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Original Article

Antipsychotic and behavior effect of the ethanolic extract from the bark of *Maytenus macrocarpa* (Ruiz &Pav.) Briq.in mice

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ABSTRACT

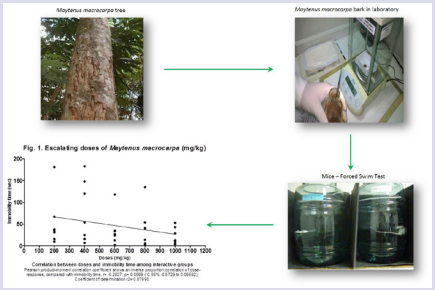
Introduction: Different studies indicate that *Maytenus* has many effects and that *M. obtusifolia* species has a central nervous system depressant effect. **Objective:** To determine antipsychotic and behavior modifying effect of the *Maytenus macrocarpa* (Ruiz & Pav.) Briq.ethanolic extract, using the forced swim test and Irwin test. **Method:** 77 albino mice were used, with an average weight of 25 g. They were split into 7 groups and they were administered with *Maytenus macrocarpa* of 200 mg/kg, 400 mg/kg, 600 mg/kg, 800 mg/kg, 1000 mg/kg, caffeine 32 mg/kg, diazepam 32 mg/kg, fluoxetine 30 mg/kg, haloperidol 5 mg/kg, distilled water (placebo) 0,1 ml/10 g, and a control group. The Irwin test was used to evaluate presence or absence of lethality, convulsions, Straub tail, sedation, excitement, abnormal gait, jumps, motor in coordination, abdominal writhes, piloerection, stereotypies, head twitches, scratching and breathing. The forced swim test was used to evaluate the antidepressant effects of each substance. **Results:** The highest immobility time was observed in *Maytenus macrocarpa*, compared with Haloperidol ($p > 0.05$) in the doses of 400 mg/kg, both of the substances having a Gaussian distribution. Irwin test for *Maytenus macrocarpa* of 200 mg/kg had sedation effect at minute 30; doses of 400 mg/kg at minutes 15 and 45, and doses of 600 mg/kg, 800 mg/kg and 1000 mg/kg at 15 minutes. **Conclusion:** Antipsychotic effects and behavior modifying effects were demonstrated in *Maytenus macrocarpa* (Ruiz and Pav.) Briq.ethanolic extract.

Key words: Antipsychotic, Antidepressive, Haloperidol, *Maytenus*, *macrocarpa*, Bark.

SUMMARY

- *Maytenus macrocarpa* demonstrated an antipsychotic effect and behavior modifying effect.
- *Maytenus macrocarpa* in doses of 200, 600, 800, and 1000 mg/kg showed a non-Gaussian distribution.
- The Irwin Test showed that sedation was the most important behavior after the use of *Maytenus macrocarpa*.
- The Forced Swim Test showed an inverse proportion correlation of dose-response compared with immobility time.

PICTORIAL ABSTRACT



Abbreviations used: CNS: Central nervous system, ATP: Adenosine triphosphate, GABA: Gamma-aminobutyricacid.

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INTRODUCTION

Peru has the fifth highest biodiversity of any country worldwide. It also has one of top number of species of plants with medicinal properties commonly used by the population.[1] *Maytenus macrocarpa* (Ruiz &Pav.) Briq, also known as "chuchuhuasi" is also called "master plant" because it is considered to have the ability to teach and show their own purposeto those who make use of it, like medicinal properties, or people's intentions,[2] which is why "shamans" employ it.

Empirically, this plant is commonly used to relieve symptoms related to gastrointestinal system, colds and rheumatic complains.[3] Researches have also demonstrated effects upon body temperature, heart and respiratory rate.[4] It is also antinociceptive and potentially anti-inflammatory.[5] Moreover, a negative effect upon mice spermatogenesis has been registered in which an abnormal morphology of spermatozoa was produced, probably due to chromosomalabnormalities.[6] In addition, the *Celastraceae* family has demonstrated to have cytostatic, antitumoral, antileukemic, anorectic and abortive effects.[7] Other species of genus *Maytenus*, for example *M. aquifolium, M. robusta* and *M. obtusifolia* MART have shown antiulcer activity.[8-10] In *Maytenus ilicifolia,* acute diuretic activity and hipotensive activity were found,[11] as well as antiprotozoal activity which was attributed to quinonemethide triterpenes.[12]

Furthermore, particular effects upon thecentral nervous system have been registered.*M. obtusifolia*'schloro form extracts have a central depressant effect,resulting in a significant decrease in spontaneous motor activity; also, it shows neuroleptic activity, shown as catalepsy, probably because of an interference with the central neurotransmission of dopamine.[10]

There are validated methods employed to evaluatethe effect of chemical substances upon CNS at a preclinical level.Theforced swim test, explores neuroleptic activity and/or antidepressant in mice, and the Irwin test, explores mice behavior.[13-14] This study was focused in evaluating the possible antipsychotic and behavior effect of the ethanolic extract from the bark of *Maytenus macrocarpa* (Ruiz & Pav.) Briq.in albino mice using forced swim test and Irwin test.

MATERIALS AND METHODS

Type of study, space and temporality

It is an analytic, experimentaland triple-blind tudy, performed at Centro de Medicina Tradicional y Farmacología, Facultad de Medicina Humana, Universidad de San Martin de Porres (FMH-USMP); between February and November of 2014.

Plant sample

Bark from *Maytenus macrocarpa* was collected in the region of Madre de Dios (Sur-east, Peru). The plant was identified by Dr. Berta Loja Herrera (Centro de Investigación de Medicina Tradicional y Farmacología, FMH-USMP, Lima12, Peru). Voucher specimens are deposited at the Herbarium Vargas CUZ from the Universidad Nacional San Antonio Abad del Cuzco, numbers 3547 and 3653.

Experimental animals

77 male albino mice were used, with an average weight of 25 g from National Health Institute's vivarium (Lima-Peru). Ethical guidelines of the *International Guiding Principles for Biomedical Research Involving Animal*[21] were followed. Mice went through an acclimation process in the vivariumof the FMH-USMP, with a temperature of 22°C or 71.6°F, humidity between 30-70%, light/darkness cycles of 12 hours and noise levels less than 70 db. During this time the mice were maintained on a balanced diet and water *ad libitum*. Finally, 12 hours before the experiment food was taken away. For the cage's distribution and groups, we used the random assignment technique.[22]

Chemistry sample

We used Fluoxetin in tablets Sanitary Registry NG-2276., LabAC Farma S.A., EXP.: 06/2015; Haloperidol 5mg/5ml solution for injection ampoules SOL Sanitary Registry.: EG 674., Lab. Sanderson S.A., EXP.: 11/2016; Diazepam 10 mg/2 ml solution for injection ampoules SOL. Sanitary Registry. I:S:P: F-6148/10, Lab. Sanderson S.A., EXP: 12/2017;Caffeine in tablets Sanitary Registry: N-24400, Lab. Naturales y Genéricos S.A.C EXP: 10/2016; and distilled water.

Ethanolic extract's preparation

The extract was prepared following the methodology described by Márquez-Vizcaíno Rita Luz *et al.*[23] Dry ground barkmaterial of *M. Macrocarpa* (Ruiz &Pav.) Briq.was macerated for one week in 70% ethanol. The mixture was filtered and reduced in arotavapor. This product was dried in a stove for 48 hours and it was milled in a mortar until a fine powder was obtained.This was stored in hermetic containers in the refrigerator for future use. The final product was dissolved in distilled water in the appropriateconcentration for a volume of oral administration not higher than 0.25ml.[21]

Irwin Test in mice

The substance that was given to mice was triple-blind and they were observed for 1 hour in time ranges of 15, 30, 45 and 60 minutes. Before the evaluation, mice were placed in special cages with dimensions of 32x14x13 cm. The variables analyzed were the presence or absenceof lethality, convulsions, Straub tail, sedation, excitation, abnormal gait (rolling or tip toe), jumping, motor in coordination, piloerection, abdominal writhes, stereotypies (smell, chew, head movements), head twitches, scratching and breathing abnormalities.

Forced swim test in mice

The forced swim test in mice is previously described by Roger D. Porsolt.[13] For this test, Macrolon glass tanks separated by opaque sheets were used. The tanks were filled with water to a height of approximately 20 cm, at a temperature of 23°C. Mice entered to the experimental room one hour before the begining of the test, for their acclimatization. The following conditions were checked: room temperature of 21° ± 3°C, humidity of 30-70%, levels of noise less than 70 dB. Mice were placed in the tanks, to measure their motion for 6 minutes. The first two minutes were for acclimatization, and the last four were accounted for measurement. The measurement parameter was the immobility time.[13]

Design of experimental groups

Eleven experimental groups were formed, made up of 7 mice each.These received the following substances: Group 1 (control), didn't receive substance;the following groups received the substances by oral administration: Group 2 or Placebo, received distilled water 0.1 ml / 10 g of body weight; Group 3, Haloperidol 5 mg / kg PO; Group 4, Diazepam 32 mg / kg PO; Group 5, Fluoxetine 30 mg / kg PO; Group 6 Caffeine 32 mg / kg PO; Group 7 to 11, ethanol extract of the bark of *M. macrocarpa* (Ruiz &Pav.) Briq. inescalating doses of 200, 400, 600, 800, and 1000 mg / kg.

Blind and control system

A triple-blind system was applied in which the person that administrated the solutions, observed the reactions, and performed the statistical analysis, didn't know the origin of these solutions.[24] Noise levels, humidity, room temperature and swimming tanks were monitored using VWR hygrometer model Thomas Traceable® Digital Humidity/Temperature Meter 35519-045 with a capacity to measure humidity from 60% to 82% and a temperature range from 5°C to 34°C, a sound level digital indicator Radio Shack 33-2055, able to measure from 60 to 120 Db, a insertion thermometer for water temperature of the forced swimming test, manufactured by Isolabwith measurement capabilities from -50°C to 300°C, two fan heaters to maintain a stable temperature of the room, brand NF15C 1500 WImaco with 2 heat intensities: 1000-2000W for an area of 15 m^2. Research assistants were trained to explore physical manifestations using the Pharmacology Lab Virtual Software,[25] and Microlabs[26] and by an *in vivo* pilot.

Ethics and research

The study was approved by the Research Institute of the FMH-USMP following the *International Guiding Principles for Biomedical Research Involving Animals*[21] and the Declaration of the Use of Animals in Biomedical Research[27]

Statistical analysis

The data, respectively, is presented as mean and standard deviation, and absolute and relative values. For quantitative variables the following statistics were applied: ANOVA 1 tail, Tukey and Newman-Keuls test, Kolmogórov-Smirnov test, and Pearson product-moment correlation coefficient. Statistical significance was established to a valuep<0.05, and a confidence interval of 95%. For qualitative variables, Fisher's statistic was applied, considering statistical significance for a value p<0.05, and a confidence interval of 95%. Microsoft Office Excel 2013 and the statistical program Graph Pad Prism version 5.01 were used as computing support.

RESULTS

ANOVA test showed a p-value <0.05 in Forced Swim Test. Tukey's range test obtained a p-value <0.05 among groups3 andgroup 5; group 5 andgroup 1, 2, 6 y 11; group 8 andgroup 6. Nevertheless, when comparing group 3 against group 8 there was no statistically significant difference (p>0.05) (Table 1).

Regarding the effect measured as immobility time in the administration of escalated doses of *Maytenus macrocarpa*, there was no linear

Table 1: Immobility time in experimental groups

Groups	Substance/Drug	N	Mean	Standard deviation	Kolmogórov-Smirnov Test (Gaussian distribution)
G1*	Control	7	17.77	12.29	YES
G2*	Placebo (Distilled water)	7	32.08	43.89	NO
G3*	Haloperidol	7	91.77	76.03	YES
G5*	Fluoxetina	7	22.77	21.93	NO
G7	*M. macrocarpa* 200 mg/kg	7	45.38	57.30	NO
G8*	*M. macrocarpa* 400 mg/kg	7	73.69	70.65	YES
G9	*M. macrocarpa* 600 mg/kg	7	36.31	37.94	NO
G10	*M. macrocarpa* 800 mg/kg	7	40.92	45.46	NO
G11*	*M. macrocarpa* 1000 mg/kg	7	22.38	19.13	NO

*Tukey's range test p<0.05, for immobility time between groups: 3 and 5; 5 and 1, 2, 11.

†Groups 4 and 6 are not shown in this table because they weren't considered for the Forced Swim Test.

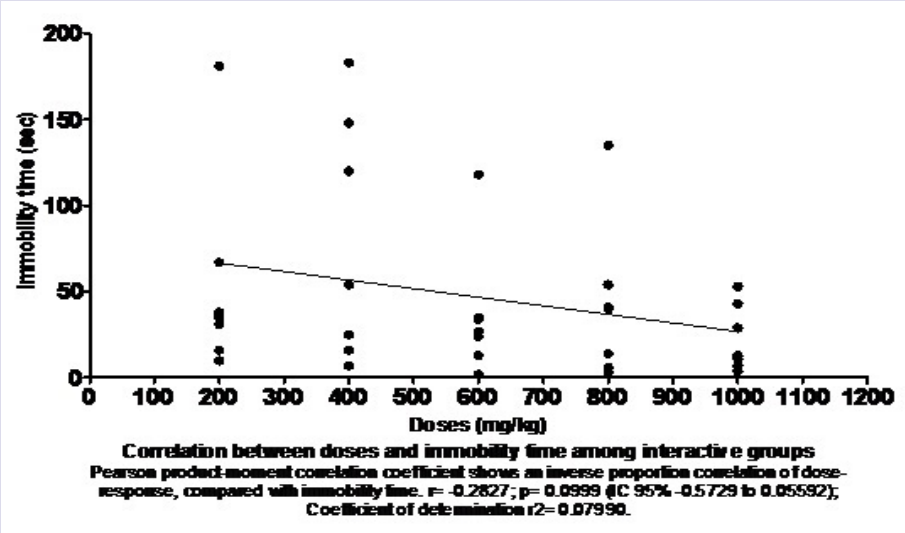

Figure 1: Escalating doses of *Maytenus macrocarpa* (mg/kg)

tendency in the dose-response relation, showing an inverse proportion correlation(r=-0.2827) (p=0.0999 IC 95%-0.5729 a 0.05592), with a determination coefficient r2=0.07990. On the other hand, the highest immobility time was registered in *Maytenus macrocarpa*dose of 400 mg/kg, which later compared with Haloperidol (positive control), according to Tukey's range test, obtained a p-value >0.05 (Figure 1).

Kolmogórov-Smirnov test, for Haloperidol and *Maytenus macrocarpa* of 400 mg/kg, presented Gaussiana distribution.

The comparison made between Haloperidol and *Maytenus macrocarpa* in doses of 200,600 and 800 mg/kg revealed ap-value >0.05. Kolmogórov-Smirnov test determined non Gaussian distribution for *Maytenus*.

However, comparison made between Haloperidol and *Maytenus macrocarpa* of 1000 mg/kg displayed a p-value <0.05 at Tukey's range test. Kolmogórov-Smirnov test obtained a non-Gaussian distribution.

Comparing Fluoxetine with *Maytenus macrocarpa* groups, Tukey's range test got a p-value >0.05. When analizing with Kolmogórov-Smirnov test, Fluoxetine presented non Gaussian distribution (K-S distance= 0.2660, p=0.0124).

In the Irwin Test, referring to control group, sedation in neurodepressant groups (Diazepam), group 9 and 11, was registered. The control group presented effects from minute 15, getting the highest effect at minute 30. On the matter of the neurodepressant group, it presented its effect on the first 15 minutes, and kept constant until minute 60. For *Maytenus macro-*

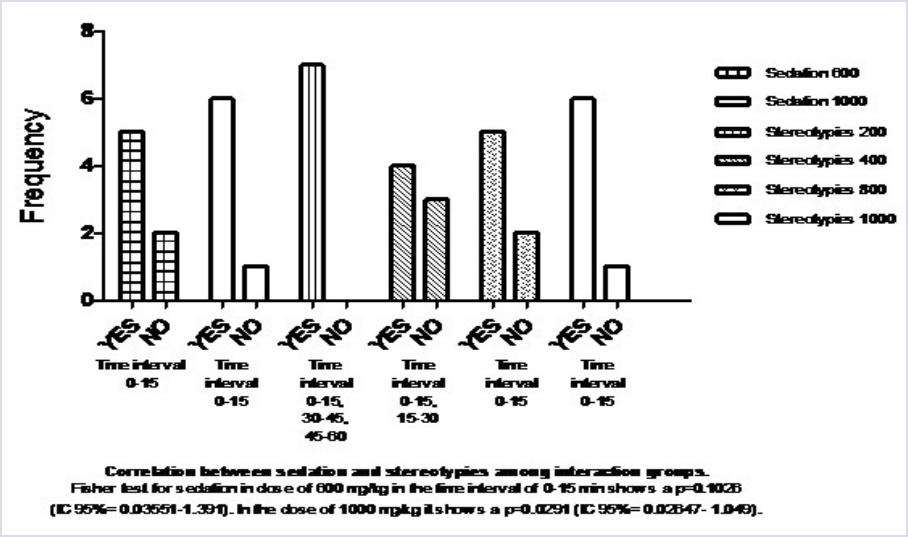

Figure 2: Irwin Test upon experimental groups

carpa groups, (7-11) every dose administrated presented sedation effect, nevertheless, not all mice from the groups were equally affected, because 56% of mice in those groups did not show sedation. Nevertheless, in 200 mg/kgdose, sedation effects were shown at minute 30; 400 mg/kg dose at minute 15 and 45; finally, doses of 600, 800 mg/kg and 1000 mg/kgin the first 15 minutes (Figure 2).

In the case of excitement, control group and neurostimulant group were the only ones who presented it. Presence of jumps was observed in group 7. Additionally, every group presented piloerection, while stereotypies were only observed in groups: neurostimulant, 7, 8, 10 and 11 (Figure 2). Finally, neurodepressant group (Diazepam) displayed poor breathing compared with the beginning of the test. Only some of these results have been considered in order to create a summary graph, because others were not significant enough.

DISCUSSION

According to the forced swim test, Haloperidol was chosen as a positive control, which has an antipsychotic effect because it is a non-selective blocker of dopamine D2 receptors in the brain.[28] When compared to doses of 200 400, 600 and 800 mg/kg of *Maytenus macrocarpa*, it didn't show a statistically significant difference, revealing a potential neuroleptic effect.

In contrast, a study of *Maytenus obtusifolia* MART root, demonstrated antipsychotic effect in the experimental model of catalepsy,[10] therefore, this study expanded the evidence of the neuroleptic activity to the *macrocarpa* species, more precisely, the bark.

This antipsychotic effect could be explained with the secondary metabolites of the genus *Maytenus*. For example, the presence of triterpenic compounds and a theircentral nervous system depressant effect, with a yet unclear mechanism of action.[29]

One of the triterpens studied in different plants, which could also be found in our plant sample, was linalool, capable of inhibiting the binding of glutamate to the membranes of the cerebral cortex.[30] Because of that, the release and uptake of glutamate is inhibited, suggesting a depressant effect on the central nervous system.[31]

In addition, the presence of other triterpens as maytenin and pristimerin has been demonstrated in the bark extract of *Maytenus ilicifolia*, which showed antioxidant activity.[32] This effect is expressed by the release of dopamine metabolites, involved in neurons damage.[33]

Another secondary metabolites present in the genus *Maytenus* are the polyphenols.[34] These could also explain the neuroleptic activity, because it has been shown that they have action upon the mitochondrial membrane potential, maintaining the transmission speed of nerve impulse, as well as the release of synaptic vesicles.[35] A previous study about green tea polyphenols demonstrated to have antidepressant effect upon mouse behavior due to the hypothalamic–pituitary–adrenal axis.[36] Another study evaluated the ability to swim of rats that were fed with polyphenols, obtaining a significant increase of swimming time, due to an increase of glycogen and ATP in rodents' muscles.[37]

In this study, for theforced swim test, a non-Gaussian distribution was observed at doses of 200, 600, 800 and 1000 mg/kg of *Maytenus macrocarpa.* This dispersion could be explained by some pharmacokinetic influence, considering that other investigations have demonstrated cytochrome P450 action upon flavonoids.[38] An investigation about cytochrome P450 action upon flavonoid metabolism indicates that humans as well as mice, show cytochrome P450 complex in the liver microsomes and membranes, specially CYP1A2. This isozyme shows a great effect as a flavonoid metabolism inhibitor.[38]

An important effect that was registered in multiple doses, at the Irwin test for *Maytenus macrocarpa*, was sedation, which matches with a study about *Maytenus obtusifolia* bark's extract. This study used amphetamine-inducedtoxicity test in order to verify the protective value of *Maytenus obtusifolia,* and described as a probable mechanism of drug action, the interference with the central neurotransmission of dopamine.[10] Furthermore, a preclinical study demonstrated that flavonoids such as kaempferol, isolated from *Maytenus ilicifolia* leaves,[8] and its glycosides, had an anxiolytic activity due to a bond with $GABA_A$ receptors.[39]

We found an interesting result in the jumping variablein the doses of 600, 800 and 1000 mg/kg of *Maytenus macrocarpa,* where there was no statistically significant difference with the neurodepressant group (Diazepam). This could have happened because of a flavonoid (hyperoside), which was found in leaves of *Maytenus ilicifolia* and *M. aquifolium (Celastraceae).* This flavonoid has depressant central nervous system activity and at the same time, anti-depressant activity through dopaminergic pathways, according to the experimental model of forced swim test in rats.[40]

We also found piloerection in all the doses of *Maytenus macrocarpa.* That might have been provoked by the action of muscarinic receptors in the piloerector muscle.[41]

Finally, we found a non significant result in the variable stereotypies, which presented a neuro stimulation pattern as it happened with the application of caffeine.The results in our investigation showed a significant neuroleptic and sedative effect in the experiment with mice. This could be an advantage against typical antipsychotic drugs as these drugs produce hypnosis but not sedation. The pharmacological therapy could improve because sedation will control the psychomotor agitation in a patient with psychotic breakdown and the patient could be calmed without the need of inducing hypnosis.

The principal limitation of our study is that it cannot delimit the action mechanism or place. Also, we evaluated the effects of the whole extract and not the effects of each secondary metabolite that belonged to the bark, so differences could be found. For future studies, examination of the metabolites effects and level of action of each one should be made.

CONCLUSION

The antipsychotic effect and behavior modifying effect were demonstrated in *Maytenus macrocarpa* ethanolic extract.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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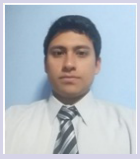

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