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# The Influence of the Hydroalcoholic Extract of *Polypodium vulgare L.* on Pentylenetetrazole-induced Seizures and Its Comparison with the Impact of Sodium Valproate in a Rat Model

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## Abstract

**Objective:** Epilepsy affects over 1% of the global population. *Polypodium vulgare L.* has emerged in studies, suggesting its potential antiepileptic effect. This study evaluated the anticonvulsant efficacy of the hydroalcoholic extract of *Polypodium vulgare L.* in a pentylenetetrazole (PTZ)-induced epilepsy model in rats. This research aimed to determine the optimal dosage for delaying seizure onset and reducing seizure severity. This study investigated whether the active compounds offer a viable alternative for epilepsy management, mainly through their potential interaction with GABAergic mechanisms.

**Methods:** We randomly selected four groups of 10 and 2 groups of 8 male Wistar laboratory rats. The reference and control groups received PTZ and distilled water, respectively. In contrast, experimental groups 1 and 2 received the hydroalcoholic extract of *Polypodium vulgare L.* at doses of 300 and 500 mg per kg of body weight. Experimental groups 3 and 4 received 150 and 300 mg per kg dose. Chemical kindling was induced in all groups via intraperitoneal injection of PTZ. Data analysis was conducted using Statistical Package for the Social Sciences version 20 software.

**Results:** The hydroalcoholic extract of *Polypodium vulgare L.* exerted a pronounced mitigating effect on convulsions induced by PTZ administration, specifically at a dosage of 300 mg/kg of body weight. It effectively prolonged the time necessary for seizure onset.

**Conclusion:** The administration of a 300 mg dose of the hydroalcoholic extract of *Polypodium vulgare L.* demonstrated superior efficacy compared with its 500 mg counterpart and both the 300 and 150 mg doses of sodium valproate in addressing PTZ-induced epilepsy. The results suggest that the hydroalcoholic extract of *Polypodium vulgare L.* has promise as an effective treatment for epilepsy.

**Keywords:** Polypodium vulgare, epilepsy, sodium valproate, valproic acid, kindling

## INTRODUCTION

Neurological disorders are the second leading cause of global mortality, accounting for 11.6% of disability-adjusted life years and contributing to 16.5% of all deaths.[1] Epilepsy is a notable neurological disorder characterized by its prevalence, and the risk of its occurrence notably increases with the progression of age.[2] The global prevalence of this disease has been reported to encompass nearly 46 million individuals.[3] Epilepsy is a chronic central nervous system (CNS) disorder that affects individuals of all ages and exhibits a global distribution. Despite the existence of various disease mechanisms that can contribute to epilepsy, the cause remains unknown in approximately 50% of cases globally.[4] Seizures resulting from epilepsy are linked to various complications and issues, including diminished quality of life,[5] job-related challenges and difficulties,[5] cognitive disorders,[6] depression,[7] and bone fractures.[8] Therefore, given the numerous consequences associated with epilepsy, the management of epilepsy should prioritize the prescription of anticonvulsant drugs, addressing the underlying causes, and mitigating the systemic consequences to prevent damage induced by seizures.[9,10]

In recent years, numerous antiepileptic drugs have been tested and utilized, with over 80% of patients experiencing epileptic seizures receiving at least one of these medications.[11] Sodium valproate is a commonly prescribed medication widely used for the treatment of epileptic seizures. Nevertheless, the use of this drug is linked to various side effects, including digestive problems (nausea, vomiting, diarrhea, etc.), drowsiness, headache, and dizziness. Several complications have been identified, leading to non-adherence by patients.[12]

The most common animal model is chemical kindling, which is used to study epilepsy. The kindling model shares strong similarities with different types of human epilepsy.[13] When applied repetitively and intermittently, a subconvulsive stimulus (either chemical or electrical) leads to a process called Kindling. It can eventually produce full-blown convulsions.[14] Goddard[15] first proposed the kindling model in the late 1960s. The kindling model is usually used for seizure development and epilepsy. Then, the behavioral involvement and duration of induced seizures increase repeatedly.[16] PTZ kindling finally leads to potentially irreversible long-lasting changes in the structure of neuronal networks.[10]

Nature is a rich source of diverse biological and chemical substances, with numerous plants exhibiting anti-anxiety, analgesic, anti-depressant, and anti-convulsant effects.[17] Among these plants, *Bisfaij* (*Polypodium vulgare L.)* stands out, and its antiepileptic effects have been mentioned in a few studies.[17,18] *Bisfaij* plant has been reported to possess various antioxidant, antimicrobial, analgesic, and antibiotic characteristics.[19] Additionally, it has been demonstrated that *Polypodium vulgare L.* exerts an effect on nerve activity by reducing the activity of the CNS.[18]

*Polypodium vulgare L.* contains organic substances such as resin, tannin, steroids, flavonoids, alkaloids, glycosides, proteins, and reducing sugar. Moreover, studies have shown that this plant also contains minerals such as calcium, magnesium, potassium, iron, sulfur, and chloride.[20,21] It has been reported that *Polypodium vulgare L.* exhibits a protective effect against drug-induced catalepsy. This suggests that it may enhance the transmission of dopamine in the CNS and could be explored for its potential impact on Parkinsonism disorders. Furthermore, considering the activity and reduction of the effect of *Bisfaij* plant extract on the CNS, the possibility of an antiepileptic impact on this plant has also been raised.[21,22] On the one hand, the butyric acid present in the stem and root of *Bisfaij* serves as a precursor to the neurotransmitter gamma-aminobutyric acid (GABA). On the other hand, sodium valproate exerts its anticonvulsant effects by increasing the levels of this neurotransmitter. Therefore, it seems plausible that the bisfaij stem extract, which contains butyric acid, could potentially contribute to seizure control through a similar mechanism.[23]

Pentylenetetrazole (PTZ) is employed as a selective antagonist of $GABA_A$ receptors, whereas phaclofen serves as a selective antagonist of $GABA_B$ receptors.[24] GABA receptors function as target sites for drugs such as benzodiazepines. Classical benzodiazepines exert their therapeutic effects by binding to the benzodiazepine site of the GABA receptor, promoting chloride flow through the ion channel complex. However, this mechanism is linked to a broad spectrum of side effects.[25] Considering that antiepileptic drugs often require prolonged usage, sometimes for a lifetime, and acknowledging the potential side effects and risks associated with chemical drugs, their extended use may result in adverse effects and even drug poisoning. However, these limitations could compromise the efficacy of these treatments in achieving the desired therapeutic outcome.[26]

Considering the medicinal and therapeutic properties traditionally attributed to the *Bisfaij* plant for the treatment of epilepsy, as well as the side effects associated with chemical drugs commonly used for this purpose, this study explored the therapeutic effects of the *Bisfaij* plant in alleviating symptoms and treating epilepsy.

**MAIN POINTS**

- One strength of our research lies in the use of the animal kindling method. To enhance the scope of our investigation, we administered two distinct doses of *Polypodium vulgare L.* extract and compared them with a standard anticonvulsant medication commonly used for this condition.
- Another highlight of our study was the thorough observation and precise recording of animal behaviors, which ensured detailed behavioral analysis.
- Additionally, a comparison between intervention groups treated with the hydroalcoholic extract of *Polypodium vulgare L.* and those treated with an anticonvulsant drug revealed a novel aspect of our approach.

## METHODS

### Plant Collection

The study was approved by the Fasa University of Medical Sciences Research Ethics Committees (approval number: e-9210, date: 01.10.2013). The plant was obtained from a local perfumery in Shiraz city and was subsequently submitted to the Department of Pharmacy at Shiraz University for purity confirmation. The pharmaceutical department endorsed and assigned voucher number PM12983- to this plant, identifying it as *Polypodium vulgare L.* of the family *Polypodiaceae*.

### Extraction

The Department of Pharmacy at Shiraz University prepared the hydroalcoholic extract. After cleaning and drying in the air, the resulting solution was ground and powdered. The second step in preparing hydro-alcohol extract was percolation with 70% ethanol, which was poured into the percolator device. The extract was then concentrated using a rotatory evaporator and dried in a vacuum oven. The Bisfaij hydroalcoholic extract was diluted with distilled water to produce two different concentrations for this study. The *Bisfaij* group 300 received an oral gavage of 300 mg/kg of body weight, whereas the *Bisfaij* group 500 received a concentration of 500 mg/kg. These doses are administered to rats.

### Laboratory Animals

For this study, 56 Wistar male rats, aged between 2 and 3 months, were randomly chosen. The rats' weights fell within the range of 180 to 220 g. The rats were housed in containers and provided ample food and water. They were subjected to a 12-hour light and 12-hour dark cycle with suitable temperature and humidity conditions.

### Pentylenetetrazole

PTZ with the commercial formula C6H10N4 was procured from Kiagene (IRAN) for use in this study.

### Sodium Valproate

The pharmacy dispenses sodium valproate in 500-mg tablets. Subsequently, it undergoes crushing using a designated mortar and is diluted in two distinct ratios with distilled water for application in the research investigation.

***In vivo* Treatments**

No manipulation was executed on the control group. The reference group was administered seven complete doses of PTZ at varying levels (25, 30, 35, 40, 45, and 50 mg per kilogram of rats' weight). These doses were dissolved in distilled water at a volume percentage of 1% and administered through subcutaneous injections every other day.

The *Bisfaij* group 300 received 300 mg/kg of the Bisfaij hydroalcoholic extract for body weight 30 minutes before PTZ administration. This dosage was prepared by diluting it with distilled water to a concentration of 1%.

*Bisfaij* group 500 received a dosage of 500 mg/kg hydroalcoholic extract of *Bisfaij* of body weight 30 minutes before the administration of PTZ. This dosage consisted of the hydroalcoholic extract of *Bisfaij*, which was orally administered using a gavage syringe and diluted to 1%. Similarly, sodium valproate groups 150 and 300 received 150 and 300 mg sodium valproate, respectively, diluted with distilled water at a concentration of 1% per kilogram of body weight 30 minutes before PTZ injection. Oral administration was performed using a gavage syringe. Subsequently, the movements of the rats within 30 min were meticulously recorded by a camera and subjected to behavioral assessments focusing on seizure severity and seizure onset time.

The severity of seizures was evaluated according to the following criteria:[27]

- *Score 0: Absence of any observable reaction.*

- *Score 1: Facial and ear twitches.*

- *Score 2: Myoclonic jerks of the body.*

- *Score 3: Clonic movements involving the front limbs.*

- *Score 4: Generalized clonic seizures, leading to the animal turning to one side.*

- *Score 5: Generalized tonic-clonic seizure.*

- *Score 6: Equivalent to animal demise.*

Ultimately, all rats were anesthetized via intraperitoneal injection of 80 mg/kg ketamine (Sigma Aldrich Co.) and sacrificed under the Helsinki Declaration of 1975.

**Statistical Analysis**

Data are presented as means±standard error of the mean. The comparison between groups was done with a one-way analysis of variance (ANOVA) test, followed by Tukey's post-hoc test. P values <0.05 were considered as statistically significant. The data were analyzed using IBM Statistical Package for the Social Sciences software version 22 (IBM Co. Armonk, NY). Additionally, Microsoft Excel (version 2013) was used to create tables and graphs to represent the results visually.

**RESULTS**

Upon examining the relationship between the average seizure onset time across the studied groups, the results indicated a lack of significant differences in seizure onset time between the second and third injections (p value >0.05). However, in the fourth iteration, the findings revealed a substantial increase in seizure onset time for the *Bisfaij* 300 (p value=0.007) and *500* (p value=0.004) compared to group sodium valproate 300. This disparity did not reach significance for any of the groups compared to the reference group. In the fifth injection round, a notable reduction in the mean and standard deviation of seizure onset time was observed in the sodium valproate 150 group compared with the bisfaij 300 group, and this difference was statistically significant (p value=0.006).

In the sodium valproate 150, the seizure onset time was 7.50±9.38 minutes. However, even during this time, there was no significant reduction in the seizure onset time compared with the reference time. In the sixth injection, groups of sodium valproate 150 and sodium valproate 300 exhibited a significant decrease in seizure onset time compared with group *Bisfaij* 300 (p values of 0.03 and 0.02, respectively). Moving on to the seventh injection, group *Bisfaij* 300 displayed a prolonged seizure time compared with group sodium valproate 150, and a statistically significant difference was observed between these two groups (p value=0.002). Furthermore, significant differences were reported between group *Bisfaij* 300 and the reference group and between group sodium valproate 300 and the reference group. Detailed information on seizure onset and its intergroup relationships is presented in Figure 1.

In the current investigation, seizure intensity was assessed using a rating scale ranging from 0 to 6, and the study aimed to explore the correlation between average seizure intensity across different groups. In the second injection round, the findings indicated no significant difference in seizure severity among the groups (p value >0.05). In the third injection round, the seizure intensity in group *Bisfaij* 300 was notably lower than in the sodium valproate 150 (p value=0.03). However, this difference was not statistically significant when comparing *Bisfaij* 300 with the reference group. During the fourth injection round, the intensity of seizures in the Bisfaij 500 group was significantly lower than that in the sodium valproate 150 and 300 (p value=0.01). There were no significant differences in seizure severity among the intervention groups for the fifth and sixth injections. However, in the seventh injection, the intensity of seizures in group *Bisfaij* 300 was significantly lower than in the reference group and the *Bisfaij* 500 and sodium valproate 150. The graphical representation illustrates that group *Bisfaij* 300 exhibited the lowest seizure intensity during the fifth and seventh injections compared with the other groups (0.7±0.47 and 0.6±0.42, respectively) (Figure 2).

**DISCUSSION**

Sodium valproate is a relatively side-effect-free compound routinely used.[28] However, its side effects, especially its teratogenicity, are attracting increasing attention. The term "fetal valproate syndrome" indicates that multiple organ malformations have been observed.[29] In one study, the authors further confirmed the teratogenetic effects of sodium valproate on fetuses when pregnant mice were exposed to this compound during the gestational period of organ formation, i.e., gestational day 6 to day 9 in mice.[30] A hypoplastic right ventricle has been reported in a fetus exposed to valproate.[31]

Sodium valproate has a broad spectrum of anticonvulsive properties. The most commonly reported side effects involve

the gastrointestinal tract, including nausea, vomiting, abdominal cramps, and diarrhea. Sodium valproate can also have transient effects on the CNS, such as drowsiness and sedation. Most patients report an overall feeling of increased alertness. Sodium valproate inhibits the secondary phase of platelet aggregation. Consequently, prolonged bleeding times and thrombocytopenia have been reported in some cases, primarily in children.[32]

The advent of newer anticonvulsants has expanded therapeutic options, with new drugs showing fewer interactions and reduced hypersensitivity compared to older medications. However, these newer drugs have not been effective in decreasing the prevalence of drug-resistant epilepsy or in preventing epilepsy in high-risk individuals. There is an urgent need for renewed efforts in antiepileptic drug development to discover more effective treatments for drug-resistant epilepsy, including severe and catastrophic forms.[33]

The use of complementary and alternative medicine, including among patients with epilepsy, is increasing. Herbal medicines, one of the most popular alternative medicines, are perceived by many users as safe and effective.[34] Although herbal treatments are widely employed in epilepsy management, solid evidence supporting their efficacy and toxicity profiles is lacking. Therefore, herbal remedies must undergo evidence-based evaluation.[35]

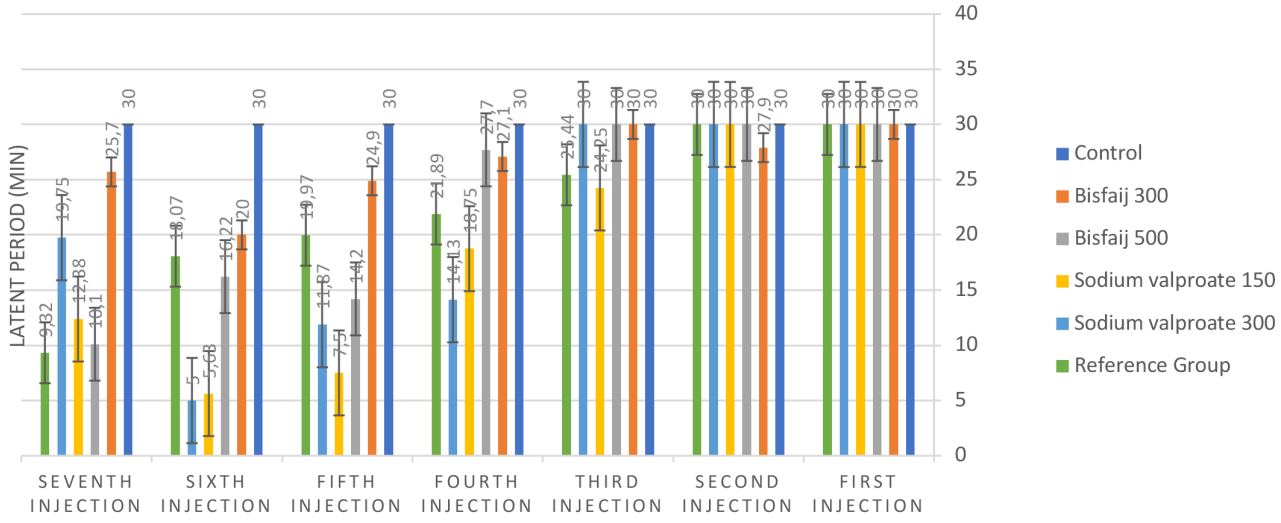
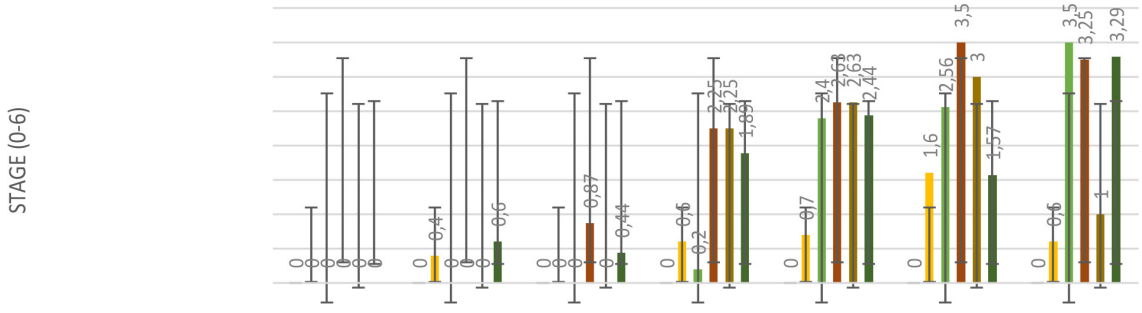



**Figure 1.** Comparison of seizure onset times in the studied groups from the first to seventh injection of pentylenetetrazole
- In the seventh injection, 19.75 and 25.7 values indicate the significance of the difference between the mean values of each group and the reference group
- In the seventh injection, values of 10.1, 12.38, and 19.75 indicate the significance of the difference between the mean values of each group and the control group




| | First Injection | Second injection | Third Injection | Fourth Injection | Fifth Injection | Sixth Injection | Seventh Injection |
|---|---|---|---|---|---|---|---|
| Control Group | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bisfaij 300 | 0 | 0,4 | 0 | 0,6 | 0,7 | 1,6 | 0,6 |
| Bisfaij 500 | 0 | 0 | 0 | 0,2 | 2,4 | 2,56 | 3,5 |
| Sodium valproate 150 | 0 | 0 | 0,87 | 2,25 | 2,63 | 3,5 | 3,25 |
| Sodium valproate 300 | 0 | 0 | 0 | 2,25 | 2,63 | 3 | 1 |
| Reference Group | 0 | 0,6 | 0,44 | 1,89 | 2,44 | 1,57 | 3,29 |

**Figure 2.** Comparison of seizure severity in the study groups from the first to seventh injection
- In the seventh injection, 0.6 and 1 indicate the significance of the difference between the mean value of each group and the reference group
- In the seventh injection, the 3.5, 3.25, and 3.29 values indicate the significance of the difference between the mean values of each group and the control group

The current study, employing a behavioral approach, investigated the impact of the hydroalcoholic extract of *Polypodium vulgare L. (Bisfaij)* on PTZ-induced epilepsy using the kindling method. The findings suggest that administration of a 300 mg dose of *Polypodium vulgare L.* extract per kilogram of body weight and time at the onset of seizures demonstrated greater efficacy regarding both the onset time and intensity of convulsive attacks than other intervention groups. The study's outcomes regarding the average seizure onset time revealed that group 1 exhibited a longer average seizure onset time than the other intervention groups. Consequently, group 1, which received a 300 mg dose of *Polypodium vulgare L.* hydroalcoholic extract, demonstrated more favorable results than the other drug groups and notably outperformed group 2. Moreover, the mean intensity of epilepsy was lower in group 1 than in the other four groups.

In light of these findings, it can be concluded that the therapeutic efficacy of the hydroalcoholic extract of *Polypodium vulgare L* is more pronounced at a dose of 300 mg/kg than at a dose of 500 mg/kg. Additionally, behavioral observations from this study indicate that the 300 mg dose of hydroalcoholic extract of *Polypodium vulgare L.* resulted in a lower average epilepsy score among the animals in the intervention groups. This suggests a more significant reduction in the intensity of explosive attacks than in the other intervention groups.

Based on these findings, it can be deduced that the therapeutic effects of the 300 mg dose of *Polypodium vulgare L.* surpass those of the 500 mg dose. Within the sodium valproate intervention groups, the study results indicated that a dose of 300 mg per kilogram exhibited greater therapeutic efficacy than a 150 mg per kilogram dosage concerning the onset time of seizures and intensity of convulsive attacks in animals. Ultimately, the 300 mg dose of *Polypodium vulgare L.* demonstrated superior effectiveness to its 500 mg dose and the 300 and 150 mg doses of sodium valproate in treating PTZ-induced epilepsy. This superiority was reflected in the reduction of convulsive attack intensity and its delayed onset.

The research on the antiepileptic effects of the *Bisfaij* plant is notably limited. Phytochemical investigations have revealed that the rhizome of the *Bisfaij* plant harbors various medicinal compounds, such as *Polypodin A* and *Polypodin B*. These compounds exert protective effects against neurological and neurodegenerative disorders. Furthermore, it has been noted that the caffeine content of *Bisfaij* exhibits a stimulating effect on adrenergic receptors and possesses antioxidant properties. While these findings suggest the potential therapeutic benefits of *Bisfaij*, further comprehensive studies are warranted to elucidate and validate its antiepileptic properties.[36]

One study suggested that *Polypodin A*, a compound found in the *Bisfaij* plant, reduces the neuroleptic effect of apomorphine in laboratory animals. This implies that *Polypodin A* may modulate or mitigate the neuroleptic effects induced by apomorphine in experimental settings.[37] GABA is a gamma-amino acid with a molecular structure that includes a butanoic acid backbone, where the amine group is substituted at the C-4 position. GABA is a neurotransmitter that plays a pivotal role in human metabolism. It serves as an inhibitory neurotransmitter in the CNS, exerting influence over diverse physiological processes, such as regulating neuronal excitability and modulating mood. GABA's inhibitory actions contribute to maintaining a balance in neural activity and preventing excessive neuronal firing.[38] Given the existence of butyric acid within the rhizomes of the *Bisfaij* plant[18] and its structural correlation with the neurotransmitter GABA, there may be a linkage between the presence of this compound and the mechanism underlying the anticonvulsant effects attributed to the *Bisfaij* plant. This observation paves the way for future research investigations, emphasizing the importance of exploring this connection in subsequent studies.

### Study Limitations

A notable strength of this study lies in its use of the animal kindling method, which provides a robust foundation for investigations. Additionally, including two distinct doses of the *Bisfaij* plant juxtaposed against conventional anticonvulsant drugs for disease management adds depth to the research design. However, certain limitations should be acknowledged. The present study did not examine serum factors implicated in epilepsy development, suggesting the need for subsequent laboratory analyses in future research.

## CONCLUSION

This study demonstrated that the hydroalcoholic extract of *Polypodium vulgare L.* (*Bisfaij*) has significant therapeutic potential for treating PTZ-induced epilepsy in animal models. These findings suggest that *Polypodium vulgare L.* may offer a promising alternative treatment for epilepsy, possibly because of its active compounds, such as *Polypodin A* and caffeic acid, which may interact with GABAergic mechanisms. However, further studies are needed to elucidate the precise mechanisms of action and explore this extract's potential clinical applications in epilepsy management. The mechanism underlying the anticonvulsant effects of bisfaij plants remains unexplored in this study, representing another avenue for future investigation.

### Ethics

**Ethics Committee Approval:** The study was approved by the Fasa University of Medical Sciences Research Ethics Committees (approval number: e-9210, date: 01.10.2013).

**Informed Consent:** Animal experiment.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.S., A.E.B., M.S., Concept: S.S., M.S., Design: S.S., M.S., Data Collection or Processing: A.E.B., M.S., Analysis or Interpretation: A.E.B., M.S., Literature Search: S.S., A.E.B., M.S., Writing: S.S., A.E.B., M.S.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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