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Immunological effect of the bufotoxin 7CH in rats

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Abstract
Aim: Determine the effect immune of bufotoxin 7CH in rats belonging to the Balb-c and Wistar strains.
Methods: An experimental study was conducted. The bufotoxin was maintained in alcoholic solution and applied to rats in doses of 20 units via intramuscular insulin syringe. Observations were recorded. 10 rats from both strains survived and were administered 9 to 10 times a butotoxin dosage.
Results: Administering 9 to 10 dosages generated an acute set of symptoms that culminated with their death, without having a meaningful difference among both strains.
Conclusions: The bufotoxin 7CH in both rat strains and intervenes in reducing mortality from effect of the bufotoxin. The death of rat strains by overdose of the bufotoxin could mean the strengthening of the immune system derived from the bufotoxin 7 CH homeopathy.

Keywords: bufotoxin, effect, bufotoxin 7CH, experimental, immunological effect

Introduction

The World Health Organization defines epilepsy as a chronic disorder of the brain of diverse etiology, characterized by spontaneous recurrent seizures due to excessive electrical discharges of a group of brain cells. Epilepsy is a public health problem; it is estimated that 50 million worldwide have a diagnosis of epilepsy, in Latin America there is a prevalence of 18 per 1000 inhabitants. It can arise since childhood or at any age, mainly children and young people (0-20 years). Its main cause is intertwined with heredity factors and even after the administration of some medicines, as well as it can result from traumatic accidents [1-4].

A seizure is a paroxysmal event that occurs by the abnormal or excessive neuronal activity in the brain. Depending on the distribution of discharges, this abnormal neuronal activity can manifest itself in different forms, ranging from dramatic convulsive activity to even subjective experience phenomena which are difficult to warn by an observer. Epilepsy refers a clinical condition resulting from recurrent unprovoked seizures that can't be explained by a medical condition such as fever or substance withdrawal [5-6].

There is evidence that immunological process are might have a role in the pathogenesis of epilepsy through the involvement of different types of antibodies, however, there are no autoantibodies found specifically in epilepsy [6-10]. Pharmacological treatments of epilepsy are usually ineffective and with hepatic toxicity, for which reason, development of a complementary and alternative medical measured can help for epilepsy treatment [11].

The bufotoxin is a toxin secreted by the skin of toads [12]. In its biochemical composition, it is a component that is formed as a result of the binding of bufofagin and a molecule arginina. The toxic action of the bufotoxin is observed at an enzymatic level, inhibiting ATPasa Na +-Pump + k of the cardiac muscle fiber, blocking activity on Na channels, increases the concentration of intracellular Ca + +, causing an increment in the contraction of the heart and a reduction in the heart rate [13-16].

A study conducted in Wistar rats shown that bufotoxin decreased brain dopamin when using such homeopathic boosted doses, this means that the most notable decrement occurred when employing the highest dynamic [17, 18].

Conducting this research had as intrinsic aim to confirm by itself the action of homeopathic medicines against epilepsy, assuming that an boosted substance causes pathogenesis and an effect, as well as to open up new treatment options for this type of patients, where apparently the therapeutic limits range from magic to the unusual, but homeopathy represents a real therapeutic for this problem. Therefore, the results of this project will expand new alternatives to treat this disease and also encourage scientific research in the experimental field in order to support homeopathic drug action.

This investigation is focused were to determine the dose of bufotoxin to induce epilepsy in Balb-c and Winstar rats and describe induced epileptic symptoms after application of such toxin, which was subsequently boosted and administered at 7 CH, CH 12 and CH 30, to demonstrate that homeopathic treatments stimulate the organisms to get heal itself.

Research Questions

Administering the bufotoxin 7CH provokes a positive effect towards the immune system of the exposed rats, which were exposed at the beginning to buffo toxin and at the end to buftoxin 7CH.?

Aim
Determine the effect immune of bufotoxin 7CH in Balb-c and Wistar rats.

Specific objective
Compare the effect immune of the bufotoxin 7CH in both strains of rats.

Hypothesis
H_I = The administration of the bufotoxin 7CH, centesimal Hahnemannian causes an important effect on the strengthening and resisting of the immune system in both strains of rat.
H_O= The administration of the bufotoxin 7CH, centesimal Hahnemannian does not causes an important effect on the strengthening and resisting of the immune system in both strains of rat.

Variables

Table 1: Relationship between the variables:

Independent Variables	Intervening Variables	Dependent Variables
The administration of bufotoxin 7CH in rates effect immune	Acquisition of an illness while in the laboratory.	They survive to more than a dose of the toxin without presenting any epilepsy symptoms.

Methods
Type of Study: an experimental.
Universe: Rat strains BALB-c and Winstar since 8 days of born up to their reproductive age.
Sample size: 10 rats of the Balb-c strain and 10 Wistar strain rats.
Sampling method: random probabilistic in blocks of 10 rodents.
Unit study: Rats of the strain BALB-c and Winstar with epilepsy induced though bufotoxin; such rats were immediately administered with boosted bufotoxin in the selected power. (7 CH).
Unit of analysis: Symptoms and mortality of rats of the strain BALB-c and Winstar with epilepsy induced though bufotoxin; such rats were immediately administered with boosted bufotoxin 7CH. Later with 20 units of toxin.
The drug 7CH was prepared in the laboratory by previously taking a drop of the diluted toxin in a mixture containing ethyl alcohol of 96 ° C and distilled water at a concentration of 70% alcohol in water which was taken gout and mixed with 99 drops of ethyl alcohol boosted by 100 strokes energetic revitalization in a phone book which was previously prepared for this purpose. The procedure was repeated up to the power 7 CH and 30 CH. During the piloting which will later serve to standardize the sample, difficulty to establish pathogenesis in the Balb-c strain was not observed. Even though pathogenesis is alike, convulsions were less perceived in the Wistar strain that in the Balb-c strain. After this, the drug was directly administered with a homeopathic and sterile dropper, each rat was given 8-10 drops, three doses every 15 minutes and then 15 drops were left in their drinking water. The optimal standardized time for the symptomatology is observed within 10 minutes after the inoculation of the toxin, the symptoms rats showed were recorded in a log for their later use in constructing a database. After receiving their doses of medication the rats that survived were placed back into their polycarbonate rooms, with food and medicine in their drinking water. The rats that did not survive had a necropsy in order to record the findings in the log. Moreover, they were kept under observation and under administration of homeopathic medicine for over a period of 4 weeks, in which no record of epileptic episode appeared. In two groups of 10 rats of both strains randomized rats previously inoculated with the toxin and then with the drug at the 7 CH and which survived were administered again with the toxin and observations were recorded [16, 17]. When the experiment was accomplished the results were analyzed in SPSS-15.
Inclusion criteria: Balb-c and Wistar rat strains of 4-45 weeks old that are under control in the laboratory and which would not show suggestive symptoms of disease.
No – inclusion criteria: rats under 4 weeks and over 45 weeks old with suggestive symptoms of some disease.
Exclusion Criteria: Excludes those rats that were not inoculated with the toxin and it was possible to administer the homeopathic medicine on time to assess its effect.
Considerations of animals: were maintained under controlled conditions in a healthy environment and were handled with respect and dignity to animals

***Source*:** UMG. [17]

Fig 1: Obtaining the bufotoxin; contained in the dorsal cutaneous glands of the toad.

Results
The results of this investigation make reference to laboratory work performed with rodents of 10 Balb-c strain and 10 of the Wistar strain in two groups of randomized rats previously inoculated with the toxin and then with the drug at the 7 CH
When comparing the rats which received the toxin, the medicine and the ones that died, as did those who received drug and toxin and survived with a p value of 47,958 and a $p \leq 0.05$ of 000, indicating that there is significant difference suggesting that the homeopathic medicine shows a significant effect on the reversal of the symptoms presented. (Table 1) [16].

Table 1: Frequency and percentage of survival under the effect of the energized bufotoxin 7 CH including necropsies [16].

Explanation of the effect	Frecuency	Percent
natural death	1	2%
Rats were killed by the effects of the toxin, and to which we fail to administer the medication.	19	38%
Rats were killed by the effects of the toxin, which if reached as to administer the medication. 7 CH	7	14%
Rats that survived the dose of toxin and reacted to the drug effect. 7 CH	23	46%
Total	50	100%

***Source*:** Direct, experiments showing the effect on survival and energized bufotoxin necropsy killing rodents.

The results below show the effects of multiple doses of the toxin in rats, which were previously treated with the same toxin but at 7CH.

The rats from the Balb-c strain show a very little neuro toxic effects along with convulsions; which are observed to have a length of 5 seconds. At the same time, they show piloerection. They do not move in any direction but show themselves wobbly in a standing position. Soon after that, they die. It seems that rats from Balb-c strains present epileptic symptoms, but in a very fast way. The rats from wistar strain, show a small increase on their eyes' size as they act wobbly; they look disoriented in their vision which denotes the neurotoxic effect which also causes them to die (Table 2).

Table 2: Immune effect on epilepsy survivors with bufotoxin 7CH exposed to 9-10 doses of toxin.

Balb/c	doses	Effect	Description of effect	Wistar	doses	effect	Description of effect
1	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	1	10	death	Fulminant death
2	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	2	10	death	Piloerection alomost unperciptible, then fulminant death.
3	9	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed before dose 10.	3	10	death	Piloerection alomost unperciptible, then fulminant death.
4	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	4	10	death	Fulminant death
5	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	5	9	death	Fulminant death
6	9	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed before dose 10.	6	10	death	Fulminant death
7	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	7	10	death	Piloerection alomost unperciptible, then fulminant death.
8	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	8	9	death	Piloerection alomost unperciptible, then fulminant death.
9	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	9	10	death	Piloerection alomost unperciptible, then fulminant death.
10	10	Death	Piloerection, they start having a fulminant convulsion and then die; this effect is observed after the 9th dose.	10	10	death	Piloerection alomost unperciptible, then fulminant death.

***Source*:** Direct, experiments showing the immune effect.

Discussion

Drug induced liver injury associated with antiepileptic medication is well-documented, reason which to development a new alternative medical for help patients with epilepsy is a necessity.

There are few reports of the effect of bufotoxin. The composition of this substance has cholesterol, gamasistosterol and ergosterol, regarding the nervous system, catecholamines, and adrenaline itself may trigger compatible symptoms within the same convulsions or seizures of cardiology type; as shown by a study conducted with an intoxicated dog by means of the bufotoxin or poison from the bufo toad [18].

In this work we evaluated the immunological effect of bufotoxin 7CH in rats the Balb-c and Wistar strains. Results shown that bufotoxin 7CH in both rat strains intervenes in reducing mortality from effect of the bufotoxin by a possible immunological effect.

It has been reported that bufotoxin decreases brain dopamine in inverse proportion to homeopathic revitalization used (6, 9 and 15 CH) [18].

The effect of epilepsy symptoms as compatible effect with epilepsy among both rat strains represents the value to use it for testing the effect of drugs on the eve of finding an effective cure against this disease [16].

Convulsions that start in extremities and in their tail mark the final phase of the rats' lives, a situation which, in the Wistar strain occurs very quickly not as characteristic as in the Balb-c strain. Therefore, it is important to establish that there is some commonality between both strains in reference

to the effect of both, the toxin and the effect observed after administering the toxin. Wistar seizures and hence it is important noted that if there is some on the effect of both the toxin as part of the effect [17].

There is no previous study with which to compare the results, but it is starkly important to note that it was possible to determine that the medicine, even prepared under the conditions described above, meet a demonstrable impact on the conditions under which the experiments were conducted.

One of the limitations we can note and that was manifested in this study consisted on the fact that the rats used were standardized by age, a situation which could be determined in terms of age. Rats that survived are in their optimal reproductive stage which is at 16 weeks old. The most vulnerable situations were rats with 4 weeks old, the youngest and the oldest 40 weeks old.

As part of the observed effect after administering the boosted toxin, it was possible to observe that after 48 hrs. the presence of a degenerative ulcerative necrotic lesion as an area of alopecia where the toxin was inoculated. This situation led to make two crop batches of the toxin in trypticase soy agar which until 72 hrs. were negative so apparently the injury could be related to the corrosivity of the toxin but not perhaps under the presence of a microorganism that could have contaminated it during processing. We can say that because experiments were carried out under controlled conditions.

Note that in strain Balb-c with an average age between 14 and 16 weeks old, after being inoculated with 25 units of the substance by means of an insulin syringe between 5 and 10 min. symptomatology began as a consistent effect with the necropsies performed at the project within the Wistar strain.

It is of utmost importance to compare the results obtained by Cazin, *et al*, 1987 where after intoxicating rats with arsenic; boosted dilutions were employed between 5 to 15 CH which achieved a better effect regarding the elimination of arsenic by means of both urine and feces under the utilization of 7 CH, which had a similar effect to what was observed even when in these experiments it was not possible to determine the presence of the bufotoxin in urine and feces as one of the major limitations on one side is that the previous study does not indicate the type of analysis and how it could have been conducted, in addition, it was not possible to accomplish such measurement in in this study.

In this current study as well as in the previous one (where the effect of the toxin resistance is observed and where a model for the study of epilepsy was generated [16] the records of analysis of 10 strains of rats denoted an important effect, thus:

It is worth noticing that rats to which the toxin was administered and which had generated a set of epileptic symptoms (meaning those rats about which the model had been generated and to which it had been previously administered the bufotoxin 7CH) received a positive effect on their immune system, since both strains showed the same resistance to overdose of such toxin, being able to stand from 9 to 10 doses with non or little epileptic symptoms and finally dying. This means that the animals employed at the experimental phase generate immunological changes against the toxin and thus their multiple resistance about its dose. Therefore, the investigation hypothesis is proved and thus the null hypothesis is rejected.

Conclusions

The bufotoxin under the conditions it was prepared provokes an important regarding the reversal of symptoms resulted from the administration of the bufotoxin. The results obtained from this research allow strengthening and verifying that a boosted substance has an important effect immunological.

The rats that survived did not present any characteristic symptoms with 9-10 doses the initial inoculation. The death of rat strains by overdose of the bufotoxin could mean the strengthening of the immune system derived from the bufotoxin 7 CH homeopathy.

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