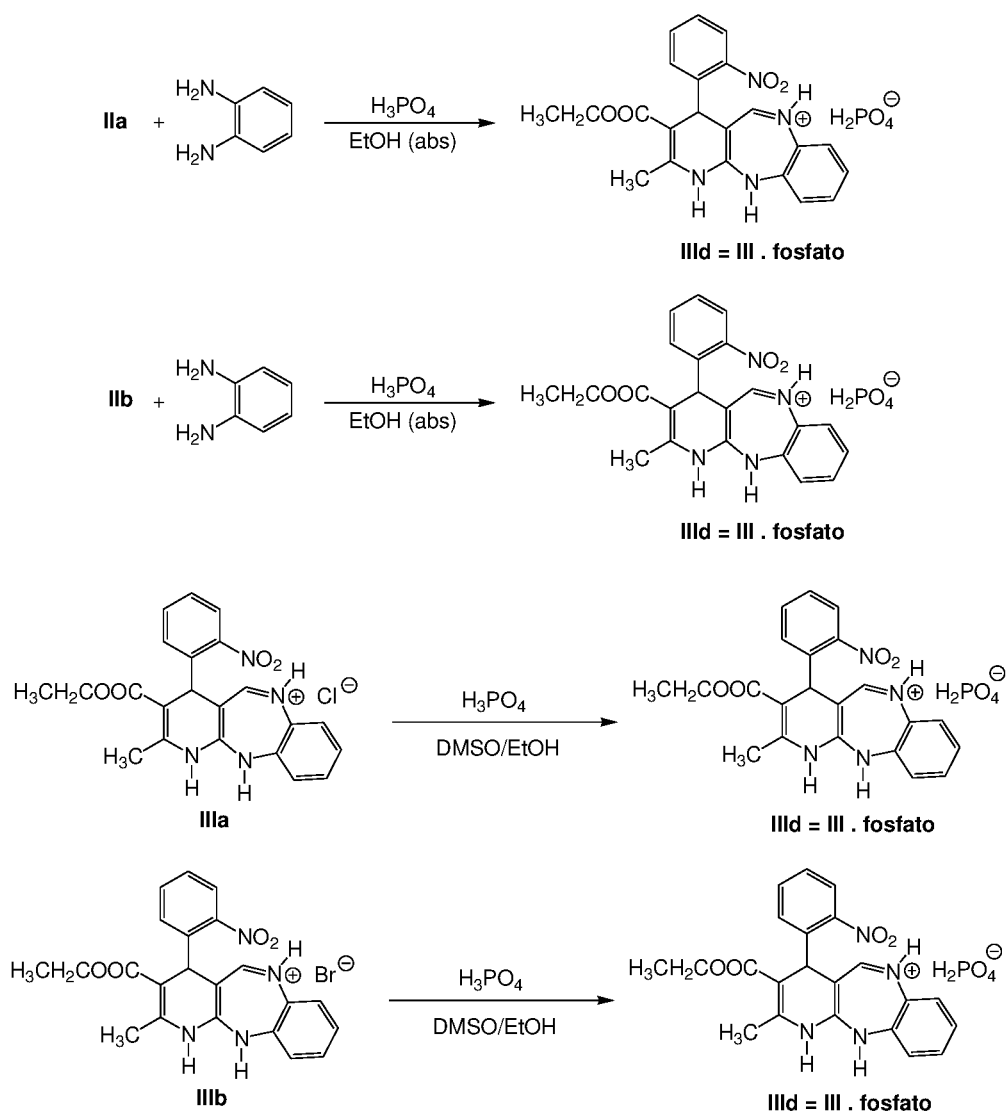


Figure 7. Obtaining phosphate salt from JM-20 (III d).

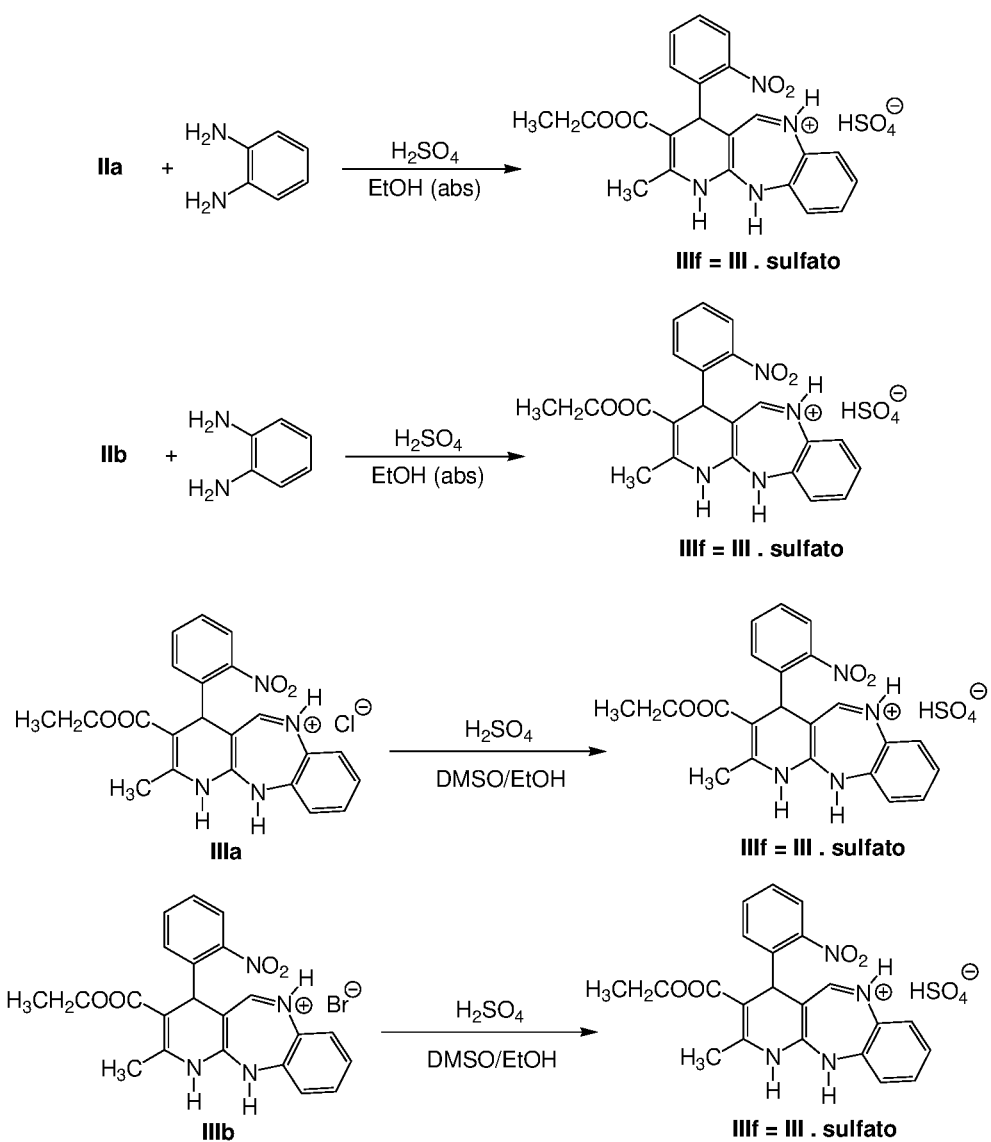
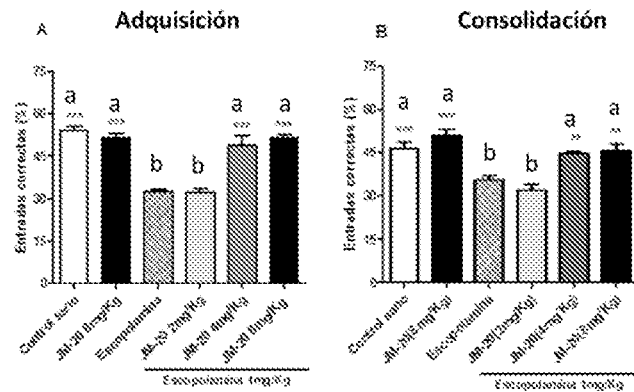


Figure 8. Obtaining sulfate salt from JM-20 (**III f**).

Memoria de Corto plazo



Memoria de Largo Plazo

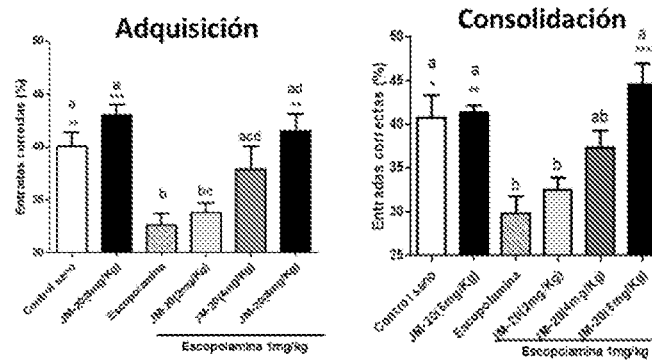


Figure 9: Effects of JM-20 on short and long term spatial memory, particularly in the processes of acquisition and consolidation. Data is expressed as the mean $\pm$ DE (n=7, per group) of the percentage of spontaneous alternation. For the statistical analysis we carried out ANOVA and Tukey multiple comparison tests. Different letters differentiate amongst themselves for $p < 0.05$.

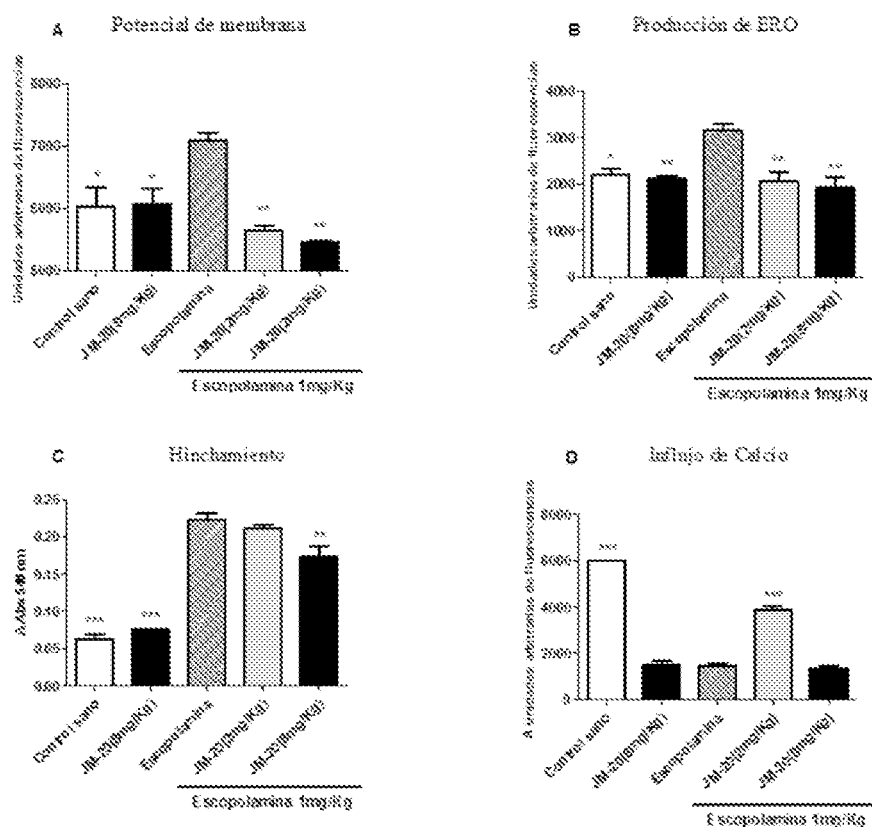


Figure 10: Effects of JM-20 on the mitochondrial dysfunction of cerebral tissue in rats treated with scopolamine. The different doses of JM-20 were administered orally 1 hour prior to the administration of the scopolamine. Brain mitochondria were isolated 1 hour after the administration of the scopolamine and we assessed membrane potential (**A**), H_2O_2 generation (**B**), mitochondrial swelling (**C**) and calcium influx to the mitochondria (**D**). The bars represent the mean $\pm$ EEM ($n = 10$). Different letters show the differences between groups: $p < 0,05$, by ANOVA and *post hoc* Tukey. (A): ***, $p = 0.0009$, (B): **, $p = 0.0034$ regarding the vehicle/scopolamine 1mg/Kg group, by ANOVA and *post hoc* Tukey group.

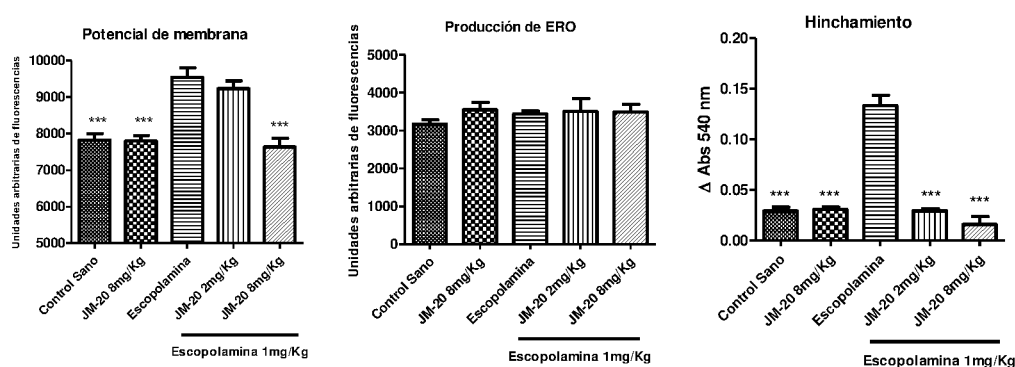


Figure 11: The effect of JM-20 on mitochondrial dysfunction of cerebral tissue in rats treated with scopolamine. The different doses of JM-20 were administered orally 1 hour prior to the administration of the scopolamine. Brain mitochondria were isolated 24 hours after administration of the scopolamine and we assessed the membrane potential (A), H_2O_2 generation (B) and mitochondrial swelling (C). The bars represent the mean $\pm$ EEM ($n = 10$). ***, $p < 0.001$; **, $p < 0.01$ y *, $p < 0.05$ in regards to the vehicle/scopolamine group 1mg/Kg, by ANOVA and *post hoc* Tukey.

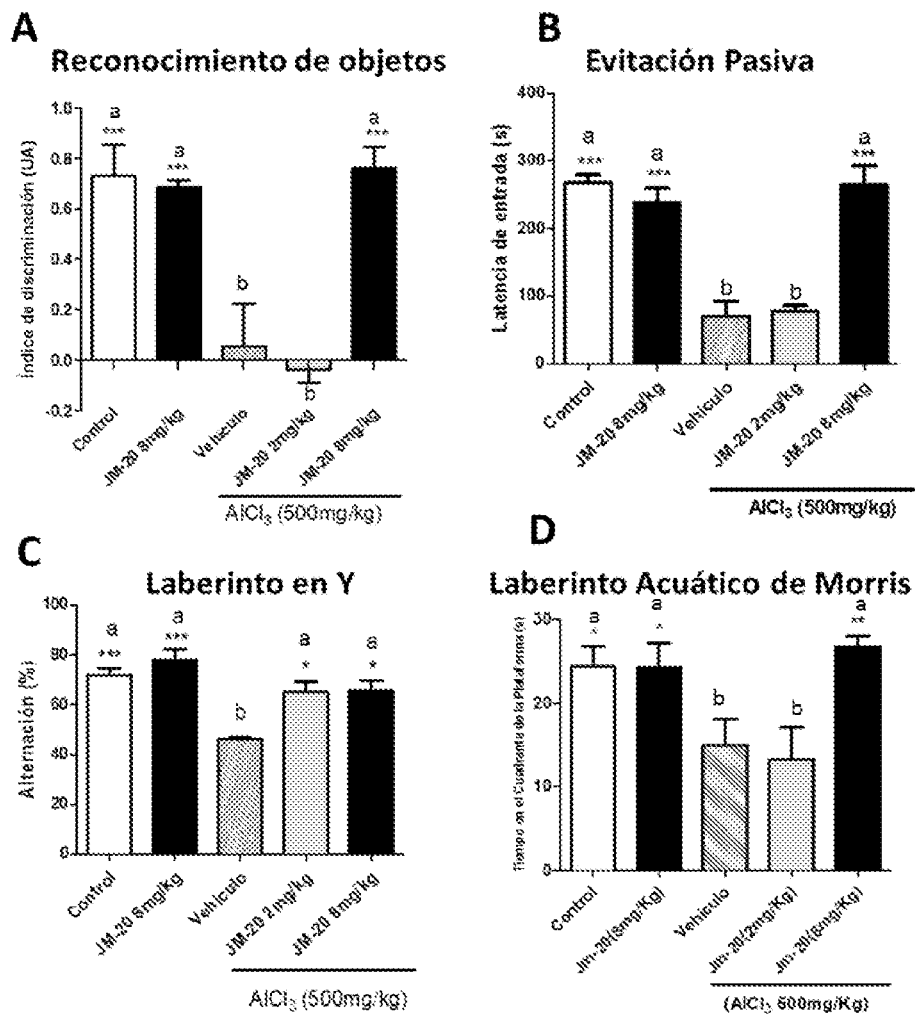


Figure 12: The effect of JM-20 on the loss of different types of memory induced by aluminum. A) Recognition of novel objects test, novel object recognition memory; B) Passive avoidance test, emotional-associative memory; C) Y-maze test, spatial working memory; D) Morris Water Maze spatial reference memory. The bars represent the mean $\pm$ EEM. Different letters show differences between groups: $p < 0.05$, by ANOVA and post hoc Tukey. ***, $p < 0.001$; **, $p < 0.01$ y *, $p < 0.05$ comparison in regards to the group treated with aluminum 500mg/Kg, by ANOVA and post hoc Tukey group.

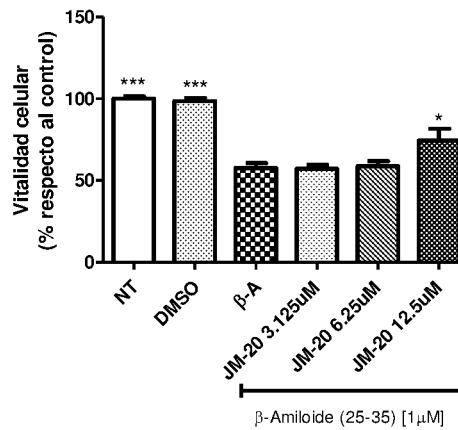


Figure 13: Effects of JM-20 on damage to cellular viability produced by beta amyloid 25-35 peptide oligomers

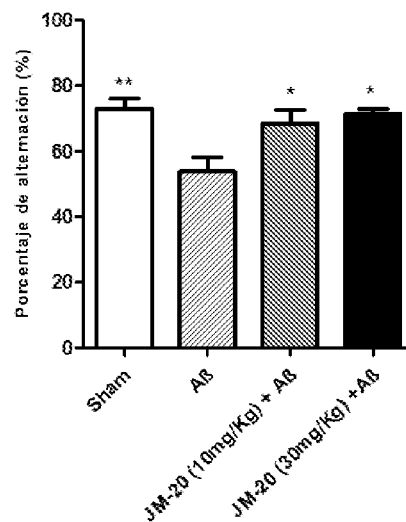


Figure 14. JM-20 reverts memory loss induced by beta amyloid 25-35 peptide oligomers. JM-20 was administered 7 days following administration of the beta amyloid. The bars represent the mean $\pm$ EEM; the comparison is done on the group treated with beta amyloid ***, $p < 0.001$; **, $p < 0.01$ y *, $p < 0.05$ by ANOVA and post hoc Tukey group.

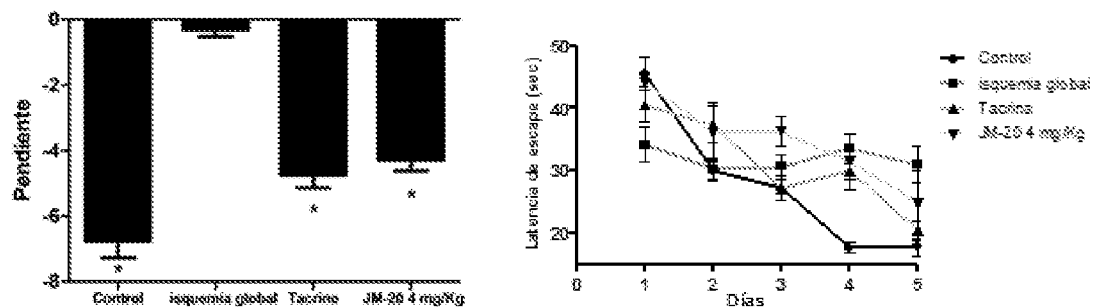


Figure 15: Effects of JM-20 on cognitive deterioration induced by the transitory occlusion of the common carotid arteries of rats. The animals were administered oral doses of 4 mg/Kg, 1 after reperfusion. Data is expressed as the mean $\pm$ DE (n=8, per group). For statistical analysis we carried out ANOVA and Tukey multiple comparison tests, * $p < 0.01$, represents significant differences in regards to ischemic animals without n treatment.

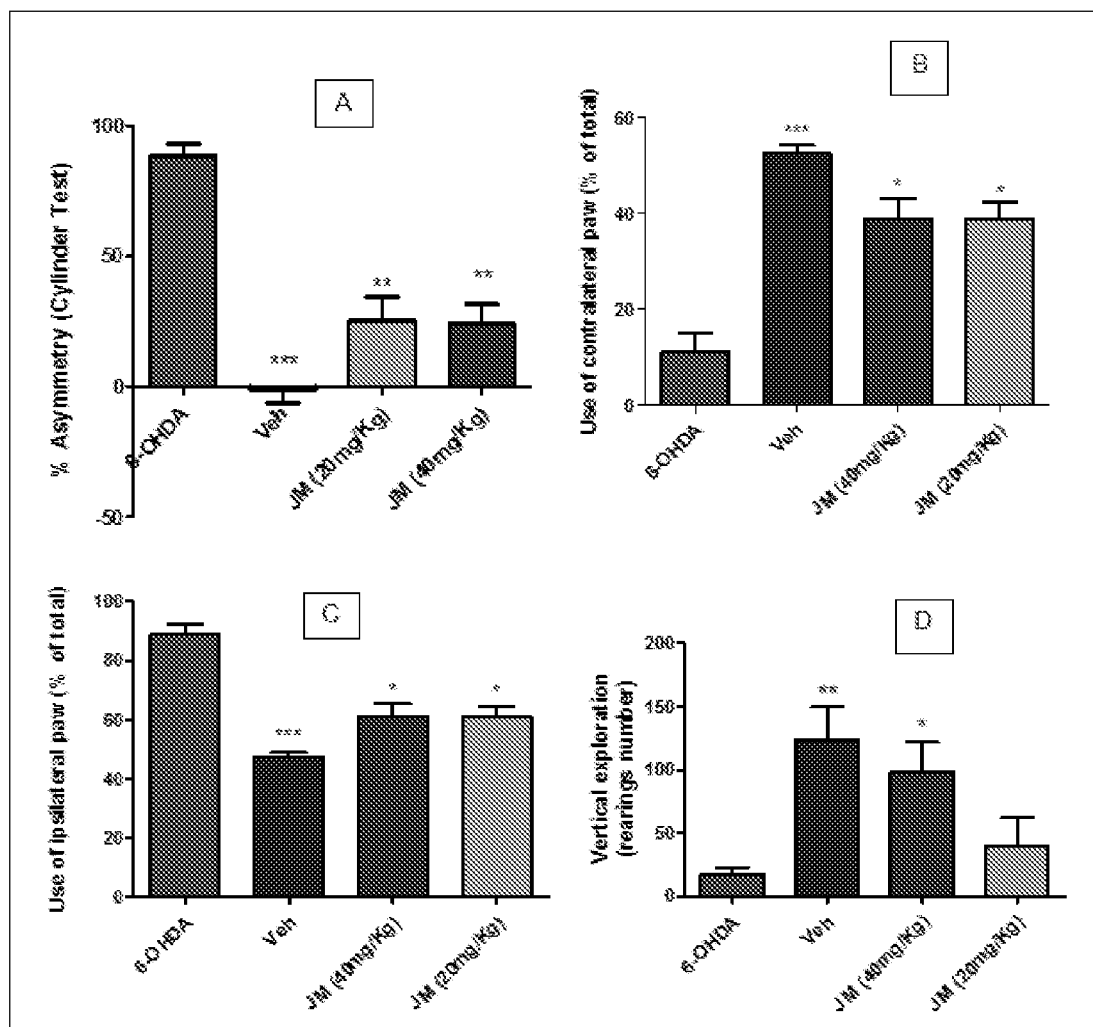


Figure 16: The neuroprotector effect of JM-20 on damage induced by 6-OHDA in male Wistar rats after 7 days of treatment. (A) reduction of the % of asymmetry (cylinder test), animals treated with JM-20 showed diminished motor damage in comparison with damaged animals without treatment. **(B)** increased use of the paw contralateral to the damage and **(C)** normal values of the use of the ipsilateral paw (50%~), in damaged animals and those treated with both doses of JM-20 (40 and 20 mg/Kg). **(D)** Animals treated with JM-20 increased their vertical exploration, being statistically different to the dose of 40mg/Kg in regards to untreated damaged animals.

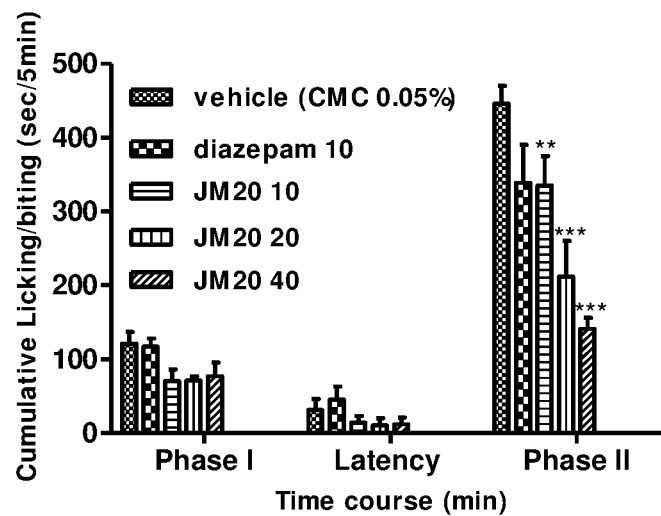


Figure 17A: Effects of JM-20 (10-40mg/Kg, 10mL/Kg, p.o.), diazepam 10mg/Kg or vehicle (CMC, 0.05%) on licking/biting behavior after injecting 5% formalin on the plantar surface of the rat's paw. The data is presented as accumulative mean of the time of licking/biting $\pm$ EEM during Phase I (0-5min), Latency (5-15min) and Phase II (15-45min) or the formalin test, $n=7$ per group, *** $p<0.001$ represents significant differences in regards to the control group treated with the vehicle (ANOVA for one route followed by Dunnett's *a posteriori*).

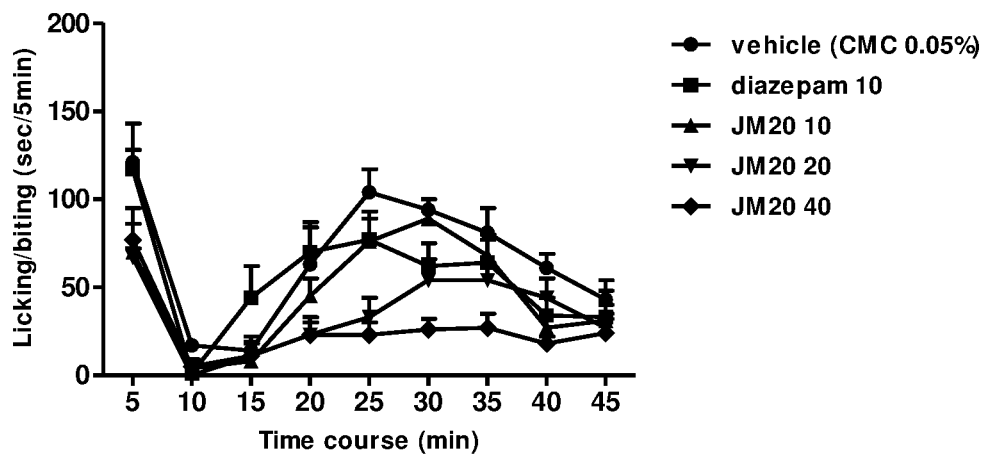


Figure 17B. The time course of the effect of JM-20 (10-40mg/Kg, 10mL/Kg, p.o.), diazepam 10mg/Kg or vehicle (CMC, 0.05%) on licking/biting behavior after injection of 5% formalin on the plantar surface of the rat's paw. Data is presented as the mean of licking/biting time (/5min/sec) $\pm$ EEM during the 45 minutes of the test, $n=7$ per group.

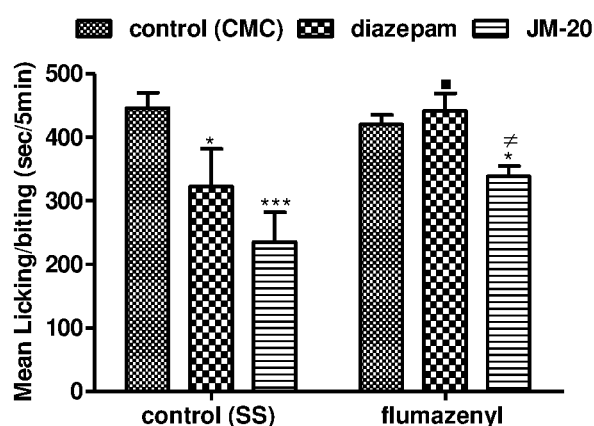


Figure 18: The influence of pre-treating animals with flumazenyl (10 mg/Kg, i.p) on the antihypernociceptive effect of JM-20 on Phase II of the 5% formalin test. Each column represents the reaction time of 6-10 animals per group as the mean $\pm$ EEM, * $P < 0.05$, *** $P < 0.001$ represents the statistical differences between treated and control groups (only vehicle), **** $P < 0.05$ represents statistical differences between groups treated with diazepam in the presence or absence of flumazenyl, # $P < 0.05$ represents statistical differences between groups treated with JM-20 in the presence or absence of flumazenyl.

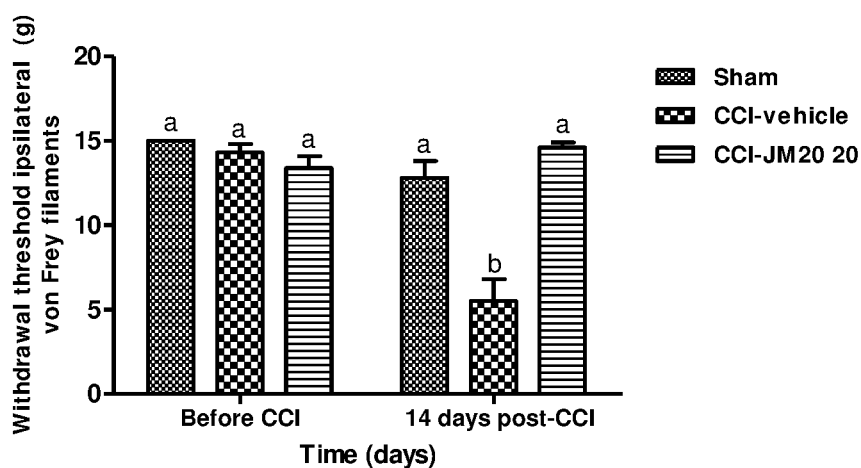


Figure 19: Effects of JM-20 (20mg/Kg, 10mL/Kg, p.o.) or the vehicle (CMC, 0.05%) on mechanical allodynia in the ipsilateral paw of the CCI rats 14 days after surgery, determined by measuring the paw withdrawal response with von Frey filament stimulation. Data is presented as the mean $\pm$ EEM of the 50% of the response threshold, $n=7$ per group. Different letters represent significant differences between the groups (ANOVA for one route followed by Bonferroni's *a posteriori*).

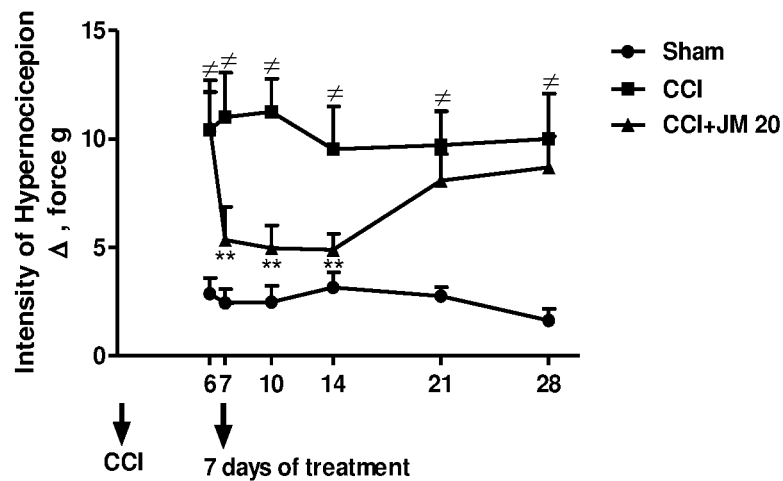


Figure 20: Effects of JM-20 (20/Kg, 10mL/Kg, p.o.) or vehicle (CMC, 0.5%) on the intensity of mechanical hypernociception of the ipsilateral paws of CCI rats determined by measuring the paw withdrawal response when stimulated with electronic von Frey. Data is presented as the mean $\pm$ EEM of the threshold difference (Δ) for withdrawal in grams calculated on the basis of subtracting the mean of the three measurements of different time intervals from the mean of the three time measurements 0, n=7 per group $^{**}p<0.01$ represents significant differences in regards to the control group treated with the vehicle, $^{#}p<0.05$ represents significant differences in regards to the Sham CCI Group (ANOVA for one route followed by Bonferroni's *a posteriori*).

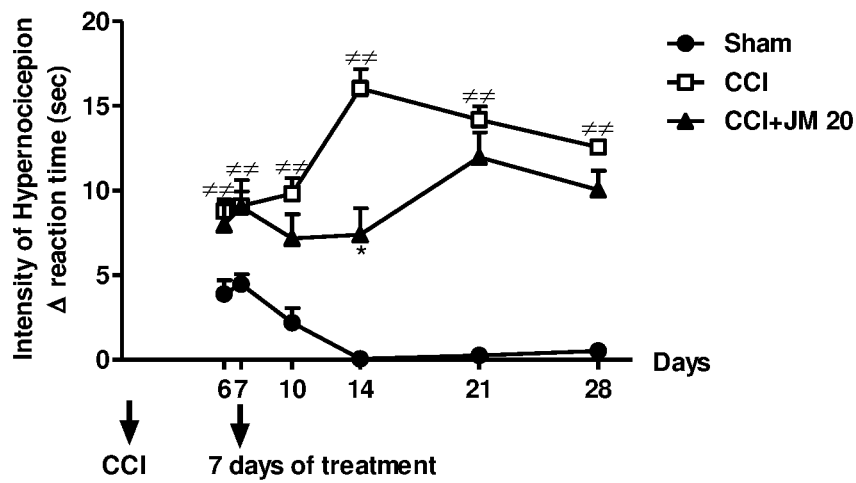


Figure 21. Effects of JM-20 (20mg/Kg, 10mL/Kg, p.o.) or vehicle (CMC, 0.05%) on mechanical hypernociception to constant pressure on the paws by a modification to the classic Randal Selito or SH Ferreira Test. Data is presented as the mean $\pm$ EEM of the difference (Δ) of the reaction time calculated by subtracting the measurement of the different time intervals from the time measurement 0, n=7 per group * $p<0.05$ represents significant differences in regards to the control group treated with the vehicle, ## $p<0.01$ represents significant differences in regards to the Sham CCI Group (ANOVA for one route followed by Bonferroni's *a posteriori*).

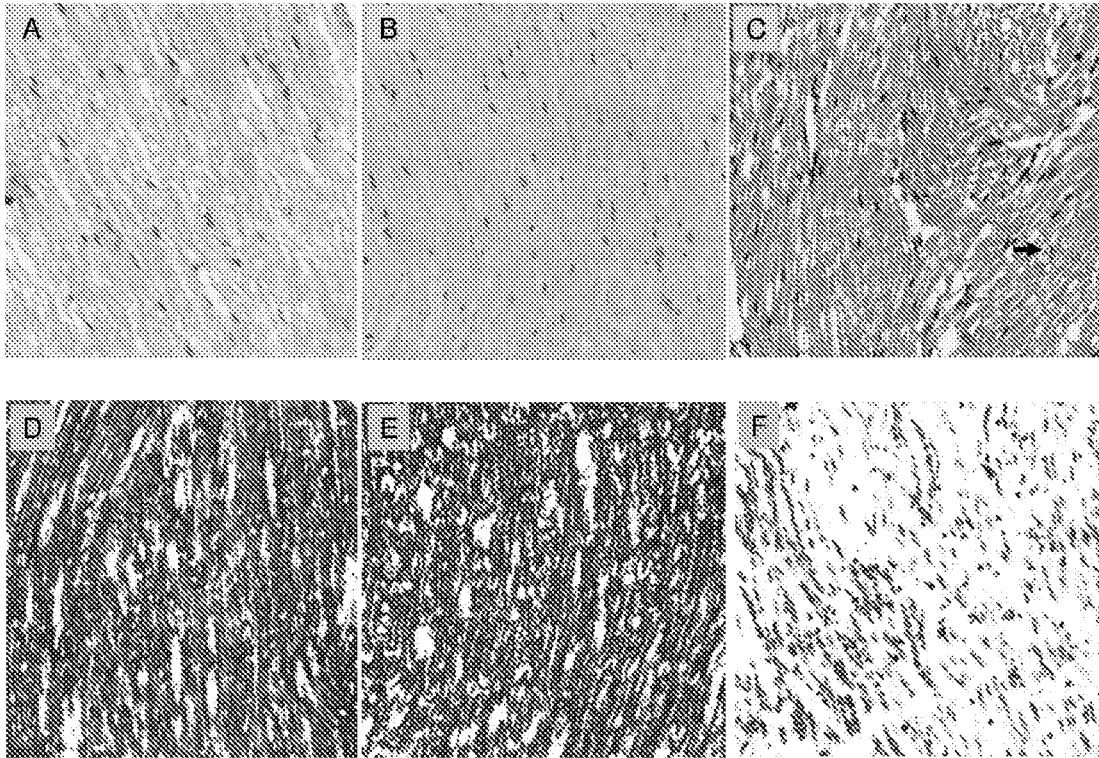


Figure 22: Effects of treatment with JM-20 (20mg/Kg, p.o) at repeated doses for 7 days starting on Day 7 Post-CCI on histological changes induced by CCI (Wallerian degeneration) at 14 days after surgery. A, B and C show the longitudinal section of the sciatic nerve 5 mm distal to the lesion on CCI Sham rats, CCI treated with JM-20 and treated with vehicle respectively (H/E coloration). We can observe the increase in cellularity in CCI animals relatively compared with sham operated animals as the result of the proliferation of Schwann cells and the infiltration of macrophages and the loss of an orderly alignment of axons associated to their myelin sheathes as compared to the sham-CCI. The arrow indicates one of the many digestion chambers of Schwann cells containing a myelin ovoid (reddish oval mass). D, E, F show sections from the same animals but they are colored by the *luxol fast blue* (LFB) technique, a specific technique for myelin tincture. B and E: reduced disorganization of the nerve fibers induced by CCI and increased number of cells and the degradation of myelin sheathes in animals treated with JM-20, particularly this last effect specified in E.

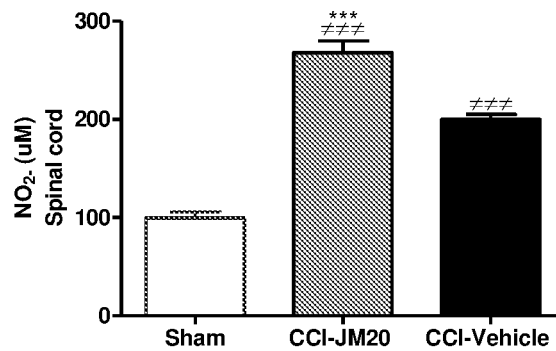


Figure 23: Effects of JM-20 (20mg/Kg, 10mL/Kg, p.o.) or vehicle (CMC, 0.05%) on spinal concentrations of nitrates as an indicator of nitric oxide concentrations. Data presented as the mean $\pm$ EEM, n=7 per group, *** $p<0.001$ represents significant differences regarding the control group treated with the vehicle, ### $p<0.001$ represents significant differences regarding the Sham CCI Group (ANOVA for one route followed by Bonferroni's *a posteriori*).

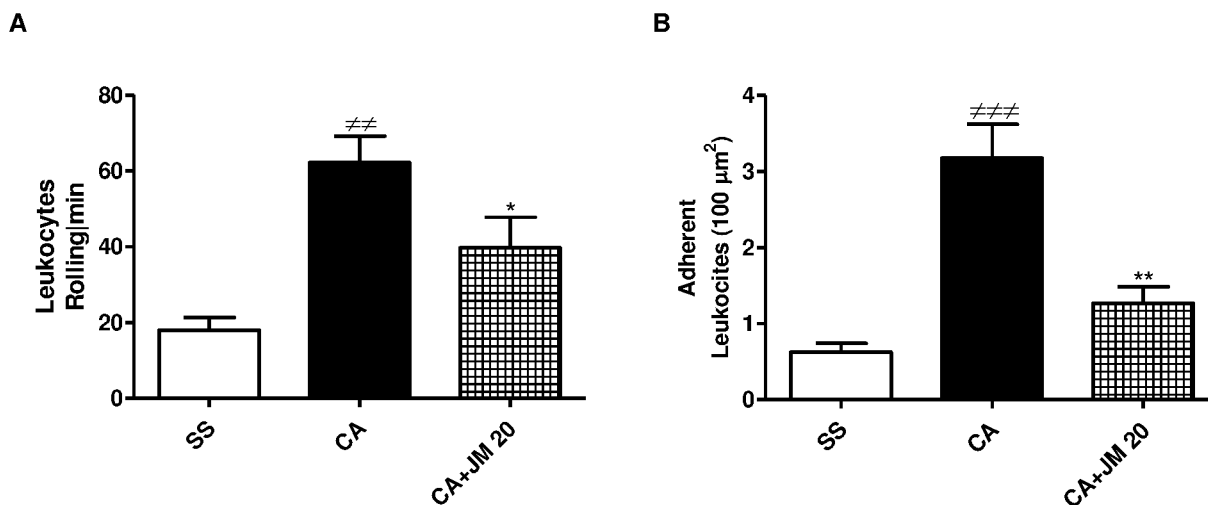


Figure 24: Effects of JM-20 (20mg/Kg, 10mL/Kg, p.o.) or vehicle (CMC, 0.05%) on peritoneal inflammatory response induced by carragenin (500 μ g/cavity) in rats. **A.** Leukocyte rolling and **B** their adhesion to the vascular endothelium in the mesentery were determined by intravital microscope 4 hours after induction of the inflammation. Data is presented as the mean $\pm$ EEM, n=6 per group, * $p<0.05$, ** $p<0.01$ represents significant differences in regards to the control group treated with the vehicle, ### $p<0.001$, ## $p<0.01$ represents significant differences in regards to the control with intraperitoneal saline solution (ANOVA for one route followed by Newman-Keuls or Bonferroni's *a posteriori* in each respective case).

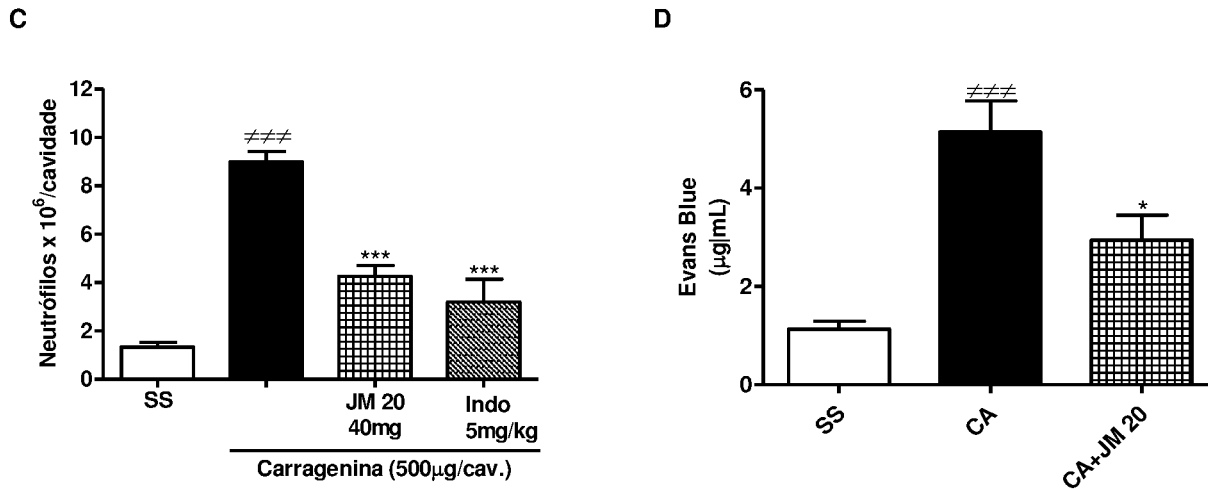


Figure 24. Effects of JM-20 (40mg/Kg, 10mL/Kg, p.o.) or vehicle (CMC, 0.05%) on the peritoneal inflammatory response induced by carragenin (500µg/cavity) C. Migration of leukocytes towards the peritoneal cavity and **D.** vascular permeability (µg/mL) were determined 4hours post-CA. Data is presented as the mean $\pm$ EEM of the number of neutrophils/cavity, n=6 per group, * $p<0.05$, *** $p<0.01$ represents the significant differences in regard to the control group treated with the vehicle, ### $p<0.001$ represents the significant differences in regard to control with the intraperitoneal saline solution (ANOVA for one route followed by Bonferroni's *a posteriori*).

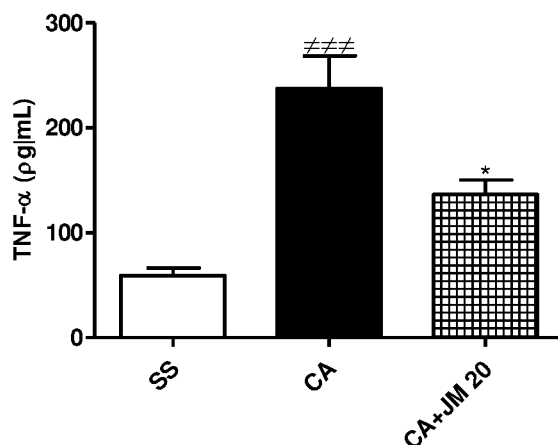


Figure 24 E: Effects of JM-20 (20mg/Kg, 10mL/Kg, p.o.) or vehicle (CMC, 0.05%) on concentrations of the tumor necrosis factor (TNF α) in the peritoneal fluid 4 hours after the carragenin injection. Data is presented as the mean $\pm$ EEM, n=6 per group * $p<0.05$ represents the significant differences in regard to the control group treated with the vehicle, ### $p<0.05$ represents significant differences in regard to control with intraperitoneal saline solution (ANOVA for one route followed by Bonferroni's *a posteriori*).