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Full Length Research Paper

Bufo-toxin effects on epileptic symptomatology stimulated according to rat strains

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This study aims to determine the effect of the boosted bufo-toxin towards epileptic symptomatology induced through bufo-toxin in the strains known as BALB/c and Wistar in order to break into the symptomatology which confers lethality to this toxin. The bufo- toxin was maintained in alcoholic solution and applied to rats in doses of 5 to 5-20 units via intramuscular insulin syringe, and observations were recorded. When the rats in question died, a necropsy was performed in order to finally describe the effect of the toxin to the internal organs. Subsequently, the bufo-toxin was boosted and administered at 7, 12 and 30 CH according to the rat strains, and observations were carried out. The rats that were inoculated with 20 units of the named toxin show epileptic symptomatology and finally died from brain stroke or heart attack. However, when administering the bufo-toxin boosted at the 7 CH, such pathogenic symptomology disappears and mortality in rats decreased to 48%. The more effective homeopathic potency was the 7 CH. This model can be used to investigate useful drugs against epilepsy and even heart diseases; the homeopathic medicine at 7 CH acts immediately regarding potencies 12 and 30 CH. The boosted toxin reduces the pathogenic symptomology generated by the bufo-toxin in both rat strains and intervenes in reducing mortality from such effect.

Key words: Bufo-toxin, effect, epilepsy, heart attack, experimental.

INTRODUCTION

Epilepsy is a public health problem; its prevalence is 18 per 1000 inhabitants. It can arise from childhood or at any age. 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 (Gracia, 2006). This type of problem affects mainly children and young people (0-20 years), but is most common in children younger than 9 years old, which truly represents a public health problem (National Library of Health and Social Security, 2011). This research aims to benefit those who have the need for treatment to alleviate or cure epilepsy due to the fact that no medications can solve this condition so far.

An epileptic crisis from the Latin “sacire” takeovers is a paroxysmal phenomenon due to abnormal, excessive and hyper synchronic discharges, of a group of neurons in the central nervous system (CNS). Depending on the distribution of discharges, this abnormal CNS activity can manifest itself in different forms, ranging from dramatic seizure activity to even subjective experience phenomena which are difficult to warn by an observer. Although a variety of factors influence the incidence and prevalence of epileptic episodes, 5 to 10% of the world population will have at least one of those during their lifetime; the incidence is highest in childhood and at

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adulthood. Epilepsy describes a condition in which a person has recurrent epileptic crises due to an underlying chronic process. This definition implies that a person, who has had a single episode or multiple crises secondary to correctable or preventable factors, does not necessarily have epilepsy (Fauci et al., 1998).

The main feature that distinguishes the different categories of epileptic crises is whether the epileptic activity is partial or generalized. Partial episodes are those in which seizure activity is confined to small areas of the cerebral cortex. Generalized crises simultaneously affect broad brain regions in a bilateral and symmetrical way. As a general rule, partial crises are typically associated with structural brain lesions. On the other hand, generalized crises can result from cellular, biochemical or structural anomalies of wider distribution. There are two types of convulsive disorders namely: an isolated episode (non-recurring) - as it can be accompanied by a febrile illness or after a traumatic brain injury; and epilepsy - a recurrent paroxysmal disorder of brain function characterized by brief and sudden crises which alter consciousness and is accompanied by motor activity, sensory phenomena or inappropriate behavior caused by excessive neuronal discharge (Beers et al., 2005).

Early concepts about epilepsy were developed in ancient India, in the period of 4500 - 1500 B.C. In Ayurvedic literature of Charakas Samhita (400 A.C.), epilepsy was described as APASMARA whose meaning was loss of consciousness. The Charakas Samhita contains many references about all aspects of epilepsy, including symptoms, etiology, diagnosis and treatment.

In tables of ancient Babylon dated 2000 B.C., records are different types of attacks associated with the name of an evil spirit, the treatment was therefore primarily spiritual. The Babylonian approach was the predecessor of the Greek concept of sacred disease.

Etymologically, the word epilepsy is derived from the Greek word “Epilambanein” meaning be moved abruptly. Hippocrates in 400 B.C. was the first to describe the disease as a brain abnormality considered “sacred disease”. He recommended physical treatments and stated that if the disease became chronic, it was then incurable.

In the eighteenth century, as soon as neurology emerged, a new discipline distinct from psychiatry, the concept of epilepsy as a brain disorder became widely accepted, especially in Europe and the United States of America (Brailowsky, 1999). In 1873 in London, the neurologist, John Hunglings Jackson, suggested that the attacks were the result of “an abnormal electrical discharge occasionally sudden, intense, rapid and repeated in the gray matter.”

In 1929, Hans Berger, a psychiatry professor at the University of Jena in Germany, published for the first time that brain electrical activity was likely to be recorded from the surface of the skull. Later, in 1931, Berger published electrographic changes associated with epilepsy; the EEG (Electro encephalogram) helped locate the download site attacks and expanded the possibilities of neuro-surgical treatments, which were made available since the 1950s onwards in London, Montreal and Paris. Finally, in 1973, the World Health Organization published a dictionary that defines epilepsy as a chronic condition of diverse etiology, characterized by recurrent convulsions, due to excessive discharge of cerebral neurons (convulsions), possibly associated with various clinical and paraclinical manifestations (Brailowsky, 1999). Convulsions usually varied from partial episodes which affect only one part of the brain, simple partial (focal) and complex partial (Kasper et al., 2005: 929-933). This should be supported on the characteristics of the EEG. Other studies such as computed tomography and/or magnetic resonance imaging are not initially required due to the fact that the patient‟s symptomatology and the encephalographic study are very accurate to diagnose the disease (Kasper et al., 2005: 933-936).

Regarding treatment of partial epilepsy crises, some medications are: felbamate (Felbatol) ®, gabapentin (Neurontin) ®, levetiracetam (Keppra) ®, lamotrigine (Lamictal) ®, oxcarbazepine (Trileptal) ®, pregabalin (Lyrica) ®, topiramate (Topamax) ® and tiagabine (Sabril) ®. For generalized episodes, the following medications are prescribed: levetiracetam (Keppra) ®, lamotrigine (Lamictal) ®, topiramate (Topamax) ®.

Anticonvulsant drugs need regular blood checks to assess the management of dose and prevent occurrence of side effects and / or toxic effects (Dictionary for Proprietary Medicinal Central Nervous System, 2007: 427). Nowadays, there is no cure for this disease and this adds value to the current experimental model.

Pharmacological treatment of crises completely eliminates in 1/3 of the patients and significantly reduces its frequency in a third part of them. Nearly 2/3 of patients with well controlled crises can discontinue medication without causing relapse.

Most patients with epilepsy are neurologically normal except for their crises, but excessive use of AEDs can decrease their alertness. The progressive mental deterioration is usually associated with the neurological disorder that seizures cause. The left temporal lobe epilepsy is associated with verbal memory alterations, whereas right temporal lobe epilepsy can cause disorders of the vise-spatial memory. The prognosis is best when there is no demonstrable brain injury (Kasper et al., 2005: 1409-1410).

Epilepsy is defined by the presence of recurrent seizures of unknown etiology (Collins, 1999). It is a disorder of the central nervous system characterized by repeating two or more unprovoked crises caused by an immediate and identifiable cause. The occurrence of a single seizure does not allow the diagnosis of epilepsia (Dictionary for Proprietary Medicinal Central Nervous System, 2007: 420).

The bufo-toxin is a toxin derived from the skin of toads (Bufotoxin, 2011). In its biochemical composition, it is a component that is formed as a result of the binding of bufofagin and a molecule arginina (Godoy et al., 2012). The toxic action of the bufo-toxin 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 (Moyano et al., 2009).

Conducting this research has an intrinsic aim of confirming by itself the action of homeopathic medicines against epilepsy, assuming that a boosted substance causes pathogenesis and an effect, as well as opening 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. It is important to point out that this project will increase the recognition of homeopathic doctors‟ assistance in the public health in a social level, with defined experimental basis, and also homeopathic therapy from social action will benefit those who have the need for treatment to alleviate or cure epilepsy. There are no barriers or limitations to this project.

In order to carry out this research project, both economic and financial resources and materials were collected. In its first phase, this research was carried out in the Laboratory of Biology at The Colegio Francisco Febres Cordero La Salle. Regarding its second phase, it was held at the facilities of the physical, chemical and biological laboratory at Bachillerato Cervantes Loma Bonita de la Universidad Marista de Guadalajara.

The genesis of homeopathy

Homeopathy is a type of medicine that guides towards medical practice; it is unique in its genre, and was conceived two centuries ago by Samuel Hahnemann. It implies an original therapeutic trajectory which is based on an empirical and conceptual approach to illnesses and diseases, on its integration to the personalized study of the sick person and his treatment (Benkemoun et al., 2000: 9). Christian Friedrich Samuel Hahnemann was born on April 10th 1755 in Meissen, a small city in Sajonia, Germany, and passed away in Paris On July 2nd 1843 (Benkemoun et al., 2000: 19).

In the spring of 1775, Hahnemann goes to Leipzig to study at the Faculty of Medicine where he obtained his studies free of charge thanks to the support of Pörner. Since 1776, the student, Hahnemann, made several translations of English and Scottish medical works. Hahnemann admires Haller and Boerhaaver‟s careers because they both use the knowledge of physics, mathematics and physiology for medical practice, and he cites numerous works about pulse written in Latin, French and English.

This intense translation activity is prolonged for more than twenty years and allowed him to meet his needs until 1800, when its exclusive medical activity was sufficient to serve his entire family (Benkemoun et al., 2000: 20). 1790 is the date in which Hahnemman issues his first statement about the principle of similarity. In the German language translation of *The lectures on the Materia Medica* of the Scottish Cullen, entitled as *Abhandllung uber die Materia Medika,* Hahnemann inserted large number of comments on the text, as if they were notes. Cullen says tonics and bitters, including quinine, have an overall effect through its local effect on the tone of the stomach. It is exactly on this theoretical controversy that Hahnemann inserted his famous note on quinine.

Hahnemann talks in this note about a new property of the quinine, which allows explaining its curative virtues: quinine in intermittent fevers cured the patient because it has the ability to produce at fairly high doses, a similar fever in a healthy individual. It is the first statement of the principle of similarity, theoretical basis of the whole homeopathic doctrine to be taken up and developed in the Organon (Benkemoun et al., 2000: 26).

Hahnemann, moved by an enlightened curiosity, sensed the discovery of a new therapeutic principle and performed himself the following experiment:

“Hahnemann said he took for several days four drams of good Cinchona twice a day, then he described his symptoms and concluded thus: For me, the symptoms were typical of intermittent fevers. I stopped taking the medicine and felt better again.”

He published the results of his experiments on the effects of drugs, just six years later, in 1796, in an essay entitled “Essay on a new principle to discover the healing power of drugs”, after which he clearly understood that the tests should be done in healthy individuals and not sick individuals. Reaching the principle of similarity, he then enunciate in Latin “Simílla Similibus curentur” so that year 1796 is considered, with all fairness, the year of the birth of homeopathy. Since then the great struggle of Hahnemann to impose his truth to the scientific world of his time began, a struggle that lasted until today.

In 1810, he published the first edition of the Organon. This is a book of one hundred and twenty-two pages preceded by an introduction of forty-eight pages. The body of this first edition consisted of two hundred and seventy-one paragraphs in which Hahnemann explains his conception of the disease, how homeopathic medicine acts, its medical prescription and therapeutic behavior. The exposure of the homeopathic doctrine is an authentic bible of reference for anyone who wants to practice homeopathy even today. Hahnemann published

four new editions of the Organon reviewed and completed in accordance with amendments to his theory, under the title Organon, The Art of Healing: 1819, 1824, 1829 and 1833. In 1921, a sixth edition was published posthumously by the publisher Haelh from Hahnemann‟s original documents.

In 1811, Hahnemann published the first tome of the *Materia Medica Pura*. *The Homeopathic Materia Medica* comprises the set of pathogenesis, for instance, experimental clinical tables of medicinal active substances in a healthy person (Benkemoun et al., 2000: 28).

In 1828, Hahnemann operates a major change in his homeopathic doctrine publishing the first volume of chronic diseases, their specific nature and their homeopathic treatment. Hahnemann introduces the concept of miasms to explain relapses for which he describes three diathesis of miasmatic origin: psora, sycosis and syphilis.

Since 1812, Hahnemann starts having some students and teaches at the University of Leipzig which leads him to capture some students who would become his first disciples: Franz, Gross, Hartmann, Hornburg, Langhammer, Ruckert, Stapf and Wislecenus who participate in meetings at the house of their old master and in the experimentation of pathogenesis. Hahnemann is conscious of his great success in his medical practice and begins to be consulted by citizens from all over the country.

The end of the life of Hahnemann is romantic enough to be told and also demonstrates the extreme vitality of this stubborn old man.

Enriqueta, Hahnemann‟s first wife died in 1830 and thereafter, Hahnemann lives with his two daughters, in their small house in Kothen. Under the pretext of illness, the young Mary Melania goes to visit him (she is 34 and he is 79), and Hahnemann was seduced by this elegant young woman who came from Paris. He was carried away by his enthusiasm and his plans to travel to France. Finally, they got married in January 1835 and departed to Paris that year. After receiving authorization to work in Paris, Hahnemann restarted a great medical activity, aided by Melania, obtaining very good results which would make him famous and increase his clientele. His stay in the French capital, key city of culture and medicine, helped the development and international distribution of homeopathy. Many of his students ended up spreading his teaching abroad.

Hahnemann died of chronic bronchitis at the age of 88, on July 2nd 1843, at his home in Paris, 1 Milan Street. His remains were buried in the cemetery of Montmartre and then transferred to the cemetery of Pere-Lachaise where they rest today (Benkemoun et al., 2000: 29).

Science should be for everyone. If homeopathy is a chimera or a worthless system, it will fall by itself. If, however, it is consider a progress, it will extend itself despite all our precautions, and the Academy should want it more than anyone else, because it has the mission to make science advance and to encourage discovery (Eizayaga, 1991: 33-34).

The philosophical basis of homeopathy

„Life Force‟ is a material but not a corporeal energy. For that condition or dynamic, it is able to “move” the material body in its physical-chemical and biological reactions. „Vital Force‟ implies life emanates from the soul to the body, from the center to the periphery, from the depth to the surface, from the spiritual to the organic, in centrifugal sense, keeping balance of what is called health.

Since life force is energy, it means that it could only be disturbed by other energies of alike nature, which would act on the same plane of action. These energies can be:

- Physical: Heat, cold, electricity, radiation, vibration and trauma.
- Chemicals: Toxic medicines and foods.
- Biological: Microbial infections and parasitic virus.
- Miasmatic: Miasmatic contagion and miasmatic inheritance unexplained by the mechanism of microbial infection.
- Psychic: Sorrows, frustrations, moral or emotional traumas, conflicts and fears.

This would explain how the life force is disrupted by the boosted homeopathic remedy that has no mass but energy released.

Healing therefore in active reaction mechanism (what nowadays is known as immune response), automatically reacts „Vital Force‟ to return to physiological balance (Eizayaga, 1991: 101-102).

Homeopathic medicinal action

Every agent that acts upon the vitality of every medicine disturbs more or less life force and causes some changes in the health of the individual for a more or less extensive period. This is called primary action. Even when this is product of power and life force conjugated, it is mainly the former. In its action, our vital force tries to oppose its own energy. This resistant action is a property, it is truly an automatic action, the power that preserves our lives and which deserves to be called secondary or reactive action (Hahnemann, 2006: 128).

While the primary action takes place, medicinal morbific agents (medicines) on our healthy body, our life force seems to behave purely passive (receptive) and, as it will be seen by the following examples, it is as it were subjected to tolerate artificial impressions which from the outside has an effect on it. Thus altering the health, even though as it seems aims to develop:

A) The health condition exactly opposite (reactive action, secondary action) to that effect (primary action) that

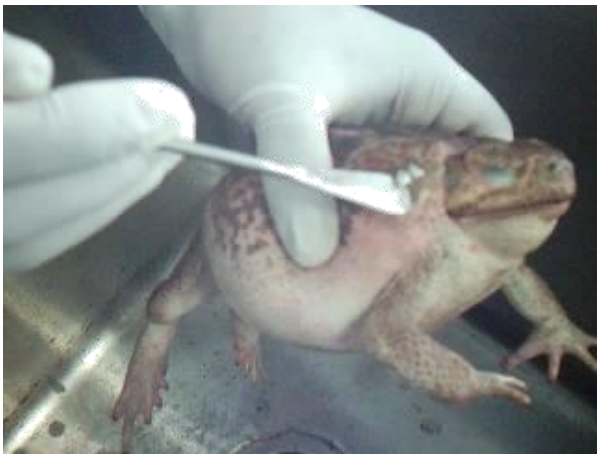

Figure 1. Obtaining the bufo-toxin considered as a poisonous oily discharge, contained in the dorsal cutaneous glands of the commonly known toad frog. Direct Source: Physical-chemical-biological laboratory, UMG, Marist University of Guadalajara.

acted upon it and where such opposite MIGHT be a proportional degree to the intensity of the effect of artificial, morbific or medicinal agent (primary action) and also proportional to its own energy.

B) If there is no state in nature exactly opposite to the primary action, it appears that it attempts to neutralize this; in other words, it means to exercise its superior power to extinguish the change imposed on it from outside (for medicine) by substituting normal state of its own (secondary action, curative action) (Hahnemann, 2006: 129).

Homeopathic experimentation

A remedy that is experienced in the healthy man causes certain symptoms; it is able to heal the sick of natural diseases with similar symptoms - “law of similar”.

In healthy human experimentation, one should carefully note the symptoms experienced, resulting in homeopathic pathogenesis. Those involved in the tests are called “experimenters”, they should be of both genres, clinically healthy and should be a maximum number (not less than 50 and better around 100).

Experimenters must have a certain level of intelligence and culture, in order to accurately and clearly describe the feelings they experience and do so in writing. They should have certain level of intelligence and culture in order to clearly and precisely describe the sensations they experiment and keep written evidence. They should also lead a quiet life while the experiment is carried out, with a moderate diet, avoiding excesses and substances with medicinal properties that may disturb the results.

Substances to experiment should be pure, authentic, well known and with all their energy.

The administration method that Hahnemann recommended for experiments is that during many days, subjects should take 4 or 6 cells for 30 CH dissolved in water and digested in fasting, we should always keep in mind the susceptibility of the experiencing subject to increase or decrease the dose to see the results (Eizayaga, 1991: 61-64).

Action of the bufo frog on brain dopamine

Bufo frog gets an oily secretion that is considered poisonous; it is contained on its dorsal glands under the skin and such animal is commonly known as toad frog. Indigenous women used this substance when they do not tolerate their husbands" assiduity in order to cause them impotence (Jacques and Sánchez, 1991: 75).

In 1992, the named substance was studied in 40 Wistar rats whose weight was between 150 and 180 g, and which were divided into four batches. One group was given bufo frog boosted at 6 CH (1×10-12), 9 CH (1×10-18) and 15 CH (1×10-30), at a dose of 25 ml every 8 h for 10 days, by means of an esophageal catheter.

Results showed that the rats in such group got sacrificed by decapitation and the brains were frozen at 40°C. Cerebral dopamine managed under the Waldeck and Carlsson technique (Eizayaga, 1991: 61-64) showed that in the control group, the average dopamine concentration was 0998 ng/g, as such, rats receiving 6 CH frog Bufo were 986 ng/g, and those administered 9 CH had lower scores of 974 ng/g. So it is concluded that Bufo frog produces decreased brain DA when using such homeopathic boosted doses, this means that the most notable decrement occurred when employing the highest dynamic (The Main Directions of Research in Homeopathy, 1995), as reported in Figure 1.

Research in homeopathy

The set of symptoms should be the principal issue to the doctor; indeed the only thing to watch for in each case and using his art to heal, should be eliminating illnesses to transform it into health. The investigation of the medicine with similar effects as a result of their experiments in healthy man, founded a real therapeutic method but whose overall approach differs, despite careful consideration of symptoms. And when he recommends the use of high dilutions whose explanation is based on a “dynamic”, introduced in pharmacology, the physicochemical paradox still continues to raise controversy and actual experimental work.

The main thrusts of this research relate to the issues raised by the scientific evaluation of the different phases of homeopathy and clinical practice of homeopathic physicians in the reality of the activity of high dilutions. Schematically, the work done can be grouped into four different axes:

- Experimental biological research: designed to demonstrate the activity of high dilutions.
- Clinical Research: designed to demonstrate the activity

Table 1. Relationship between the variables.

Independent variable	Intervening variable	Dependent variable
Bufo-toxin poisoning.	Acquisition of an illness while in the laboratory.	Epilepsy induced through bufo-toxin action.
The administration of bufo-toxin boosted by 7, 12 and 30 CH		The reduction or elimination of epilepsy symptoms through boosted bufo-toxin.

of homeopathic medicines on sick individuals.
- Physical-chemical Research.
- Research that integrates the "holistic" dimension of homeopathy [28].

Two fundamental aspects on which this study focused were to determine the dose of diluted bufo-toxin to induce epilepsy in rats of the BALB/c and Wistar strains and describe induced epileptic symptoms after application of such toxin, which was subsequently boosted and administered at 7, 12 and 30 CH, and observations on the effect were recorded. Apparently, the effect of bufo-toxin has not been studied in rats, so the realization of this project will be based on answering the following questions:

Research questions

What is the effect of bufo-toxin in the two strains of rat? Is it possible to implement an experimental animal model for research on new drugs against epilepsy?

Research objectives

Objective

This study aims to determine the effect of diluted and boosted bufo-toxin towards induced epilepsy in rats belonging to the BALB/c Wistar strains.

Specific objectives

This study has the following specific objectives:

- To determine the dose of the diluted bufo-toxin in order to induce epilepsy in rats of the BALB/c and Wistar strains.
- To describe induced epileptic symptoms before and after administering the boosted bufo-toxin in both rat strains.
- To determine the boosted power of the bufo-toxin to the effect that occurs in the decrease of symptoms between the powers of: 7, 12 to 30 CH.
- To compare the effect of the boosted bufo-toxin towards epilepsy induced in both strains of rats.
- To statistically compare mortality rate under the effect of the previous boosted poisoning bufo-toxin, within both rat strains.

Hypotheses

H_1 - The administration of the bufo-toxin boosted to 7 CH, according to centesimal Hahnemannian eliminates epilepsy symptoms in rats, which was induced by the same toxin in both rat strains.
H_0 - The administration of the bufo-toxin boosted to 7 CH, according to centesimal Hahnemannian does not eliminate epilepsy symptoms in rats, which was induced by the same toxin in both rat strains.

Variables

Different variables were employed in this study and they include the independent, intervening and dependent variables. Table 1 shows the relationship between the variables.

MATERIALS AND METHODS

An experimental or intervention design was carried out due to the fact that the researcher intervenes in modifying the conditions under which this research was conducted. Rat strains of BALB/c and Wistar were used from 8 days of birth to their reproductive age. The sample size consists of 50 rats each of the BALB/c strain and Wistar strain. Random probabilistic sampling method was used in blocks of 10 rodents. Rats of both strains BALB/c and Wistar that were though induced with bufo-toxin but still have epilepsy were immediately administered with boosted bufo-toxin in the selected power (7 CH). Rats of both strains BALB/c and Wistar though induced with bufo-toxin but still show symptoms and mortality of epilepsy were immediately administered with boosted bufo-toxin in the selected power.

Rats were maintained in polycarbonate rooms with chips, food and water, such rats were fed with kibbles and their health was constantly checked. They remained healthy in space conditions and proceeded to inoculation of the toxin, placing each rat in a separate space from the rest of the rats.

Two frogs from the family of Bufonidae were bought in order to carry out the extraction of the bufo-toxin; such toads were placed in a bowl with some water so they

Table 2. Gender distribution in both rat strains.

Gender	BALB/c strain (%)	Wistar strain (%)
Male	80	22
Female	20	78

Direct source: Physical-chemical-biological laboratory, UMG, Marist University of Guadalajara, and Biology laboratory, La Salle.

were wet. Apple slices were added to their bowl so that they could attract other insects and they could eat. The toxin is extracted under controlled conditions from the toads" glands located on the sides of their necks in the head region. The toxin was stored in a test tube, previously sterilized by autoclaving and diluted in alcohol for preservation, such tube was saved at -4°C, it was warmed in warm water for its use. Each rat was weighed to track and observe whether or not the weight was unrelated to the amount of toxin administered. In a sterile area of the laboratory, the rat was placed and given 20 units of the medicine through an insulin syringe; such amount of medicine was previously hinted in the pilot test which at the same time showed that such medicine provoke more symptomatology without compromising the life of the rat in a short period of time.

The drug 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 of 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 than 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 min and then 15 drops were left in their drinking water. The optimal standardized time for the symptomatology was observed within 10 min 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. Rats previously inoculated with the toxin and then with the drug at 7 CH and which survived were administered again with the toxin and observations were recorded. When the experiment was accomplished, the results were analyzed in SPSS-15.

BALB/c and Wistar rat strains of 4 - 45 weeks old that are under control in the laboratory and which did not show suggestive symptoms of disease were included in this study.

Rats less than 4 weeks old and over 45 weeks old with suggestive symptoms of some disease were not included in this study.

This study excludes those rats that were not inoculated with the toxin and was not 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.

RESULTS

The results of this investigation made reference to the laboratory work performed with rodents of 50 BALB/c strain and 50 Wistar strain, from which the BALB/c strain for 40 rats (80%) were female and 10 (20%) were male, while for the Wistar strain, 39 (78%) were female and only 11 (22%) were male (Table 2).

Regarding the age of rodents used in this research, for BALB/c strain it was between 14 and 16 weeks of age on average. The results obtained with the BALB/c strain denote that when administering between 10-15 units of the toxin, the symptoms take about 30 min to appear, but it is undetectable in some cases. Therefore, 20 units were administered and the symptomatology increased its manifestation, approximately 1 to 10 min effect is clearly observed. Initially, they stay quiet with bristling hair and tremors in their limbs. In a few minutes, the tremor in their muscles is widespread, and it was observed that the eyes both bulged and started with convulsive movements of limbs. For instance, the movement was done inwards as the hands of a clock in the left upper extremity, then to the right, then to the left lower extremity, and finally to the right lower limb, showing the same mobility pattern in the tail. During the convulsive phase, rats take a supine position; on average, they die after 30 min, except in some cases where death of the rodent was not verified, but apparently can occur between about 2 and 15 h.

The autopsies revealed that brain stroke occurs; myocardial infarction occurs as the main cause of death,

Table 3. Percentage of symptoms with greater presence in rodents with bufotoxin administration.

Symptom	Percentage	Symptom	Percentage
Pilo-erection	85.4	Tearing	20.8
Hyperventilation	54.2	Toxin effect of defecates	20.8
Hypersensitivity to noise	45.8	Toxin effect of urine	20.8
Scratches in the head	41.7	Hyperactivity	18.8
Trembling jaw drooling	37.5	Sialorrhea	14.6
Stupor	35.4	Clonic movements of the head	12.5
Whiskers extended forward	29.2	Difficulty opening the eyes	10.4

Direct source: Physical-chemical-biological laboratory, UMG, Marist University of Guadalajara, and Biology laboratory, La Salle.

Table 4. Percent findings at necropsy.

Finding	Percentage	Finding	Percentage
Heart attack	100	Splenomegaly	32
Hepatomegy	84	Testicular hypertrofy	11
Distended colon	68	Collapsed lung	5
Stroke	53		

Direct source: Physical-chemical-biological laboratory, UMG, Marist University of Guadalajara, and Biology laboratory, La Salle.

Table 5. Comparison between BALB/c and Wistar strains regarding survival.

Strain	Living with toxin and drug	Toxin and drug deaths	Toxin without drug deaths	Natural death
BALB/c	25 (50%), 19 (38%)	6 (12%)	0	0
Wistar	23 (46%)	7 (14%)	19 (38%)	1 (2%)

apparently the effect is neurotoxic, but is finally followed by myocardial infarction. Regarding the Wistar strain, the same symptoms were observed and in this case they were quantified (Tables 3 and 4).

In reference to the Wistar strain, it was observed that symptoms that manifested during the pilot phase were similar to the BALB/c strain. It was found that 2 rats (4%) showed no symptoms and a rat presented 23 symptoms, 17 presented symptoms of 3 to 6 which represents 34% of the sample. In reference to the necropsy performed to the rodents, a necropsy was performed to a rat, even when its death was not related to administration of the toxin; at 31 (62%) no necropsy was performed. Autopsies indicate neurotoxic effects, especially cardiovascular effects on their liver (Tables 3 and 4). In the Wistar strain, convulsions occur very rapidly as compared to the BALB/c strain. Apparently, the Wistar strain is more sensitive to the toxin, but there is some agreement between the two strains as compared to the effect observed after the administration of the toxin.

The results about the survival of rats which were inoculated with the toxin and which on the onset of symptoms were given the boosted bufo-toxin in the proposed experiment, showed 44 rats (88%) of which 25 survived representing 50%. The sample denotes a significant recovery in all their physiological functions after the first shots of the medicine and continued well without any apparent problems. 19 (38%) of the remaining rats that survived had an injury or loss of hair or zone of alopecia in the area where the toxin was inoculated. 6 (12%) rats died regardless of receiving the toxin or even 12 h of being under the administration of the homeopathic medicine.

The results about the survival of the 50 rats belonging to the Wistar strain show that rats representing 23 (46%) showed a significant recovery in all their functions after spending 12 h under the administration of the homeopathic medicine, but all of them had a ulcerative lesion on the thigh where they were inoculated. 7 (14%) rats died even if they received the toxin and the medicine, but 19 (38%) of the rats died without being first administered with the medicine; one rat died of natural causes even without receiving the toxin (Tables 5 and 6).

In the rats that survived the effects of the toxin and that

Table 6. Frequency and percentage of survival under the effect of the energized bufotoxin 7 CH including necropsies.

Explanation of the effect	Frequency	Percent
Natural death	1	2
Rats were killed by the effects of the toxin, due to the fact that the medication was not administered	19	38
Rats were killed by the effects of the toxin, even though the medication of 7 CH was administered	7	14
Rats that survived the dose of toxin and reacted to the drug effect of 7 CH	23	46
Total	50	100

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

were under the homeopathic medicine, one of them presented an ulcerative lesion in the left lower extremity which was so corrosive that caused it to lose mobility and sensitiveness; however, the rat continued living without any further complication, until it was decided to sacrifice it three days later.

None of the rats, after being submitted to convulsion crises and administered the medicine, showed an epileptic episode. This shows a confirmation of the hypothesis of this research.

In general, it was observed that all symptoms of rats when presenting epileptic crises was that they first stare for a few seconds, after that they presented tonic clonic movements which began in the upper extremities from right to left in order to reach the tail, which rotated anti-clockwise in a few rats. The symptoms lasted an average of 1-15 min, but the homeopathic medicine was inoculated.

It was observed that the frequency of survival regarding the rats" gender was that 65% are females and 35% are males, which survived the toxin under the administration of medication for treatment.

When comparing the rats which received the toxin, the medicine and the ones that died, as did those who received the drug and toxin and survived with a p-value of 47,958 and a $p \leq 0.05$ of 000, the study indicated that there is significant difference suggesting that the homeopathic medicine shows a significant effect on the reversal of the symptoms presented, although a necrotic lesion was presented on the area where the toxin was inoculated (Table 6).

DISCUSSION

The achievements in the laboratory regarding the knowledge generated about the effect of the bufo-toxin in rodents of the BALB/c strain as in the Wistar strain are of great importance, as they represent an option to test drugs against epilepsy and myocardial infarction because these diseases represent the leading cause of death under the effect of bufo-toxin. Studies on the poisoning of this substance on dogs, which live approximately 40 min show that among the symptoms described, there exists a negative effect over the heart. This occurs because it manifests a negative deflection of the complex QRS, atrial fibrillation, and death if the animal is not treated. The symptoms are classified into three: mild, moderated and severe. Among those last symptoms, convulsions arise without giving any explanation of how they are and how they disappear. Apparently there are no publications where the effect of this substance is described and this denotes an area of opportunity for further research on epilepsy and not on cardiology. The composition of this substance has even 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 bufo-toxin or poison from the bufo toad (Moyano et al., 2009).

Note that in the publication of Resendis (1991) entitled *Action on bufo frog brain dopamine* the study was performed only with 40 rats which were divided into 4 groups of 10 rats each and where the drug effect was studied in doses boosted at 6, 9 and 15 CH to study the effect of cerebral dopamine. The cerebral dopamine detected that the drug produces decreased brain dopamine in inverse proportion to the homeopathic revitalization used. For instance, the most notable decrement was achieved with the highest boosted medicine showing a significant effect of bufo frog; however it is important to establish that the medicine acquired was previously prepared, opposite to the toxin employed in this experiment which was directly prepared from the toxin. It should be noted that the medicine was prepared in the laboratory by previously taking a drop of the diluted toxin from a mixture containing ethyl alcohol of 96°C and distilled water to a concentration of 70% alcohol in water from which a drop was taken and mixed with 99 drops of ethyl alcohol shaking in order to boost it through 100 strong strokes in a telephone directory previously prepared for this goal. It was not possible to prepare the substance according to the rule 8 of the pharmacopoeia which indicates that such action must be

carried out by grinding, and shaking the dilution, such procedure was not possible due to the lack of contamination-free mortars.

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 verge of finding an effective cure against this disease.

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 are observed hence it is important to note that both strains have an effect on the toxin.

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 when prepared under the conditions described above, meet a demonstrable impact on the conditions under which the experiments were conducted.

One of the limitations observed and manifested in this study is 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 which are 4 weeks old (the youngest) and 40 weeks old (the oldest).

As part of the observed effect after administering the boosted toxin, it was possible to observe that after 48 h, the presence of a degenerative ulcerative necrotic lesion as an area of alopecia is where the toxin was inoculated. This situation led to the makeup of two crop batches of the toxin in trypticase soy agar which until 72 h 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. This can be said because experiments were carried out under controlled conditions.

Note that in strain BALB/c with an average age of 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) 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 in these experiments. It was not possible to determine the presence of the bufo-toxin 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 this study.

Conclusions

The following conclusions were drawn in this study:

1. The effect of the toxin is lethal.
2. Average rats that were 16 weeks old responded more clearly to the effect of the boosted toxin and those which were 40 weeks old were the ones which more often survived the impact of the toxin and revealed more clearly induced epilepsy.
3. The administration of the bufo-toxin allows this research to have an animal model to study epilepsy and myocardial infarction, and even to find the therapeutic effect in animals, plants or minerals as it represents a model for the study of any type of medication.
4. The boosted toxin under the prepared conditions has an important effect regarding the reversal of symptoms that resulted from the administration of the bufo-toxin.
5. The results obtained from this research allow strengthening and verification of the fact that the boosted substance has an important effect over a specific pathogenesis.
6. There is significant difference between rats that survived and those which died under the effect of the toxin and medication, plus symptoms of epilepsy as well as other symptoms characterized by the experiment which were not manifested and observed again in both strains.
7. The rats that survived and which were inoculated on a random selection of 5 rats of the BALB/c strain did not present any characteristic symptoms with doses of up to 10 times superior to the initial inoculation. Thus, an effect was shown on the immune system of the rat.

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