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Research Article

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Psychopharmacological Studies of 5-(4-Methylphenyl)-3-Phenylimidazolidine-2,4-dione (HPA-05) in Mice

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ABSTRACT

A significant proportion of the pharmaceutical market is currently made up of organic compounds, the majority of which are heterocyclic. Of these, the hydantoins merit particular attention due to their numerous biological. The purpose of our research was to study the acute toxicity and the effects of the imidazolidine derivative 5-(4-methylphenyl)-3-phenylimidazolidine-2,4-dione (HPA-05), obtained by organic synthesis, on the central nervous system of mice. The psychopharmacological study of HPA-05 (50, 100 and 200 mg/kg, i.p.) on mice was assessed in the open field test, rota-rod test, elevated plus-maze test, pentylenetetrazole induced seizure test and maximal electroshock seizure test. The median lethal dose (LD50) could not be determined since it exceeds the maximum dose used in this study (1000 mg/kg, i.p.). In the open field test, HPA-05 was found to have the profile of Central nervous system depression. HPA-05 (50, 100 and 200 mg/kg, i.p.) did not alter the animals' motor coordination, as evaluated in the rotarod test. In the elevated plus maze test, no behavioral change indicative of a possible anxiolytic effect was found. The results with HPA-05 also showed that this compound effectively increases the latency time until the onset of seizures induced by pentylenetetrazole or in the maximal electroshock seizure test. Therefore, considering this set of results, it is reasonable to suggest that HPA-05 has a profile similar to that of depressants and anticonvulsants, without affecting motor function.

Keywords: 5-(4-methylphenyl)-3-phenylimidazolidine-2,4-dione; Anticonvulsant; HPA-05; Psychopharmacology; Imidazolidine

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INTRODUCTION

Since antiquity, natural products have been used to treat a wide variety of diseases. Nevertheless, at the end of the nineteenth century, synthetic substances were introduced with the objective of identifying less toxic forms of treating diseases, with their use becoming widespread in the twentieth century [1]. Around 85% of the pharmacological drugs available today are of synthetic origin, not including those derived from semi-synthetic processes [2]. In this scenario, various substances can be obtained by synthesizing new compounds or modifying the structure of already known molecules when their chemical structure relationship and their biological effects are taken into consideration [3]. A significant proportion of the pharmaceutical market is currently composed of organic compounds, 62% of which are heterocyclic, i.e., they possess heteroatoms (atoms of elements other than carbon) in their rings [2]. Of these atoms, nitrogen, a component of hydantoin, considered a prototype substance for the

development of other imidazole drugs, is particularly noteworthy [4]. The reactivity of the ring system and the various biological activities reported have encouraged investigators to continue with studies and with the synthesis of hydantoin (i.e., imidazolidine- 2,4-dione) derivatives [5]. Some of the most important biological effects of hydantoin derivatives include anti-inflammatory [6,7], antifungal [8], schistosomicidal [9], herbicidal [10] and tuberculostatic activities [11]. With respect to their central action, 5,5-diphenylhydantoin, generically referred to as phenytoin, is largely used worldwide as the anticonvulsant of choice for epileptic seizures, specifically partial and tonic-clonic seizures, but not for absence seizures. Its anti-epileptic effect occurs principally due to its ability to block the voltage-gated sodium channels, with greater affinity for those channels in an inactive state, thus preventing a return to the resting state, which is required to generate new action potentials [12]. HPA-05, 5-(4-methylphenyl)-3-phenylimidazolidine-2,4-dione (Figure 1) is an imidazolidine derivative structurally similar to the above-mentioned prototype, which has a central effect. Therefore, this study investigated the possible inherent psychopharmacological effects of HPA-05.

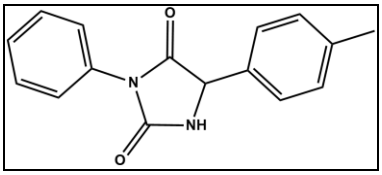

Figure 1: Chemical structure of 5-(4-methylphenyl)-3-phenylimidazolidine-2,4-dione (HPA-05)

EXPERIMENTAL SECTION

Animals

Male Swiss albino mice (*Mus musculus*) of 2-3 months of age, weighing 25-35 g and obtained from the Prof. Dr. Thomas George Animal Laboratory of the Federal University of Paraíba, Brazil, were used in the study. The animals were housed in polyethylene cages at a temperature of 21 ± 1^{o}C and had free access to chow and water. They were kept in a 12-hour light-dark cycle. All the experimental procedures were previously analyzed by the institute's animal research ethics committee, with approval being granted under number 0305/10.

Acute Toxicity

Determining the median lethal dose (LD50) enables the possible toxic effects of substances/ extracts, and the dose responsible for the death of 50% of the animals in a given study to be established [13], thus allowing pharmacological tests to be performed with safe doses. In this study, HPA-05 was given intraperitoneally (i.p.) at doses of 25, 50, 100, 200 and 1,000 mg/kg, and the animals were observed daily for fourteen days to quantify the number of deaths.

Open Field Test

The mice were divided into three groups with eight mice in each group. The experimental group received HPA-05 (50, 100 and 200 mg/kg, i.p.), while the control group received the vehicle alone and the standard treatment group received diazepam (1 mg/kg, i.p.). Thirty minutes after administration, each animal was allowed to explore the open field freely for five minutes. The following parameters were observed during that 5-minute period: ambulation, rearing, grooming and the number of fecal boluses deposited.

Evaluation of Motor Coordination

In the rotating rod (rotarod) test, first proposed by Dunham and Miya [14], mice are placed on a bar that rotates at 7 rpm. The animal's ability to remain on the rod is then assessed. This study divided the animals into a control group and an experimental group, with eight animals in each group. The animals in the experimental group received HPA-05 (50, 100 and 200 mg/kg, i.p.), while those in the control group received only the vehicle. The mice were placed on the rotating rod at 30, 60 and 120 minutes following administration of the treatment, and the total time they managed to stay on the bar was recorded up to a total of three minutes. A maximum limit of three falls was permitted for each animal [14].

Elevated Plus Maze Test

The mice were divided into a control group, an experimental group and a standard treatment group, with eight mice in each group. The mice in the experimental group received HPA-05 (50, 100 and 200 mg/kg, i.p.), while those in the control group received only the vehicle and those in the standard treatment group received diazepam (1 mg/kg,

i.p.). Thirty minutes after treatment administration, each animal was submitted individually to the test for a 5-minute period, with the following parameters being evaluated: the number of times the animal went into the open and closed arms of the device and the amount of time it remained there.

Pentylenetetrazole Induced Seizure Test

The PTZ-induced seizure test is widely used to evaluate the anticonvulsant effect of drugs in animals [15]. Thirty minutes after administration of the vehicle, HPA-05 (50, 100 or 200 mg/kg, i.p.) or diazepam (2 mg/kg, i.p.), all the groups were given PTZ (60 mg/kg, i.p.). The animals were then observed over a 20-minute period immediately following administration of the drug and the following parameters were recorded: the time until the animal had a seizure, i.e., the time from the administration of PTZ until the onset of the first seizure, the duration of the seizures and the number of deaths.

Maximal Electroshock Seizure Test

This test is based on the application of repetitive electric pulses to induce a pattern characteristic of epileptic activity in different neuronal structures. The resulting self-sustained seizure activity is usually referred to as after discharge [16]. Thirty minutes after treatment with the vehicle, HPA-05 (50, 100 or 200 mg/kg, i.p.) or phenytoin (25 mg/kg, i.p.) all the animals were submitted to electric shocks of 150 pulses/second for 0.5 seconds, administered via auricular electrodes (ECT UNIT 7801 electroconvulsive therapy unit). The principal parameters observed were: the number of animals that suffered tonic seizures, the number of deaths and the time the animal spent flexing and stretching its paws.

Statistical Analysis

The data were compared using analysis of variation (ANOVA) followed by Dunnett's test or contingency table analysis with Fisher's exact test was performed. The results were expressed as means ± standard error of the mean (SEM). A P-value of $p<0.05$ was considered statistically significant.

RESULTS

Acute Toxicity

HPA-05 at doses of 25, 50, 100, 200 and 1000 mg / kg, i.p. did not cause mortality in mice for a period of 14 days of observation. Based on these results, it could not determine the LD50, since it is above 1000 mg/kg. Thus, HPA-05 has low toxicity.

Effect of HPA-05 on Open Field Test

All the doses of HPA-05 (50, 100 and 200 mg/kg, i.p.) significantly reduced the parameters of ambulation**,** rearing, grooming and the number of fecal boluses compared to the animals in the control group. As expected, diazepam (1 mg/kg, i.p.) decreased all parameters analyzed on open field test (Table 1).

Table 1: Effect of HPA-05 on the open field test in mice

Treatment	Dose (mg/kg)	Parameters			
		Ambulation	Rearing (number)	Grooming (s)	Defecation (number)
Vehicle	-	71.3 ± 4.9	7.8 ± 0.8	15.1 ± 1.9	2.5 ± 0.2
HPA-05	50	27.8 ± 5.6***	0.8 ± 0.5**	3.3 ± 1.4***	0.1 ± 0.1***
	100	24.5 ± 2.9***	0.3 ± 0.2***	4.7 ± 1.7***	0.0 ± 0.0***
	200	23.0 ± 4.9***	0.3 ± 0.3***	0.0 ± 0.0***	0.1 ± 0.1***
Diazepam	1	21 ± 3,1***	0.2 ± 0.1***	0.0 ± 0.0***	0.1 ± 0.1***

Values are expressed as means ± SEM; **p<0.01, *** p<0.001 versus control group. One-way ANOVA followed by Dunnett's test

Effect of HPA-05 on Motor Coordination

There was no statistically significant difference in the time spent on the rotating bar (motor coordination) by the mice treated with HPA-05 (50, 100 and 200 mg/kg, i.p.) or diazepam (4 mg/kg, i.p.) compared to the animals in the control group. This result was found at the three time points after treatment. However, mice treated with diazepam (4 mg/kg, i.p.) presented a significantly altered time of performance on the bar at 30 min, when compared to control group (Figure 2).

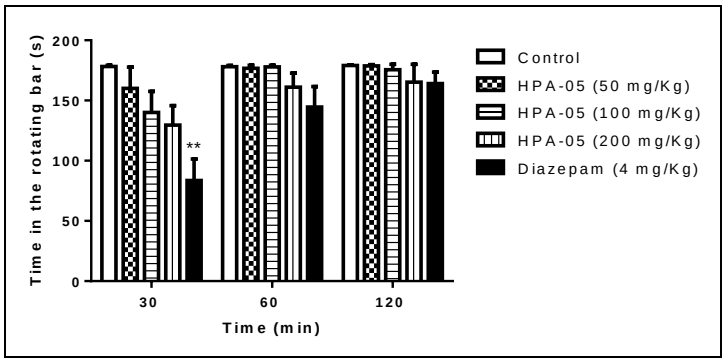

Figure 2: Effect of HPA-05 on the time the animal spent on the rotating bar in the rotarod test in mice. The values are expressed as means ± SEM; **p<0.01 versus control group. One-way ANOVA followed by Dunnett's test

Effect of HPA-05 on Elevated Plus Maze Test

The results presented in Table 2 shows the animals treated with HPA-05 (50, 100 and 200 mg/kg, i.p.) no significant changes in the number of entries and time spent in the open arms when compared to the negative control. As expected, diazepam (1 mg/kg, i.p.) reduced time spent in the open arms.

Table 2: Effect of HPA-05 in the elevated plus maze test in mice

Treatment	Dose (mg/kg)	Open arms	
		Number of entries	Time spent
Vehicle	-	3.5 ± 0.7	25.4 ± 5.2
HPA-05	50	1.6 ± 1.1	9.4 ± 5.2
	100	1.6 ± 0.4	13.5 ± 3.5
	200	2.2 ± 0.5	18.8 ± 5.8
Diazepam	1	3.9 ± 1.0	149.8 ± 39.7***

The values are expressed as means ± SEM; *** p<0.001 versus control group. One-way ANOVA followed by Dunnett's test

Effect of HPA-05 on Pentylenetetrazole Induced Seizure Test

During the PTZ-induced seizure tests, clonic and tonic/clonic seizures were seen in animals in all the groups. At the same time, a reduction was found in the percentage of tonic/clonic seizures in the animals that received HPA-05 (50, 100 or 200 mg/kg, i.p.) compared to the control group. Treatment with HPA-05 at the doses of 100 or 200 mg/kg i.p. increased the latency until the onset of seizures chemically induced by PTZ. These findings were compared to the negative control group in which only the vehicle was given and to the positive controls treated with diazepam (2 mg/kg, i.p.) (Table 3)

Table 3: Effect of HPA-05 on the type of seizure in the pentylenetetrazol-induced chemical seizures test in mice

Treatment	Dose (mg/kg)	Type of convulsion		Latency (s)
		Clonic (%)	Tonic/Clonic (%)	
Vehicle	-	100	30	99.3 ± 17.8
HPA-05	50	100	15#	149.4 ± 16.7
	100	100	5###	295.0 ± 12.2**
	200	100	1###	412.5 ± 32.4***
Diazepam	2	0###	0###	825.0 ± 64.8***

(%) represent the percentage of animals that had a seizure; Values expressed as means ± SEM; ** p<0.01 and *** p<0.001 versus the control group. One-way ANOVA followed by Dunnett's test. Contingency table analysis with Fisher's exact test was performed (#p<0.05, ###p<0.001, as compared with control values)

Effect of HPA-05 on Maximal Electroshock Seizure Test

Table 4 shows that all the animals had tonic seizures when submitted to the maximal electroshock seizure test, both in the negative control group (vehicle) and in the animals treated with HPA-05 at the dose of 50 mg/kg, i.p. In the groups treated with 100 and 200 mg/kg, i.p., 37% of the animals had tonic seizures. Mortality was zero up to 48 hours after the experiment in all the groups tested. Phenytoin (25 mg/kg, i.p.) protected the animals from the electroshock-induced seizures by reducing seizure duration.

Table 4: Effect of HPA-05 on the type of seizure and mortality recorded in the maximal electroshock seizure test

Treatment	Dose (mg/kg)	% Animals exhibiting convulsions	Time of convulsions	% Surviving animals
Vehicle	-	100	16.3 ± 0.5	100
HPA-05	50	100	16.4 ± 0.7	100
	100	37.5###	4.4 ± 2.9***	100
	200	37.5###	3.1 ± 2.0***	100
Phenytoin	25	0###	0.4 ± 0.2***	100

Values are expressed as means ± SEM; *** p<0.001 versus the control group. One-way ANOVA followed by Dunnett's test. Contingency table analysis with Fisher's exact test was performed (###p<0.001, as compared with control values)

DISCUSSION

The derivative HPA-05 has been the focus of a wide variety of studies because hydantoin compounds exert various pharmacological effects including an anticonvulsant effect. In addition, they are effective for the treatment of neuropathic pain, as has been shown with the drug phenytoin [17]. Furthermore, HPA-05 is similar in structure to other compounds of this same class that have been shown to exert antinociceptive activity [7,18].

While respecting the ethical principles for research studies in laboratory animals, acute toxicity was evaluated together with the behavioral and pharmacological assessment. Preliminary data were thus obtained on the possible effects of HPA-05 on the central nervous system, and the doses to be used in the subsequent tests were defined. In this evaluation, no deaths occurred in the animals up to the maximum dose of 1000 mg/kg. Based on these results, the toxicity of HPA-05 would appear to be low, unlike that found with the IM-3 [18] and IM-7 [19] hydantoin derivatives, whose LD50 were 1358.9 mg/kg and 1024 mg/kg, respectively. This suggests that in both cases (IM-3 and IM-7), toxicity was greater than with HPA-05, which did not cause the death of any of the animals at any of the doses tested. The results of these acute toxicity studies emphasize the differences that exist between substances that are of the same chemical class, but that will have different toxicity profiles even when the changes in their molecular structure are small. With the imidazolidine derivative IM-7 [19], which is structurally analogous to HPA-05, the same depressive-like behavior found in the present study was reported; however, IM-7 induced additional behavioral changes characteristic of substances with high levels of toxicity, which were not found with HPA-05.

In the open field test, there was a significant reduction in ambulation compared to the control group with all the doses of HPA-05 tested. The same occurred for rearing. The inhibition of these parameters suggests that this imidazolidine derivative exerts an effect similar to that shown by substances that cause central nervous system depression [20]. The same response pattern was found with the hydantoin derivative IM-7 [19], confirming the depressant effects of substances containing an imidazolidine nucleus. These findings suggest that the hydantoin derivative HPA-05 has a profile similar to that of a depressant. This finding indicates that HPA-05 may exert an anxiolytic effect, since a high rate of emotionality is associated with the increase in the number of fecal boluses, whereas anxiolytic drugs reduce this behavior [21]. The rotarod test was performed to enable a better evaluation to be conducted of the muscle-relaxant effect or motor incoordination resulting from treatment with HPA-05. The depressant effect of HPA-05 had no effect on the animals' motor coordination, thus eliminating the possibility of a muscle-relaxant or neurotoxic action. The data obtained in the rotarod test in the present study conflict with the findings reported by Carvalho [19] in a study conducted with the hydantoin derivative IM-7 (5-(4-isopropylphenyl)-3-phenyl-imidazolidine-2,4-dione) in which the occurrence of motor incoordination was reported.

No change in the animals' behavior indicative of a possible anxiolytic effect was found in the elevated plus maze test, since anxiolytic substances cause the animal to increase the number of times it goes into the open arms of the device and/or the amount of time it spends there [22]. The results suggest that there was no difference with respect to the number of times the animals treated with HPA-05 (50, 100 or 200 mg/kg, i.p.) went into the open and closed arms of the device and the time they remained there compared to the mice in the negative control group.

Several studies have reported the anticonvulsant activity of imidazolidine derivatives in animal models [23]. In order to evaluate the possibility that HPA-05 could have an anticonvulsant effect, two different methodologies were used: the pentylenetetrazol (PTZ)-induced seizure test and the maximal electroshock seizure (MES) test. These classic models are still widely used in the research of new anticonvulsant drugs [24,25].

PTZ acts by inhibiting the chloride channels associated with the GABAA receptors [26]. Therefore, it is known that the blockade of seizures induced chemically by PTZ in rodents is a characteristic of some CNS depressant drugs belonging to class anticonvulsant [27]. HPA-05 reduces the incidence of seizures induced by PTZ, as well as a qualitative change that can be demonstrated by the significant increase in latency to the onset of seizures. The increase in the latency until the onset of seizures is a strong indication of an anticonvulsant effect and drugs such as diazepam and clonazepam are able to increase this parameter [28]. Diazepam, used as a positive control, increased

the latency until the onset of seizures. This is a drug with a known anticonvulsant effect, which results from its action on the GABAA/benzodiazepine receptor.

Maximal electroshock seizure test (MES) is based on the observation that stimulation through repetitive electrical pulses, and using appropriate parameters, it is able to induce a characteristic pattern of epileptic activity in different neuronal structures [28]. Drugs that are effective in the MES test are effective for the treatment of generalized and partial seizures by blocking the voltage-gated sodium channels or through GABAergic neurotransmission [29].

The results of this test demonstrate, therefore, that the HPA - 05 has a similar profile to anticonvulsant drugs, promoted as a protection of the auricular electroshock-induced seizures in the described parameters, as compared to animals treated with vehicle only. The results corroborate the data reported in the literature, where phenytoin is the best known hydantoin, being used as a drug of first choice in the treatment of partial seizures and generalized tonic-clonic type, but contraindicated in absence seizures [26].

CONCLUSION

From these preclinical psychopharmacological studies on HPA-05 in mice, it is reasonable to conclude that its toxicity is low, and it has a profile similar to that of central nervous system depressants. Although HPA-05 proved effective in the anticonvulsant tests, further studies are necessary to characterize its mechanism of action and to determine its clinical applicability. Other methodologies involving chemical agents such as pilocarpine and strychnine could be used in order to determine the possible mechanisms of the anticonvulsant effect of this promising substance.

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