

Drawings

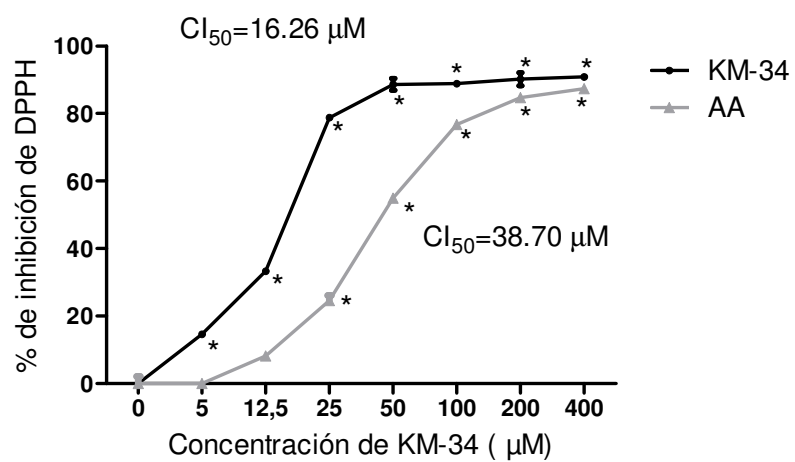


Figure 1. Percentage reduction of DPPH (100 μM) by KM-34 (5-400 μM) was determined by changing the absorbance at 550 nm from a target containing only DPPH and vehicle (zero concentration of KM -34 or AA), after 30 minutes of reaction. Values are expressed as the mean $\pm$ SD (n = 3). * P < 0.05 with respect to the vehicle.

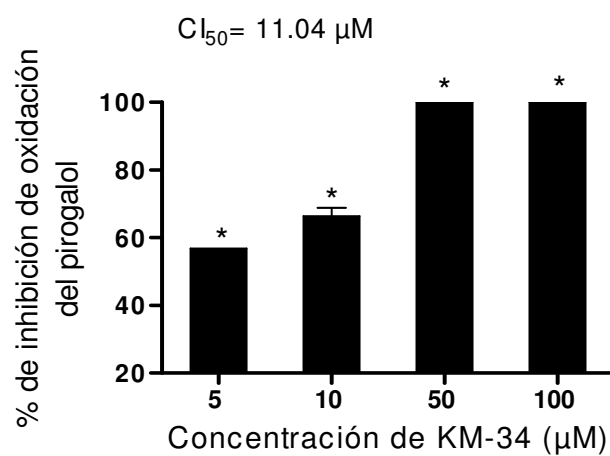


Figure 2 Activity of KM-34 (5-100 μM) as radical scavenger O_2^- expressed as the percentage inhibition of autoxidation of pyrogallol. The same was obtained by varying the absorbance at 420 nm for 1 minute with respect to a blank (uninhibited reaction, without KM-34; the slope was calculated for each concentration of the sample and for the target with a $R^2 > 0.98$. The reaction takes place in 50 mM Tris-HCl pH 8.2 buffer and was initiated by the addition of 0.134 mM Pirogalol * $P < 0.05$ to the carrier (uninhibited reaction).

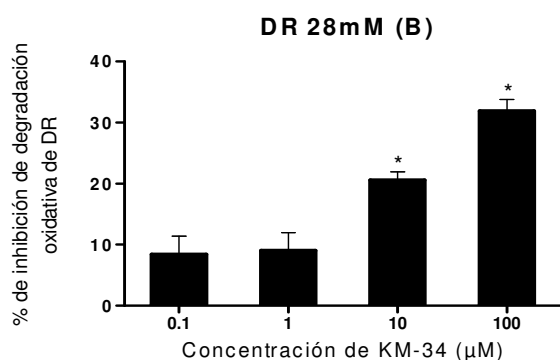


Fig.3. Effect of KM-34 (0.1, 1.10, 100 μM) on oxidative damage to 2.8 (A) and 28 mM (B) DR respectively, induced by Fe³ + - EDTA and ascorbate. The solutions were incubated 30 minutes at 37 ° C and contained 10 mM phosphate buffer pH 7.4, 100 μM EDTA, 25 μM FeCl₃, DR (2.8 or 28 mM). Reactions were initiated with 100 μM AA. The results are expressed as the percent inhibition of DR degradation relative to a control containing only absolute ethanol, obtained by changes in absorbance at 532 nm. The bars show the mean ± SD (n = 3). * P <0.05 with respect to control.

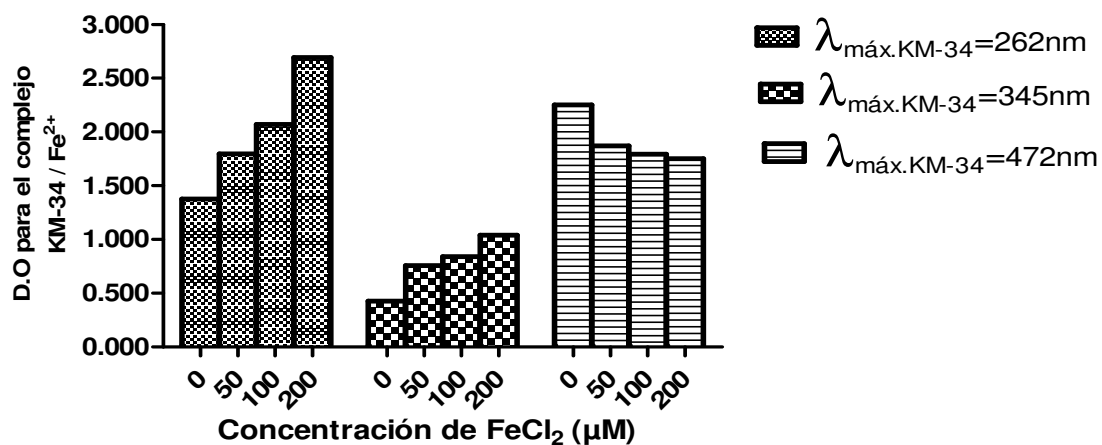


Fig.4 Maximum absorption data on UV-VIS (200-500nm) of the 100 μM KM-34 in a mixture of 10 mM phosphate buffer (KH_2PO_4 / KOH) pH 7.4 and absolute ethanol 14: 1 (v: v) and The absorbance in the presence of FeCl_2 (50,100 and 200 μM) dissolved in 10mM HCL.

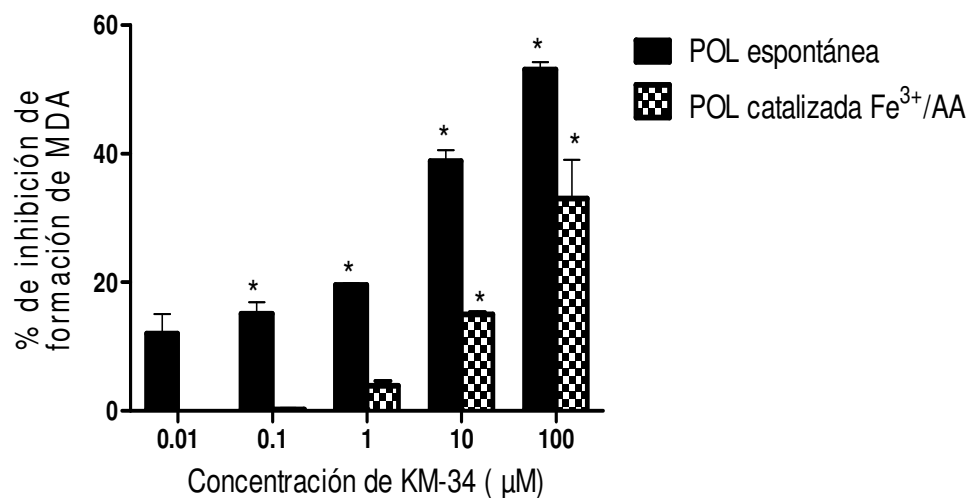


Fig.5 Effects of KM-34 (0.01-100 μM) on spontaneous lipid peroxidation and e induced by 100 mM FeCl₃ and 100 mM AA. The control contains absolute ethanol instead of KM-34. The samples were incubated at 37 ° C for 1 hour and then the MDA levels were quantified by thiobarbituric acid (ATB) and the absorbance was determined at 532 nm. Values are expressed as the mean ± SD (n = 3). * P <0.05 with respect to the control.

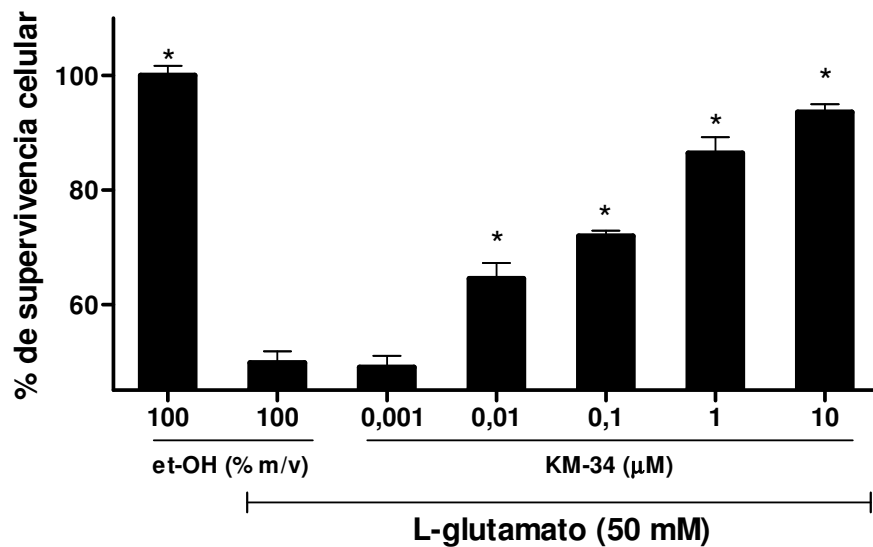


Fig.6. Cytoprotective effect of KM-34 (0.001-10 μ M) against damage induced by L-glutamate (50 mmol / L) in cultures of PC12 cells. Viability (% live cells) was determined by the MTT assay. Cells were seeded at a density of 5×10^5 cells / mL in a 96-well plate. KM-34 was added simultaneously to the addition of L-glutamate. The culture was then allowed to incubate at 37 ° C, 95% O₂ and 5% CO₂ for 4 hours. After this time the supernatant was removed and the cells were incubated with 20 μ g of MTT for an additional 2 hours and the absorbance at 450 nm was measured with reference to 630 nm. The results are expressed as percentage of live cells relative to the control (cells without damage, 100% of live cells. * Represents significant differences ($p < 0.05$) with respect to the L-glutamate control.

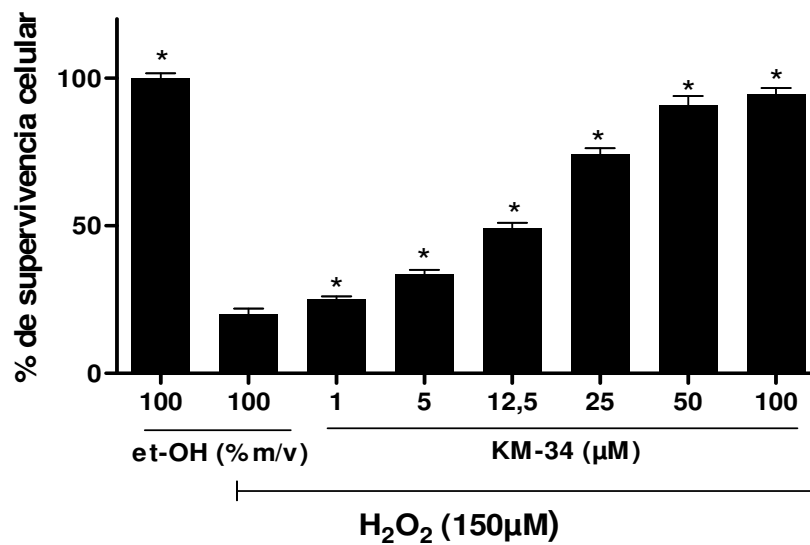


Fig.7. Cytoprotective effect of KM-34 (1-100 μ M) against damage induced by H₂O₂ (150 μ M) in cultures of PC12 cells. Viability (% live cells) was determined by the MTT assay. Cells were seeded at a density of 5×10^5 cells / mL in a 96-well plate. The KM-34 was added simultaneously to the addition of the H₂O₂ mixture. The culture was then allowed to incubate at 37 ° C, 95% O₂ and 5% CO₂ for 4 hours. After this time the supernatant was removed and the cells were incubated with 20 μ g of MTT for an additional 2 hours and the absorbance at 450 nm was measured with reference to 630 nm. The results are expressed as percentage of live cells relative to the control (undamaged cells, 100% live cells). The * represents significant differences ($p < 0.05$) with respect to the H₂O₂ control.

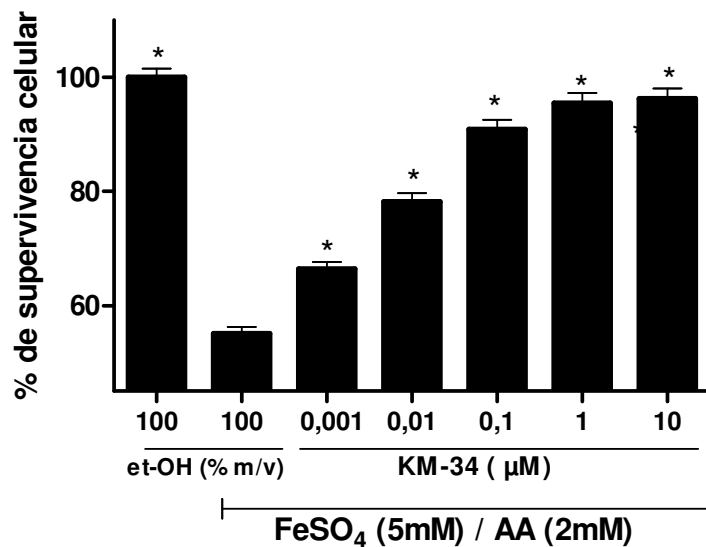


Fig.8. Cytoprotective effect of KM-34 (0.001-10 μ M) against damage induced by FeSO₄ (5 mmol / L) and ascorbic acid (AA, 2 mmol / L) in cultures of PC12 cells. Viability (% live cells) was determined by the MTT assay. Cells were seeded at a density of 5 x 10⁵ cells / mL in a 96-well plate. The KM-34 was added simultaneously to the addition of the mixture of FeSO₄ and AA. The culture was then allowed to incubate at 37 ° C, 95% O₂ and 5% CO₂ for 4 hours. After this time the supernatant was removed and the cells were incubated with 20 μ g of MTT for a further 2 hours and the absorbance at 450 nm was measured with reference to 630 nm. The results are expressed as percentage of live cells relative to the control (undamaged cells, 100% live cells). * Represents significant differences (p <0.05) with respect to FeSO₄ / AA control.

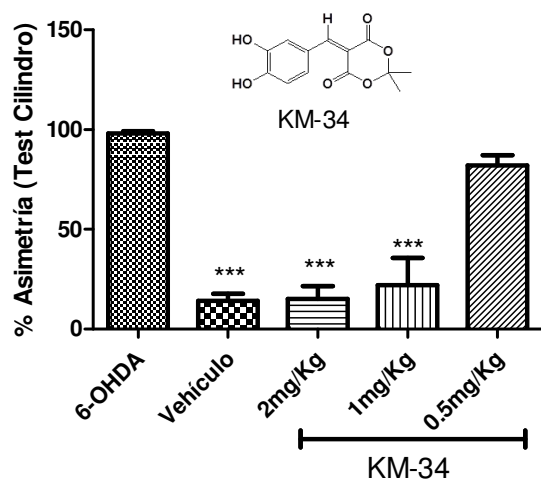


Fig. 9 Effect of KM-34 on damaged animals with 6-OHDA, cylinder test. The graph shows the different treatment groups, evaluated 7 days after the damage. In this case, the neuroprotective capacity of the doses of 2mg and 1mg in the Parkinson's model was observed, as there were statistically significant differences with respect to the animals without treatment. Not being so for the dose of 0.5mg, when not presenting statistically significant differences with respect to the damaged animals without treatment. The values represent the mean percentage of asymmetry, relative to the control group damaged $\pm$ SD. Data were analyzed using statistical methods ANOVA and Tukey's * $p < 0.05$.

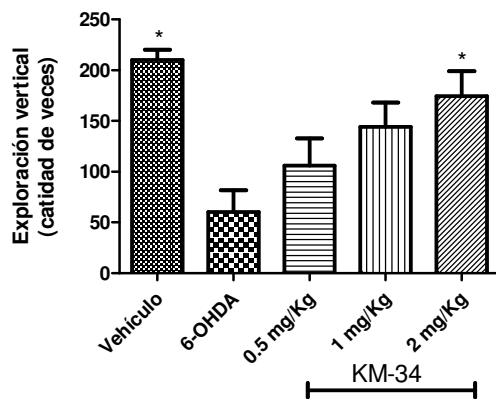


Fig. 10 Effect of KM-34 on animals damaged with 6-OHDA, exploratory behavior. . The graph shows the different treatment groups, evaluated 7 days after the damage. Here the vertical scan values of the different treatment groups are shown. The damaged group has a significantly lower vertical scan than the vehicle control (healthy animals). As the doses of KM-34 are increased, a tendency to increase vertical exploration is observed, being for the group treated with the highest dose statistically significant with respect to the damaging control. The values represent the mean percentage of asymmetry, relative to the control group damaged $\pm$ SD. Data were analyzed using statistical methods ANOVA and Tukey's * $p < 0.05$. The results indicate a potential neuroprotective effect of KM34 in Parkinson's disease.

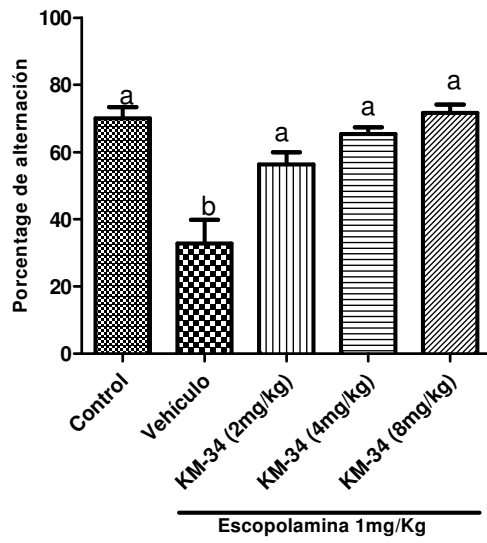


Figure 11: Effect of KM-34 on spatial memory. In the T labyrinth, KM-34 (2, 4 and 8mg / kg, p.o) was given 1 hour prior to scopolamine (1 mg / kg, i.p.). Data are expressed as mean $\pm$ SD (n = 7, per group) of the percentage of spontaneous alternation. For statistical analysis, ANOVA and Tukey's multiple comparison test were performed. Different letters differ from each other for $p < 0.01$.

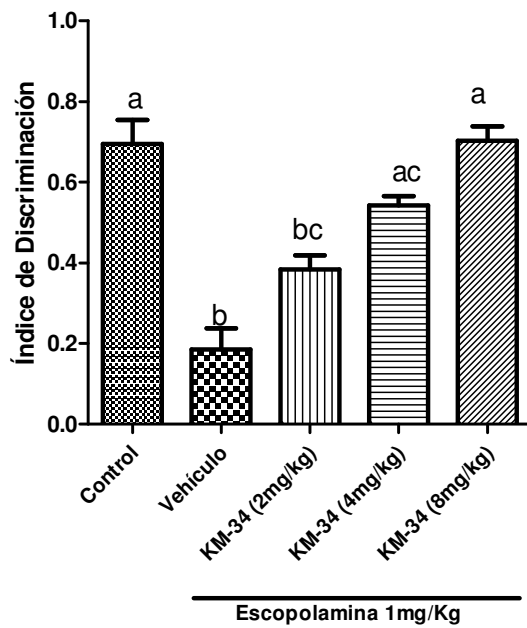


Figure 12: Effect of KM-34 on the recognition memory of new objects. In the new-object recognition test, KM-34 (2, 4 and 8mg / kg, p.o) was administered 1 hour prior to scopolamine (1 mg / kg, i.p.) prior to the training phase. The results are expressed as the discrimination rate of the total time by scanning the object N with respect to F. The data are expressed as mean $\pm$ SD (n = 8, per group). For statistical analysis, ANOVA and Tukey's multiple comparison test were performed. Different letters differ from each other for $p < 0.01$.

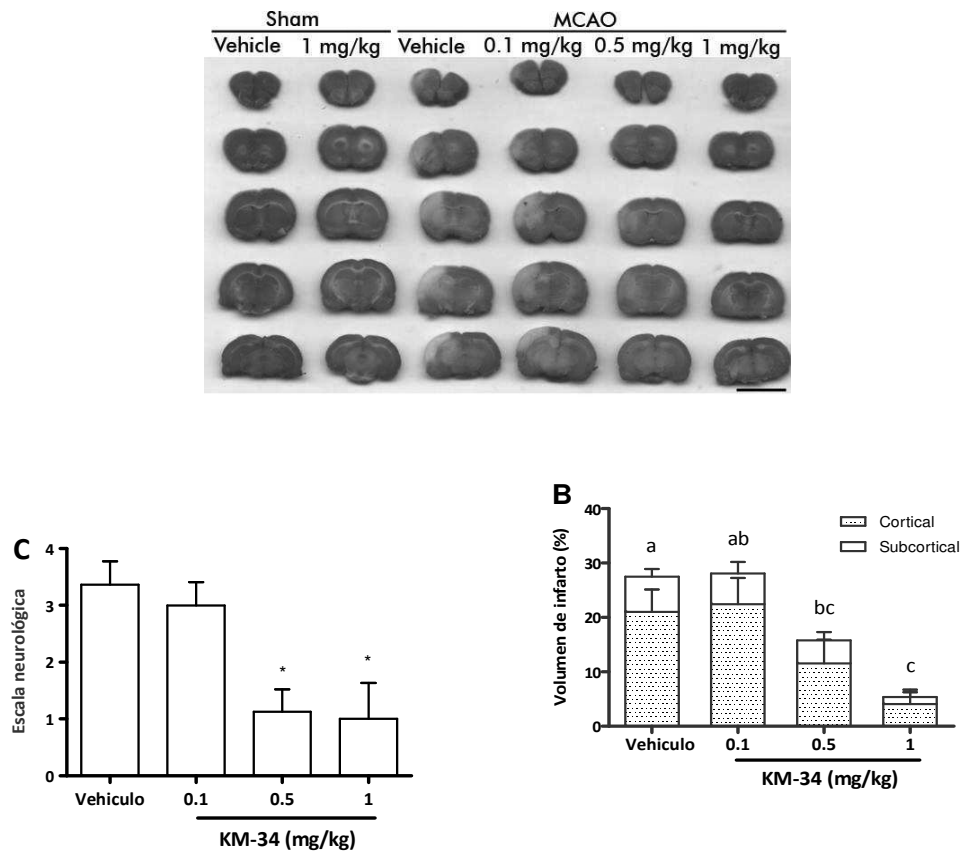
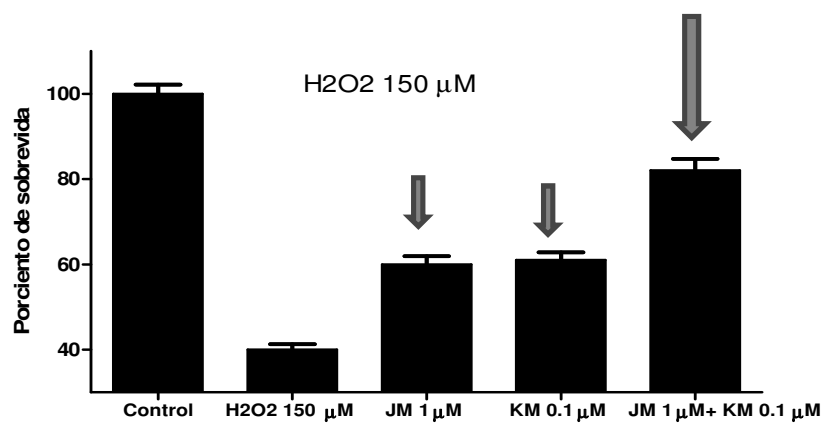
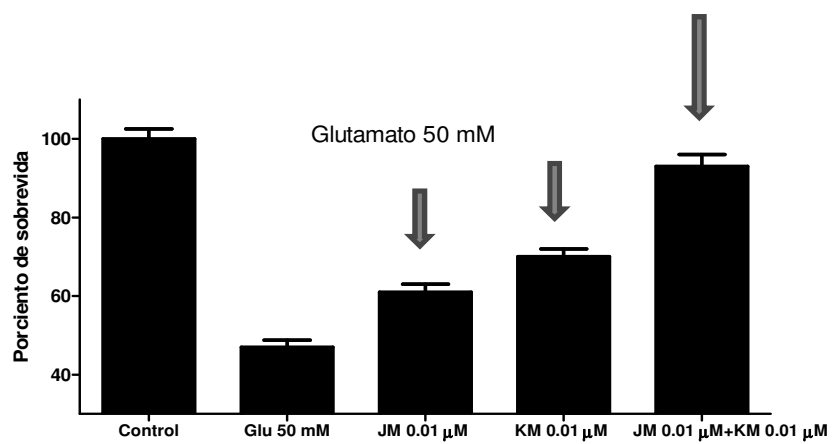


Figure 13. Acute treatment with KM-34 reduced the volume of infarction, edema and deficit in rat behavior after 90 min of middle cerebral artery occlusion (MCAO) and 23 hours of reperfusion. The different doses of the compound were administered orally 1 hour after reperfusion. (A) Representative brain coronal sections (2 mm thick) of the sham and ischemic groups treated with vehicle or KM-34 (0.1, 0.5 or 1 mg / kg) and stained with 2,3,5-triphenyltetrazolium (TTC) 2 %. The red regions correspond to undamaged tissue and white to ischemic regions. (B) Quantitative analysis of cortical, subcortical and total lesion size. (C) Neurological deficit. The bars represent the mean $\pm$ SEM (n = 8). Different letters in (A) and * in (B): p < 0.05, by ANOVA and post hoc of Newman-Keuls. Scale = 10 mm.



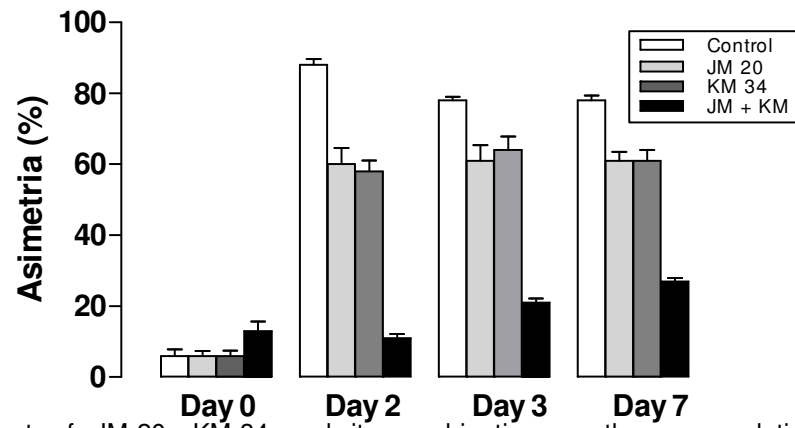


Figure 14. Effect of JM-20, KM-34 and its combination on thermocoagulation-induced asymmetry of cortical arteries