**Effectiveness of Hypertonic Saline, Polidocanol, and Glycerol as Sclerosing Agent: An Experimental Study in Javan Rabbits (Lepus nigricollis)**

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**ABSTRACT**

**INTRODUCTION:** Therapeutic approaches to varicose veins include sclerotherapy, laser ablation, and surgery. Using sclerosing agents such as hypertonic saline, polidocanol, and glycerol can be an option in cases of recurrent varicose veins. This study aimed to assess the effectiveness of hypertonic saline, polidocanol, and glycerol as sclerosant agents in an in vivo study.

**MATERIAL AND METHODS:** This was an experimental study on 24 Javan rabbits, divided into three treatment groups: hypertonic saline (group I, n=8), polidocanol (group II, n=8), and glycerol (group III, n=8). All animals received treatment by injection into the vein behind the ear, then vein damming 10 minutes later. Punch tissue samples for standard histopathological examination were taken from blood vessels at 1 hour, 24 hours, 7 days, and 45 days post-treatment across all groups. The histopathology changes were scored based on inflammation, proliferation, luminal narrowing, and fibrosis.

**RESULT:** No differences were observed in the degrees of inflammation, proliferation, luminal narrowing, or fibrosis at different observation intervals. However, a significant and positive correlation was found between inflammation, vascular proliferation, and fibrosis with all sclerosing agents (p<0.005). No significant correlation exists in the scoring of luminal narrowing among any sclerosing agent (p>0.005).

**CONCLUSION:** Hypertonic saline, polidocanol, and glycerol demonstrated comparable efficacy as sclerosing agents in vivo concerning fibrosis, vascular proliferation, and inflammation.

**Keywords**  
Varicose Veins, Sclerosing Solutions, Glycerol, Sclerosing Solutions.

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**INTRODUCTION**

Varicose veins are a manifestation of chronic venous disease, including reticular veins, telangiectasia (spider veins), hyperpigmentation, venous ulcers, lipodermatosclerosis, and edema.\(^1\) Varicose veins have a frequency of 33% of the entire population, with predominantly occurring in the lower leg.\(^1,2\)

Therapeutic approaches to varicose veins consist of sclerotherapy, laser ablation, and surgery. As reported by the European Society for Vascular Surgery, sclerotherapy is still the main treatment for varicose veins that are not caused by saphenous vein insufficiency.\(^3\) Sclerotherapy has less effectiveness in contrast to endovascular or surgical therapy, with a recanalization rate of 8.5% over six months.\(^4\)–\(^7\)

The use of sclerosing agents such as hypertonic saline, polidocanol, and glycerol can be an option in cases of recurrent varicose veins because they can be used as adjuvant therapy and can be applied repeatedly at a cheaper cost than other therapies.\(^6\) Goldman and Guex showed that hypertonic saline with or without heparin generates a great result with minimal negative effects.\(^8\) Polidocanol is a sclerosing agent that has been reported to have up to 94% efficacy at 4 years after treatment.\(^9\) Glycerin appears to be a more successful treatment for...
Tissue Biopsy and Histopathology Examination

Following the intervention, each experimental animal group was divided equally into four subgroups according to four observation periods: 1 hour, 24 hours, 7 days, and 45 days. At each time point, tissue samples from four ears (two animals) were harvested. The samples were collected using a 4-mm punch biopsy procedure under general anesthesia with ketamine. Then, for histological analysis, the samples were fixed in a 10% formaldehyde solution and stained with hematoxylin and eosin. All histopathological changes in each tissue were noted, including inflammation, proliferation, luminal narrowing, and fibrosis. These changes were graded by the previous study scoring system. The final scoring was based on the best score for each histopathological change at all observation periods.

Statistical Analysis

Statistical data analysis was performed using SPSS version 17.0 (Armonk, NY: IBM Corp.) with a 95% confidence interval (α=0.05). The quantitative data obtained are expressed as mean ± standard deviation. The Kruskal-Wallis test was used to compare the histopathological parameters between treatment groups. The Spearman rank correlation test was used to determine the relationship between each sclerosing agent's administration and each histopathological parameter at various measurement times. A p-value of less than 0.05 was considered significant.

RESULTS

Frequency Distribution of Histopathological Changes Based on Treatment Groups

The frequency distributions for the degree of inflammation, proliferation, luminal narrowing, and fibrosis are presented in Table I. For the inflammation (Figure 1A), the glycerol group showed inflammation in all specimens after 45 days, whereas the polidocanol group showed inflammation at 24 hours, 7 days, and 45 days. In terms of vascular proliferation (Figure 1B), the majority of samples showed inflammatory cells covering 1 high-power microscopic field (HPF) in the saline, glycerol, and

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polidocanol groups at all measurement times. For the luminal narrowing (Figure 1C), the majority of samples did not show luminal narrowing in the group that received saline, and two of 16 samples showed narrowing of ≤25% in the first hour and after 45 days. The glycerol group showed variation in narrowing, with a narrowing of 76–100% at 24 hours. The polidocanol group also showed variations in narrowing, and narrowing of 76–100% occurred at 7 days and 45 days. For the fibrosis (Figure 1D), the majority of samples did not show fibrosis in the group that received saline. In the glycerol group, fibrosis occurred at 1 hour and 24 hours, whereas in the polidocanol group, fibrosis occurred at 7 days and 45 days.

**Correlation between Histopathological Changes and Treatment**

Histopathology with glycerol agents after 1 hour and 24 hours showed the best results in this study. As presented in Figure 2, glycerol histopathology after 1 hour showed the presence of inflammatory cells in 2 HPF, 4 blood vessel proliferation in 1 HPF, 2 luminal narrowing of 76–100%, and 2 fibrosis in >2 HPF. Glycerol histology after 24 hours showed inflammatory cells in 2 fields of view, 4 proliferation of blood vessels, narrowing of 76–100%, and 1 fibrosis in >1 HPF.

The best results occurred with hypertonic saline agents after 1 hour and 45 days. As presented in Figure 3, the

**Table I: Frequency distribution of histopathological scoring based on treatment group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Inflammation</th>
<th>Vascular Proliferation</th>
<th>Luminal Narrowing</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>1 HPF</td>
<td>2 HPFs</td>
<td>Absent</td>
</tr>
<tr>
<td>Saline</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>1 hour</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>-</td>
<td>4 (100)</td>
</tr>
<tr>
<td>24 hours</td>
<td>4 (100)</td>
<td>-</td>
<td>-</td>
<td>4 (100)</td>
</tr>
<tr>
<td>7 days</td>
<td>4 (100)</td>
<td>-</td>
<td>-</td>
<td>4 (100)</td>
</tr>
<tr>
<td>45 days</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>-</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>1 hour</td>
<td>2 (50)</td>
<td>-</td>
<td>2 (50)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>24 hours</td>
<td>-</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>7 days</td>
<td>4 (100)</td>
<td>-</td>
<td>-</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>45 days</td>
<td>-</td>
<td>4 (100)</td>
<td>-</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>Polidocanol</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>1 hour</td>
<td>4 (100)</td>
<td>-</td>
<td>-</td>
<td>1 (25)</td>
</tr>
<tr>
<td>24 hours</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>-</td>
<td>4 (100)</td>
</tr>
<tr>
<td>7 days</td>
<td>2 (50)</td>
<td>1 (25)</td>
<td>1 (25)</td>
<td>2 (50)</td>
</tr>
<tr>
<td>45 days</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>-</td>
<td>4 (100)</td>
</tr>
</tbody>
</table>

**Figure 1.** Differences in scoring of inflammation (A), vascular proliferation (B), luminal narrowing (C), and fibrosis (D) induced by sclerosing agents over time.

**Figure 2.** Representative image on histopathological findings following glycerol injection: (A) 1 hour, (B) 24 hours (Haematoxylin & Eosin staining, magnification 40x).
Table II: Differences in histopathological scoring based on treatment group

<table>
<thead>
<tr>
<th>Group</th>
<th>Inflammation</th>
<th>Vascular proliferation</th>
<th>Luminal narrowing</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Min</td>
<td>Max</td>
<td>Median</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Polidocanol</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: *Kruskal–Wallis test

histopathological picture of hypertonic saline after 1 hour showed 2 inflammatory cells per field of view, the proliferation of blood vessels, ≤26% narrowing of the lumen, and fibrosis in 1 HPF. The histopathological picture of hypertonic saline after 45 days showed 4 inflammatory cells per field of view, the proliferation of blood vessels, ≤26% narrowing of the lumen, and fibrosis in 1 HPF. These findings indicate that saline hypertonic agents had lower-lumen narrowing potential than other agents.

**DISCUSSION**

This study showed a significant difference in the total score based on the different treatments. The median score for the saline treatment was 1.0, with a maximum value of 4.0. The median value for the glycerol treatment was 3.50, and the maximum was 9.0. The median total score for the polidocanol treatment was 2.50, with a maximum of 9.0. The p-value was 0.027. Glycerol thus produced the highest median total score compared to other treatments. The results of this study are supported by McGregor et al. who showed that the glycerin intervention significantly reduced postprocedural hyperpigmentation, swelling, and bruising.

In the polidocanol group, the best results were shown at 7 days and 45 days. As presented in Figure 4, the histopathological picture of polidocanol after 7 days showed the presence of inflammatory cells in 2 fields of view, 2 proliferation of blood vessels, 51–75% narrowing, and fibrosis in >1 HPF. The histopathological picture of polidocanol after 45 days showed the presence of 1 inflammatory cell in one field, 2 blood vessel proliferation, 51–75% narrowing, and fibrosis >1 HPF.

Figure 3. Representative image on histopathological finding following hypertonic saline injection: (A) 1 hour, (B) 45 days (Haematoxylin & Eosin staining, magnification 40x).

Figure 4. Representative image on histopathological findings following polidocanol injection: (A) 1 hour, (B) 24 hours (Haematoxylin & Eosin staining, magnification 40x).

Glycerin also shows faster and better cleansing of telangiectasia. Some individuals may respond much better to one type of sclerosant than another based on their mechanisms of action. Often, glycerin works very well on telangiectasias and fragile reticular veins in the diameter range of 0.55 to 0.95 mm, so it is frequently the treatment of choice. Different results were obtained in the systematic review by Nakano et al., which assessed the effectiveness of any treatment modality for telangiectasis and reticular veins. 35 studies involving 3632 patients were included. The systematic review found no evidence that one type of sclerosant was more effective but evidence of sclerotherapy's superiority over placebo. The evidence did not show increased patient satisfaction with one agent versus another, but evidence existed that patients were less satisfied with a placebo. Kurniawan et al. showed that various sclerosis agents such as bleomycin, polidocanol, and ethanol showed the same good results, with no significant differences regarding their effectiveness in vivo.

The best score related to inflammation was shown for glycerol sclerosant agents, with a maximum value for glycerol of 2.0. No significant difference was found in lumen narrowing based on treatment. In the fibrosis comparison test, the best results were obtained with the sclerosant agent polidocanol, with a maximum value of 2.0 and a median of 0.50. Comparing inflammation, vascular proliferation, narrowing of the lumen, and fibrosis based on time showed no significant differences at the four different measurement times.
Kurniawan et al.\textsuperscript{16} also showed no significant differences in results at different time measurements. In the degree of inflammation, no difference existed between all measurement times in all treatment groups: ethanol, polidocanol, and bleomycin ($p=0.115$, $p=0.424$, and $p=0.373$, respectively). Regarding the degree of vascular proliferation, no difference was found over the measurement time in all treatment groups ($p=0.392$). Concerning the degree of fibrosis, no difference was found over the measurement time in all treatment groups ($p=0.134$, $p=0.375$, and $p=0.798$, respectively).

Glycerol caused moderate inflammation in four (25.0\%) samples, mild inflammation in six (37.5\%) samples, and no inflammation in six (37.5\%) samples. It caused vascular proliferation in all 16 samples (100.0\%). In the samples treated with glycerol, seven (43.8\%) did not experience narrowing, and nine (56.2\%) experienced narrowing; of these, two (12.5\%) samples each experienced narrowing of $\leq 25\%$ and $26–50\%$, four (25.0\%) had 51–75\% narrowing, and one (6.3\%) had 76–100\% narrowing. Glycerol agents did not cause fibrosis in nine (56.3\%) samples and only caused fibrosis in seven (43.7\%).

In a study by Kern et al.\textsuperscript{17}, the effectiveness of glycerin in vessel clearance reached 78\%, with fewer side effects, especially those related to bruising, swelling, and postprocedural hyperpigmentation. Glycerin is a hypertonic agent with mild sclerosing properties. Glycerin sclerotherapy is rarely associated with serious complications and is well-tolerated. Embolization, hyperpigmentation, ulceration, and tissue necrosis are rare but should be considered in all patients undergoing sclerotherapy with any agent.\textsuperscript{18}

The glycerin usually used is chromic glycerin, a chemical irritant with a weak sclerosing effect. Chromated glycerin has been used since 1933 to treat telangiectasias. Chemical irritants damage cell walls by destroying epithelial cells directly from their caustic effect. Osmotic agents damage cells by shifting the water balance through cell membrane denaturation and cellular (osmotic) gradient dehydration.\textsuperscript{19,20}

Glycerin dichromacy may cause post-treatment tissue necrosis, telangiectatic matting, or hyperpigmentation if extravasation is present. On the other hand, these agents are highly allergenic due to chromium and can cause hematuria and ureteric colic, especially after high-dose administration. Chromium is one of the ten most important sensitizers. The risk of chromium allergy is severe reactions in chromium-sensitive patients and inducing sensitivity in patients not allergic to chromium before sclerotherapy. Temporary visual disturbances have been reported after sclerotherapy with chromatin glycerin.\textsuperscript{21}

Saline agents caused mild inflammation in three (18.8\%) samples, and 13 (81.3\%) samples did not experience inflammation. Saline agents caused vascular proliferation in all 16 (100.0\%) samples. Saline agents caused lumen narrowing in four (25.0\%) samples that were included in the $\leq 25\%$ lumen narrowing category, whereas 12 (75.0\%) samples did not experience lumen narrowing. Saline agents did not cause fibrosis in 15 (93.8\%) samples and only caused fibrosis in one (6.3\%).

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Hypertonic saline is a nonspecific osmotic agent that can be effective but carries a risk of tissue necrosis even when only a small volume is extravasated. It has not received Food and Drug Administration approval as a drug sclerosing agent in the United States. Concentrations of hypertonic saline and polidocanol injected as a solution formula had the same effectiveness as a sclerosing agent, but hypertonic saline was significantly more inconvenient to use and was followed by tissue pigmentation.\textsuperscript{22}

Hyperosmotic agents dehydrate target cells, leading to cell damage and death. Ionic solutions such as saline maximise the number of dissolved particles by splitting into their ionic constituents (Van't Hoff effect). Osmosis is concentration-related and cannot be targeted specifically; its use is limited by the effect on nontarget cells that will also become dehydrated (e.g., red blood cells in the parenchymal, vasculature, or stromal cells in the vicinity),
However, some complications are associated with polidocanol treatment, such as pigmentation, injection site pain, superficial thrombophlebitis, local injection induration, and transient dry cough. The European Association recommends foam sclerosing therapy at a 6 to 8 mL safe dose. Serious adverse reactions can be avoided by controlling the dosage.

The strengths of this study are the direct demonstration of the three sclerosing agents, the direct histological observation of effects, and the ability to compare the effects of the three agents in samples. However, this study has several limitations, the first being that only histopathological examination was used. Secondly, the side effects and toxicity of sclerosing agents were not assessed. Further studies comparing histopathological examination with ELISA assay (C-reactive protein, basic fibroblast growth factor, interleukin 6, interleukin 10, and vascular endothelial growth factor) and monitoring side effects should be carried out to further evaluate the effectiveness of glycerol, hypertonic saline, and polidocanol.

CONCLUSION

Significant differences between glycerol, hypertonic saline, and polidocanol on the total score based on treatment with glycerol, which had the largest median value and the same maximum value as the polidocanol agent. Specifically, significant differences were found in inflammation, vascular proliferation, and fibrosis. Glycerol showed the best efficacy against the level of inflammation. Saline and glycerol agents showed similar effects on the rate of vascular proliferation. Polidocanol showed the best effect on the level of fibrosis. At different time measurements (1 hour, 24 hours, 7 days, and 45 days), no significant differences between glycerol, hypertonic saline, and polidocanol in terms of inflammation, proliferation, luminal narrowing, and fibrosis.

FUNDING

No funding was received for this study.

CONFLICTS OF INTEREST

None
**INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)**

This experimental study used an animal model, the *Lepus nigricollis* rabbit. It was conducted in the Animal Laboratory at Hasanuddin University, Makassar, Indonesia. This protocol followed research ethics for experimental animals based on the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine at the Universitas Hasanuddin–Dr. Wahidin Sudirohusodo Hospital (approval number: 293/UN4.6.4.5.31/PP36/2022).

**REFERENCES**


