ORIGINAL ARTICLE

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, Saline Solution, Glycerol, Sclerosing Solutions.

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Received: 16th November 2023; Accepted: 19th June 2024

Doi: https://doi.org/10.31436/imjm.v23i03

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

reticular veins and spider veins than polidocanol, with fewer side effects but increased pain.[10] However, there is no evidence that this agent is considered superior to others in terms of effectiveness and patient satisfaction.[11] Therefore, this study aimed to compare the effectiveness of hypertonic saline, polidocanol, and glycerol as sclerosing agents in an *in vivo* study.

MATERIAL AND METHODS

Animal Preparation

Twenty-four *Lepus nigricollis* rabbits were included in this study. The inclusion criteria were visible blood vessels behind the ear, male, age between 12–16 weeks old, active, healthy, and a body weight of 2,000–3,000 grams. Damaged tissue samples or animals that died during the study were excluded.

The animals were acclimatised in cages for one week to minimise stress before the experimental study began. The animals were kept with the following conditions: the iron cage measured 30 cm high, 40 cm wide, and 50 cm long; each cage contained two rabbits; the cages were cleaned every day; adequate sunlight; temperature of 18–27 °C; sufficient air circulation and was not humid. Throughout the study, the rabbits were fed with dry feed (17% of their body weight) and provided with approximately 70-90 mL of mineral water daily.

Treatment Protocol

Two personnel were involved in administering the treatments: one researcher injected an intravascular sclerosant agent into the dorsal vein behind each animal's ear, while the other person restrained the rabbit and stabilised the ear. The experimental animals were divided into three groups. The first group was injected with 20% hypertonic saline (n=8), the second group was treated with 1% polidocanol (n=8), and the third group received glycerol (n=8). Ten minutes after injection, the blood vessels were occluded at the distal and proximal parts to avoid the systemic flow of the agent.

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.[12] 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

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

Group	Inflammation			Vascular Proliferation		Luminal Narrowing					Fibrosis		
	Absent	1 HPF	2 HPFs	Absent	1 HPF	Absent	≤25%	26–50%	51–75%	76–100%	Absent	1 HPF	>2 HPFs
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Saline													
1 hour	2 (50)	2 (50)	-	-	4 (100)	3 (75)	1 (25)	-	-	-	3 (75)	1 (25)	-
24 hours	4 (100)	-	-	-	4 (100)	4 (100)	-	-	-	-	4 (100)	-	-
7 days	4 (100)	-	-	-	4 (100)	4 (100)	-	-	-	-	4 (100)	-	-
45 days	3 (75)	1 (25)	-	-	4 (100)	1 (25)	3 (75)	-	-	-	4 (100)	-	-
Glycerol													
1 hour	2 (50)	-	2 (50)	-	4 (25.0)	1 (25)	-	1 (25)	2 (50)	-	1 (25)	1 (25)	2 (50)
24 hours	-	2 (50)	2 (50)	-	4 (25.0)	2 (50)	1 (25)	-	-	1 (25)	3 (75)	-	1 (25)
7 days	4 (100)	-	-	-	4 (25.0)	3 (75)	1 (25)	-	-	-	3 (75)	1 (25)	-
45 days	-	4 (100)	-	-	4 (25.0)	1 (25)	-	1 (25)	2 (50)	-	2 (50)	2 (50)	-
Polidocanol													
1 hour	4 (100)	-	-	1 (25)	3 (75)	3 (75)	1 (25)	-	-	-	4 (100)	-	-
24 hours	3 (75)	1 (25)	-	-	4 (100)	3 (75)	-	-	1 (25)	-	2 (50)	2 (50)	-
7 days	2 (50)	1 (25)	1 (25)	2 (50)	2 (50)	2 (50)	-	-	-	2 (50)	2 (50)	-	2 (50)
45 days	3 (75)	1 (25)	-	-	4 (100)	-	1 (25)	-	1 (25)	2 (50)	-	3 (75)	1 (25)

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.

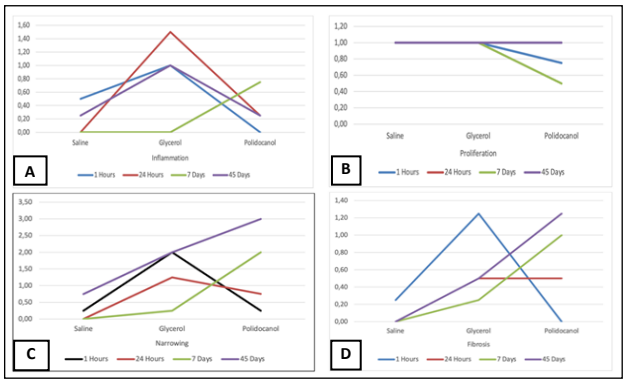

Figure 1. Differences in scoring of inflammation (A), vascular proliferation (B), luminal narrowing (C), and fibrosis (D) induced by sclerosing agents over time

Differences in Histopathological Changes Based on Treatment Groups

After determining that the data were not normally distributed using the Shapiro-Wilk normality test, the Kruskal-Wallis test was performed to identify any significant differences between the three treatment groups within each group to differentiate between various measurement times. The highest average degree of inflammation was found in the glycerol group, and the smallest was found in the polidocanol group (p=0.013). The highest mean vascular proliferation was in the saline and glycerol groups, and the smallest was in the polidocanol group (p=0.004). The average luminal narrowing was greatest in the polidocanol group, and the smallest was in the saline and glycerol groups (p=0.099). The highest mean fibrosis was in the glycerol group, and the smallest was in the saline and polidocanol groups (p=0.43; Table II).

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 blood vessels in 51–75%, 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

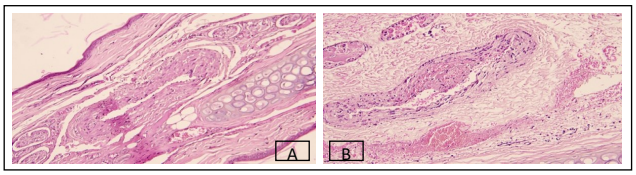

Figure 2. Representative image on histopathological findings following glycerol injection: (A) 1 hour, (B) 24 hours (Haematoxylin & Eosin staining, magnification 40x)

Table II: Differences in histopathological scoring based on treatment group

Group	Inflammation			p-value	Vascular proliferation			p-value	Luminal narrowing			p-value	Fibrosis			p-value
	Median	Min	Max		Median	Min	Max		Median	Min	Max		Median	Min	Max	
Saline	0	0	1	0.013*	1	1	1	0.004*	0	0	1	0.099*	0	0	1	0.043*
Glycerol	1	0	2		1	1	1		1	0	4		0	0	2	
Polidocanol	0	0	2		1	0	1		0.5	0	4		0.5	0	2	

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.

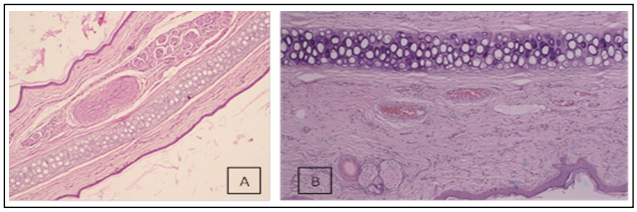

Figure 3. Representative image on histopathological finding following hypertonic saline injection: (A) 1 hour, (B) 45 days (Haematoxylin & Eosin staining, magnification 40x).

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.

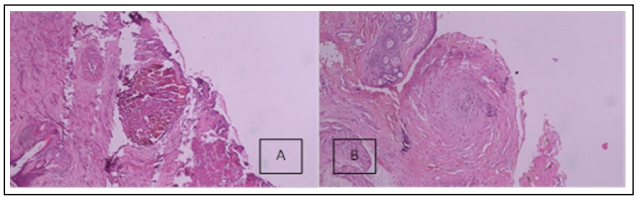

Figure 4. Representative image on histopathological findings following polydocanol injection: (A) 1 hour, (B) 24 hours (Haematoxylin & Eosin staining, magnification 40x).

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.

Glycerin also shows faster and better cleansing of telangiectasia.[13] 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[14]. 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.[15] Kurniawan et al.[16] 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.[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 (all p=0.392). Regarding the degree of luminal narrowing, no difference existed over the measurement time in all treatment groups (p=0.157, p=0.631, and p=0.686, respectively). 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 ≤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.[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.[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.[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.[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 ≤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%).

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.[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),

the presence and concentration of nearby fluids, and the presence of airtight obstacles. The absent barrier causes a series of gradients, and water flowing into the hypertonic solution is replaced by fluid in the interstitium and other, more distant cells. In addition, the hyperosmotic agent's diffusion and flow can dilute the agent and even carry it away from the intended target.[23]

Hyperosmotic stress on cells can have a variety of results. Hypertonic saline produces sclerosis of small vessels in animal models and suicidal effects with a resolution of cysts in humans with echinococcosis. The physiological processes responsible for this effect, related to apoptosis versus necrosis versus fibrosis, have yet to be fully elucidated.[24]

Polidocanol caused vascular proliferation in 13 (81.2%) samples, and only three (18.8%) samples did not experience proliferation. In the polidocanol group, eight (50.0%) samples did not experience lumen narrowing, and eight (50.0%) had lumen narrowing; of these, four (25.0%) had 76–100% narrowing, two (12.5%) had narrowing of ≤25%, and two (12.5%) had narrowing of 51–75%. In the polidocanol group, eight (50.0%) did not experience fibrosis, five (31.3%) experienced mild fibrosis (1 HPF), and three (18.8%) experienced mild fibrosis (>2 HPF).

Polidocanol is injected into varicose veins to damage the venous endothelium. It then forms a thrombus and results in secondary inflammation of the vein wall hypoxia, granulation tissue followed by fibrous growth, varicose vein closure, and, finally, the formation of fiber cords to achieve the purpose of sclerotherapy treatment. *Polidocanol* is an ether compound with an anesthetic effect. It can reduce the body's pain response. Sclerotherapy was originally used in liquid form but has gradually evolved to foam sclerotherapy. The liquid quickly mixes with blood to dilute the preparation and become inactive. Unlike liquid preparations, foam preparations can avoid mixing with intravascular blood and dislocating target vessels.[25–27]

Polidocanol in foam form is prepared by mixing liquid polidocanol with a proportion of air or carbon dioxide. 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.[25]

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).

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