Herbal Haemorrhoidal Cream for Haemorrhoids

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Abstract

Although hemorrhoids are one of the most common diseases in the world, the exact etiology underlying the development of hemorrhoids is not clear. Many different ointments are currently used to treat hemorrhoids; however, there is little evidence of the efficacy of these treatments to support their use. The aim of this study was to compare different herbal creams used for the treatment of hemorrhoids. Twenty-eight male Wistar albino rats, 6-8 weeks old and weighing 160-180 g, were used in this study as 1-control, 2-croton oil, 3-croton oil+fig leaves+artichoke leaves+walnut husks and 4-croton oil+fig leaves+artichoke leaves+walnut husks+horse chestnut fruit. After 3 days of croton oil application, rats were treated with 0.1 ml of cream or saline twice a day for 15 days by syringe. Tissue and blood samples were collected for histological, immunohistochemical and biochemical studies. Statistical significance was determined using one-way ANOVA followed by Tukey’s multiple comparison tests. Croton oil administration resulted in severe inflammation. The third group showed partial improvement in inflammation; however, the greatest degree of improvement was seen in the fourth group, and some recovered areas were observed. Myeloperoxidase immunoreactivity was found to be decreased in the third and fourth groups compared to the second group. Additionally, biochemical analyses (Myeloperoxidase, Malondialdehyde, nitrate/nitrite and nitrotyrosine levels and Superoxide Dismutase activity) were in agreement with the histological and immunohistochemical results. In conclusion, croton oil causes inflammation in the anal area and results in hemorrhoids. Treatment with our herbal hemorrhoid creams demonstrated anti-inflammatory and anti-oxidant effects in this model.

Key Words: croton oil, hemorrhoid, herbal cream, myeloperoxidase, rat

Introduction

Hemorrhoids are normal vascular structures in the anal canal that aid in stool control (4, 25). There is a network of small veins within the inner lining of the anus and lower rectum. These veins occasionally become wider and engorged with more blood than usual. These engorged veins and the overlying tissue may then develop into one or more small areas of swelling, called hemorrhoids. The exact mechanism underlying these changes that lead to the formation of hemorrhoids is not clear*. Hemorrhoids usually present with itching, rectal pain, or rectal bleeding (25). In most cases, symptoms will resolve within a few days. The symptoms of pathological hemorrhoids depend on their type. External hemorrhoids are painful, while internal hemorrhoids usually are not painful unless they become thrombosed or necrotic...
Conservative treatment typically consists of increasing dietary fiber, oral fluids to maintain hydration, non-steroidal anti-inflammatory drugs (NSAIDs), sitz baths, and rest. While many topical agents and suppositories are available for the treatment of hemorrhoids, there is little evidence to support their use (17).

Many different ointments are being used for hemorrhoid treatment. A bland soothing cream, ointment, or suppository may ease discomfort (19). A cream that contains an anesthetic may ease pain better, and one that contains a steroid may reduce inflammation and help to reduce any swelling that may occur around the hemorrhoid. All these may help to ease the itchiness and pain associated with hemorrhoids**.

In our study, a hemorrhoid model was induced by applying croton oil to the recto-anal area of rats (21). Our goal was to compare different herbal creams used for the treatment of hemorrhoids and to develop an ointment for hemorrhoid treatment.

### Materials and Methods

**Plant Material and Preparation of the Herbal Hemorrhoid Cream**

All herbs and plant oils used in this study were obtained from certified herb sellers. Generally, the herbal hemorrhoid cream (New European Patent Application 07015624.5-2107/2022504 (K10432-EP); International Patent Application PTC/EP 2009/000148 (K10432-PTC)) is comprised of an aqueous-based liquid containing herbal extracts, vegetable oils and gelling agents according to the invention application (Table 1).

The most active ingredients in the first group are the following: fig leaves (*Ficus carica*), walnut husks (*Juglans regia*), and artichoke leaves (*Cynara scolymus*) and horse chestnut fruit (*Aesculus hippocastanum*). The preferred proportion of these active ingredients within the aqueous base liquid is 30-75% by volume, with 50-70% being better and 55-65% being optimal.

The second group of assisting ingredients consists of three subgroups: a subgroup of highly desirable ingredients, including pomegranate skin (*Punica granatum*), eggplant stems and stalks (*Solanum melongena*), acorns (*Quercus macrolepis*), and pine cones (*Cupressus sempervirens*); a second subgroup of somewhat less important ingredients, including cypress cones (*Cupressus sempervirens*), juniper berry seeds (*Juniperus communis*), oak tree skin (*Quercus*), nettle leaves and seeds (*Urtica urens*), myrtle leaves (*Myrtus communis*), dragon’s blood or *Sanguis draconis* (*Dracaena draco*), and balsam apple fruits (*Momordica charantia*); and a third subgroup of even less critical ingredients, from which one or more may be selected if desired, including *Nigella sativa*, *Aloe vera*, milfoil (*Achillea millefolium*), quince leaves (*Cydonia vulgaris*), *Solidago officinalis*, ginger (*Zingiber officinale*), fennel (*Foeniculum vulgare*), rosemary (*Rosmarinus officinalis*), and cassia (*Senna corymbosa*). The preferred proportion of the entire group of secondary ingredients is 10-50% by volume, with 20-40% being

<table>
<thead>
<tr>
<th>I. Base Liquid (30-33%)</th>
<th>a) Active Ingredients (60% of I)</th>
<th>Very Desirable Ingredients (60% of b), Less Important Ingredients (30% of b), Least Important Ingredients (10% of b)</th>
</tr>
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<tbody>
<tr>
<td>b) Assisting Ingredients (30% of I)</td>
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<tr>
<td>c) Helping Ingredients (10% of I)</td>
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<tr>
<th>II. Vegetable Oils (30-33%)</th>
<th>a) Most Important Oils (70% of II)</th>
<th>Storax Oil, Cade Oil, Balsam Apples, <em>Nigella Sativa</em> Oil</th>
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<tbody>
<tr>
<td>b) Less Important Oils (24% of II)</td>
<td>Sesame Oil, Cocoa Oil, Ricine Oil, Almond Oil, Olive Oil</td>
<td></td>
</tr>
<tr>
<td>c) Least Important Oils (6% of II)</td>
<td>Sun Flower Oil, Hazelnut Oil</td>
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<th>III. Gelling Agents (30-33%)</th>
<th>a) Lanolin (75% of III)</th>
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<tr>
<td>b) Vaseline (25% of III)</td>
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| IV. Analgesics | The amounts are preferably selected to be pharmaceutically acceptable. |

| V. Chemical Agents | The amounts are preferably selected to be pharmaceutically acceptable. |

**http://www.cks.nhs.uk/CksContent/TopicReview/PreviousVersions/haemorrhoids.pdf [20 June 2011]**
more favorable and 25-35% being the optimal concentration.

The third group of helping ingredients, from which one or more may be selected if desired, includes fern leaves, common buckthorn, mallow, Melissa officinalis, Acanthus dioscoridis, Cichorium endivia, hawthorn, leek, carob, ziziphora, borage, asa foetida, Plantago, Sambucus nigra, buttercup, oleander, coconut skin, mullein, Lesser celandine, coriander, arbor vitae, anis, flax seed, and Vaccinium myrtillus. The preferred proportion of this group of ingredients is 1-25% of the aqueous base liquid by volume, with 2-20% being more favorable and 5-15% being the optimal concentration.

The cream is further composed of liquid vegetable oils, with cade oil, storax, balsam apple extract, and Nigella sativa oil being the most important. Any of these vegetable oils may be present in an amount between 0.0001-20% by weight based on the total amount of ointment, although a concentration of 0.001-10% is preferable. However, it is best if the oil volume contains 5-30% of storax oil, with 10-20% being more favorable and 12.5-17.5% being the optimal concentration. A total of 2-25% of the oil volume can be composed of an olive oil extract of balsam apples, with 5-15% being more favorable and 7.5-12.5% being optimal. Cade oil can be found in an amount of 10-40% of the oil volume, with 20-30% being more favorable and 22.5-27.5% being optimal. Nigella sativa oil preferably comprises between 2-25% of the oil volume, with 5-15% being more favorable and 7.5-12.5% being optimal. Other less important oils, namely resin oil, sesame oil, cacao oil, almond oil, castor oil and olive oil, are each preferably contained in an amount of 1-25% of the oil volume, with 2-15% being more favorable and 5-10% being optimal. Even less important oils, such as sunflower oil and hazelnut oil, are each preferably contained in an amount of 0.1-20% of the oil volume, with 1-10% being more favorable and 2-6% being optimal.

The gelling agents, namely lanolin and Vaseline, are gently heated in a water bath to increase fluidity and then slowly poured into the basic liquid. The total concentration of gelling agents can range from 20-40% by volume, with 30-33% of the total by volume being preferred. Lanolin comprises 50-90% of the gelling agent volume, with 65-85% being more favorable and 75-80% being optimal, whereas Vaseline comprises 10-50% of the gelling agent volume with 15-35% being more favorable and 20-25% being optimal.

The analgesics added to some creams may include metamizole sodium (Novalgin) and Lidocaine, although it is preferable to use more Lidocaine than Novalgin (optimal ratio is 3:2). Lidocaine may be used as a 5% solution, such as in Jetocain. The amounts of analgesics used are selected to be pharmacologically acceptable and to alleviate unpleasant sensations.

The group of chemicals in hemorrhoid creams, from which one or more may be selected if desired, includes Alum (M^1Al(SO_4)_2), where M^1 represents a monovalent ion, such as ammonium or an alkali metal, preferably potassium), boric acid, salicylic acid, zinc oxide, calcium carbonate, sodium benzoate, and a solution of basic aluminum acetate (liqueur alumini subacetatis). The total amount of chemicals preferably comprises 0.1-20% of the total volume, with 1-10% being the most preferable concentration. The herbal ingredients and stabilizing chemicals may be used in comminuted form, e.g., by crushing and/or milling, if desired.

**Experimental Protocol**

Twenty-eight male Wistar albino rats, 6-8 weeks old, and weighing 160-180 g were used in this study. Animal experiments were performed following the recommendations of the Experimental Animal Care and Use Committee of Istanbul University Experimental Medicine Research Institute (Application Date/Number: 08.06.2010/87). The rats were kept in plastic cages in a temperature-controlled room (22 ± 3°C). They were fed a commercial pellet feed and allowed to drink water ad libitum. The animals were separated into four groups as Control (n = 7), Croton oil (n = 7), Croton oil + fig leaves (Ficus carica), + artichoke leaves (Cynara scolymus) + walnut husks (Junglans regia) (LF + LA + HW) (n = 7) and Croton oil + fig leaves + artichoke leaves + walnut husks + horse chestnut fruit (Aesculus hippocastanum) (LF + LA + HW + HCF) (n = 7). The irritant consisted of 3% croton oil, 20% pyridine and 5% distilled water in diethyl ether (28). Cotton soaked with 0.5 ml of physiological saline or croton oil was placed in each animal’s rectum every morning for 3 days for 30 seconds. After 3 days of applications animals were then treated with 0.1 ml of physiological saline or herbal hemorrhoid creams for 15 days in both the morning and evening using a syringe. All animals were anesthetized using thiopental sodium (Pental Sodyum 90 mg/kg, IE; Ulagay, Istanbul, Turkey) on the day of the experiment. Tissue and blood samples were collected for further study.

**Light Microscopy and Immunohistochemical Analyses**

Recto-anal rat tissues were excised under anesthesia (thiopental sodium) and subsequently fixed for 24 h with 10% neutral formalin. A routine paraffin-embedding method was then used to obtain 4-µm-thick histological sections that were subsequently
stained with hematoxylin-eosin (HE) to evaluate morphological changes using a light microscope. The 10% neutral formalin-fixed rat recto-anal tissues were stained using a streptavidin-biotin complex (StrepABC) immunohistochemical method (13). Images were obtained using Kameram 390 CU Software (Mikro Sistem Ltd. Sti, Istanbul, Turkey).

Biochemical Analyses

We measured several inflammation biomarkers to demonstrate the level of inflammation and the biochemical effects of the treatments, both in recto-anal tissue and in blood plasma. Tissue samples, each sample had 0.250 g wet tissue weight, were lysed in ice-cold lysis buffer: 200 mM NaCl, 5 mM EDTA, 10 mM Tris, 10% glycerine, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/ml leupeptin, 28 µg/ml apro tinin (pH 7.4) and homogenized by a tissue homogenizer on ice using standard methods to open cell membranes. After that samples were centrifuged twice (1,500 × g at 4°C for 15 min) to avoid contamination with cellular debris. The supernatants were taken to the polypropylene eppendorfs. Blood was drawn from the heart of rats in tubes with EDTA. After blood sampling, plasma was separated by centrifugation: 1,500 × g at 4°C for 15 min. Plasma was removed and transferred to fresh polypropylene eppendorfs. All samples (tissue and plasma) were stored at -20°C until the day of study. Specifically, we determined the levels of nitrate/nitrite (NO\textsubscript{3}^-/NO\textsubscript{2}^-) (Nitric Oxide-OxisResearch, Portland, OR, USA), myeloperoxidase (MPO) (Hycult, Uden, The Netherlands), and nitrotyrosine (Hycult, Uden, The Netherlands) in both tissue and plasma samples, in addition to superoxide dismutase (SOD) (Cayman Chemical Company 1180 East Ellsworth Road, Ann Arbor, Michigan 48108 USA) in tissue samples only using enzyme-linked immunosorbent assay (ELISA) kits. Additionally, we determined malondialdehyde (MDA) levels in tissue samples using a standard colorimetric method.

Statistical Analyses

The results are expressed as the means ± SD. Statistical significance was calculated by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using GraphPad Prism (GraphPad Prism, Version 5, Software Program, San Diego, CA, USA). A \( P < 0.05 \) was considered to be statistically significant.

Results

Histological Analyses

Rats receiving croton oil for three days exhibited severe inflammation of the recto-anal area that was observed both macroscopically and histopathologically. During preparation of the tissues for histological studies, we also imaged the entire recto-anal area and the excised anal tissues. In control animals, the anal areas were normal and healthy relative to those of animals receiving croton oil whose recto-anal areas were bloody and necrotic (Fig. 1). Following treatment with LF + HW + LA or LF + HW + LA + HCF, the recto-anal areas of these rats showed an improvement. Although both treatments resulted in an improvement, the LF + HW + LA + HCF therapy yielded the best results as demonstrated by the near recovery of the recto-anal area and anal canal to control levels (Fig. 1).

When cross sections of the recto-anal areas of all groups were examined, the tunica mucosa (TM), tunica submucosa (TS), tunica muscularis (TMu) and
tunica serosa were normal, and all mucosal glands were clean in the control animals. The lamina propria and lamina muscularis mucosa (mm) also had normal structures. Rats who received croton oil for three days exhibited severe inflammation and necrosis, as seen macroscopically in Fig. 2. Numerous inflammatory cells were present in the tunica mucosa. The tunica mucosa and mucus glands of this layer were severely damaged. Dilated vessels and bleeding areas were also present in this layer. The tunica muscularis had a normal structure; however, the recto-anal area of this layer was less thick than that in the control animals. The tunica serosa was not affected by the application of croton oil and had a normal connective tissue structure. The fig leaves + artichoke leaves + walnut husks (LF + LA + HW) group showed partial improvement; however, they did not revert completely back to the control state.

The LF + LA + HW + HCF treatment group showed the best improvement. The inflammatory damage observed in the tunica mucosa was less severe in this group. Furthermore, the glands in this layer recovered from the induced damage and had a structure similar to that of the control group. Inflamed areas and bleeding spaces were also decreased in this group (Fig. 2).

**Myeloperoxidase (MPO) Immunohistochemical Analyses**

In this study, we demonstrated increased MPO immunoreactivity in the croton oil application group compared to the control group. In contrast, MPO immunoreactivity decreased in the LF + LA + HW and LF + LA + HW + HCF groups compared to the croton oil group. The greatest improvement was observed in the LF + LA + HW + HCF group (Fig. 3).

**Biochemical Analyses**

Recto-anal tissue homogenates and blood samples collected from animals who received croton oil showed slight increases in nitrate/nitrite levels. LF + LA + HW + HCF and LF + LA + HW treatment significantly decreased the nitrate/nitrite levels in both tissue and blood (Fig. 4) samples; however, the best improvement was observed in the animals treated with LF + LA + HW + HCF.

Nitrotyrosine levels were increased in the croton oil group compared to the control group, whereas they were significantly decreased in tissue samples from the LF + LA + HW + HCF and LF + LA + HW groups compared to the croton oil group. In contrast, nitrotyrosine levels in blood samples were higher in the LF + LA + HW group and lower in the LF + LA + HW + HCF group relative to both the control and croton oil groups (Fig. 5). However, these differences were not statistically significant. The LF + LA + HW + HCF treatment yielded the greatest effect on tissue nitrotyrosine levels.

We observed noticeable effects on free radical
scavenging enzymes in the treatment groups. SOD activity was significantly decreased in the recto-anal tissues of animals in the croton oil-only group and was less than that observed in all other groups, including the control group. Tissue SOD activity in the LF + LA + HW and LF + LA + HW + HCF groups increased significantly and was even greater than that detected in the control group (Fig. 6). Similar to other inflammatory markers, MDA levels were also increased in the croton oil group. In contrast, MDA tissue levels were significantly decreased in the LF + LA + HW + HCF and LF + LA + HW groups (Fig. 7).

Finally, myeloperoxidase (MPO) levels in blood samples were correlated with tissue MPO levels. The application of croton oil alone caused a small increase in MPO levels in tissue and blood samples compared to levels resulting from treatment with LF + LA + HW + HCF and LF + LA + HW (Fig. 8).

**Discussion**

Hemorrhoids are graded according to the degree of hemorrhoidal prolapse. For patients with first- and second-degree hemorrhoids, the treatment options are usually nonsurgical, including dietary manipulation, oral flavonoids and topical anti-hemorrhoidal
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**Fig. 5.** Tissue and plasma nitrotyrosine levels (LF + LA + HW: Fig leaves + artichoke leaves + walnut husks; LF + LA + HW + HCF: Fig leaves + artichoke leaves + walnut husks + horse chestnut fruit) (**: $P < 0.01$ relative to the croton oil group).

**Fig. 6.** Tissue superoxide dismutase levels (LF + LA + HW: Fig leaves + artichoke leaves + walnut husks; LF + LA + HW + HCF: Fig leaves + artichoke leaves + walnut husks + horse chestnut fruit) (*: $P < 0.05$ relative to the croton oil group).

**Fig. 7.** Tissue malondialdehyde levels (LF + LA + HW: Fig leaves + artichoke leaves + walnut husks; LF + LA + HW + HCF: Fig leaves + artichoke leaves + walnut husks + horse chestnut fruit) (*: $P < 0.05$, **: $P < 0.01$ relative to the croton oil group).

**Fig. 8.** Tissue and plasma myeloperoxidase levels (LF + LA + HW: Fig leaves + artichoke leaves + walnut husks; LF + LA + HW + HCF: Fig leaves + artichoke leaves + walnut husks + horse chestnut fruit).
medication (27). The treatment of hemorrhoidal disease depends on the stage of the disease and the symptoms. Surgical hemorrhoidectomy is indicated for the treatment of third- and fourth-degree symptomatic hemorrhoids. However, surgery is associated with severe postoperative pain that is a source of so much anxiety that some patients decide not to undergo the operation (23).

_Ruscus aculeatus_ L. is a member of the Liliaceae family and is native to Mediterranean Europe and Africa. It has been widely used as a laxative and diuretic agent as well as a vasoconstrictor in the topical treatment of varices and hemorrhoids (5). Accordingly, our herbal hemorrhoid cream is also derived from herbal extracts, including fig leaves, artichoke leaves, walnut husks, and horse chestnut fruit.

_Aesculus hippocastanum_ L. (Hippocastanaceae), or horse chestnut, is a common tree found in Turkey. In Turkish folk medicine, tea prepared from the crushed seeds of horse chestnut fruit was used to pass kidney stones and to treat stomach aches, while a fraction of seeds were swallowed to alleviate hemorrhoid symptoms (15). Additionally, the seeds from this plant have long been used in Europe to treat venous disorders, particularly varicose veins, and hemorrhoids, in addition to inflammatory ailments, such as arthritis, back aches, strains, tendonitis, and sports injuries (15). The seeds contain a complex mixture of triterpene saponin glycosides (escin) and several other active ingredients, including high levels of coumarins, such as esculetol, and flavonoids, including glycosides of quercetin and kaempferol. Studies have shown that the anti-exudative, anti-edematous, and vasoprotective effects of horse chestnut extracts are exclusively due to escin, a complex mixture of triterpene saponins (26).

In addition to being used as a remedy for cardiovascular and respiratory ailments, _Ficus carica_ Linn., commonly known as edible fig, has traditionally been used for its medicinal benefits as a laxative as well as an anti-spasmodic and anti-inflammatory agent (10). The leaves are claimed to be effective in treating various inflammatory conditions, including painful or swollen piles and insect stings and bites (1). The consumption of _Ficus carica_ may contribute to the prevention of diseases in which homeostasis is impaired by oxidative features. Additionally, because the leaves are characterized by high quantities of psoralen and bergapten, their use in the treatment of some dermatologic diseases, such as psoriasis and vitiligo, may warrant further investigation by the cosmetic and pharmaceutical industries (22).

Antioxidant properties of an extract from leaves of the walnut tree (_Juglans regia_) have also been reported. The extract suppresses functional insufficiency of liver-associated synthesizing enzymes, increases the anti-toxic action of hepatocytes, and improves the functional insufficiency of kidneys. The extract is recommended for the prevention of diabetes mellitus and its late-stage complications (6). Results from these studies indicate that the walnut tree is a chemopreventive agent and is an excellent source of effective natural antioxidants (3).

Artichoke leaf extract was reported to have a cholesterol-reducing effect on hypercholesterolemic subjects (14, 30). Additionally, artichoke leaf extract decreased the production of reactive oxygen species, the oxidation of low-density lipoproteins and lipid peroxidation in _in vitro_ experiments (2, 9, 31). Artichoke contains caffeoylquinic acid derivatives and flavonoids (16, 29). Our herbal hemorrhoid cream includes extracts from these four plants. As seen above, these plants have beneficial effects in treating inflammation, and they are also used as vasoprotective, anti-edematous and anti-oxidant agents.

There are many herbal remedies for hemorrhoid treatment in almost every culture. Turkey has a rich flora, and numerous plants have been reported for the treatment of hemorrhoids in Turkish folkloric medicine. Additionally, hemorrhoids are one of the major health problems that lead to the use of herbal medication (12).

The application of croton oil to the anal canal can cause tissue inflammation and damage, especially to vessels by increasing their permeability, which leads to leukocyte increment (20). In our study, after croton oil application, animals showed inflammation and damaged areas in the recto-anal canal including damaged tunica mucosa layer and the mucus glands of this layer. The LF + LA + HW + HCF group showed the best improvement in symptoms, and the inflammatory damage in the tunica mucosa was reversed. Although the LF + LA + HW group also showed improvement, this treatment was less effective than the LF + LA + HW + HCF treatment.

Recto-anal tissue homogenates and blood samples from animals who received croton oil showed a small increase in the level of oxidant markers, nitrate/nitrite and nitrotyrosine, which were decreased by our herbal hemorrhoid cream. Nitrotyrosine has been identified as an indicator of cell damage, inflammation, and the production of NO (7). It is believed that measuring the concentration of nitrotyrosine will serve as a marker for damage caused by NO in the cell. Previous studies have shown that nitrotyrosine has been found in inflammatory conditions, such as atherosclerotic plaques, rheumatoid arthritis, and many other inflammatory disorders (11, 18).

Oxidative damage to biological compounds, especially lipids through lipid peroxidation, has been shown to play an important role in various diseases.
Superoxide dismutase (SOD) is an enzyme that repairs cells and reduces the damage caused by superoxide, which is the most common free radical in the body. Free radicals can lead to lipid peroxidation in organisms. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acid peroxidation in cells, and an increase in free radicals causes an overproduction of MDA. Malondialdehyde levels are commonly used as a marker of oxidative stress and the anti-oxidant status of patients (8). We observed noticeable changes in the levels of free radical scavenging enzymes in the treatment groups. SOD activity was significantly decreased in the recto-anal tissue of animals who received croton oil. Despite this decrease in SOD activity, MDA levels increased in this group similar to the increase seen in other inflammatory markers. In contrast, MDA levels decreased and SOD activity increased following treatment with our herbal hemorrhoid cream. These results demonstrate that our herbal hemorrhoid cream, which consists of various plant extracts, has prominent anti-oxidant effects as discussed above. Another biomarker that we used to determine the extent of free radical tissue damage is myeloperoxidase (MPO) activity. MPO is secreted in extracellular matrix phagocytic vacuoles by granulocytes and converts \( \text{H}_2\text{O}_2 \) into highly reactive free radicals, including HOBr, HOI, and HOCl, to eliminate microorganisms. MPO levels in the plasma were correlated with tissue MPO levels. Also, the results of immunohistochemical analyses of MPO in recto-anal tissues were well-matched with biochemical results. All of the results were in accordance with each other. Croton oil administration caused an increase in MPO levels in the tissue and blood samples, but these levels were decreased by treatment with LF + LA + HW + HCF and LF + LA + HW. These results also support the observation that the application of croton oil increases all of the inflammatory markers that were measured, and this inflammatory response was associated with a decrease in superoxide radical scavengers.

Guillaume and Padioleau (11) reported that enzymatic and non-enzymatic lipid peroxidation was both inhibited by horse chestnut seed extract in vitro in a dose-dependent manner. In another study, Japanese horse chestnut seed extract (Aesculus turbinata Blume) was reported to scavenge DPPH (1, 1-diphenyl-2-picrylhydrazyl) radicals and superoxide anions in vitro (15). Similarly, in our study, the cream that included horse chestnut fruit was more effective than the cream containing only LF + LA + HW.

Pain in patients with anal fissures is reported to be substantially reduced after the application of nifedipine and lidocaine ointment (23). Therefore; we hypothesized that the addition of nifedipine to lidocaine would improve pain control in a large population of patients undergoing open hemorrhoidectomy. However, a previous study demonstrated that the anorectal application of an ointment containing nifedipine (0.3%) and lidocaine (1.5%) showed neither pharmacologically relevant serum levels of the active ingredients nor any hemodynamic effects in healthy volunteers (23). Consequently, we included lidocaine in our herbal hemorrhoid cream to alleviate pain.

Several glucocorticoids were applied to the recto-anal area of the rats with croton oil-induced hemorrhoids in a cream formulation. Among the steroids tested, namely diflucortolone valerate, prednisolone, hydrocortisone caproate, and hydrocortisone, diflucortolone valerate was found to suppress inflammation most effectively. Therapeutic effects of several anti-hemorrhoid drugs were also examined using a hemorrhoid model utilizing abrasive irritation and compared to those resulting from the croton oil model. In one study, microscopic observation showed that destruction of the mucus epithelium, necrosis of the mucus layer, infiltration of inflammatory cells and vasodilatation in the croton oil model were also markedly suppressed by glucocorticoids, including drug application (20). In this study, we observed remarkable improvement of hemorrhoids using our herbal cream. Additionally, our cream is completely natural and can be highly recommended for use because it does not include any glucocorticoids and their associated side effects.

In this study, we have demonstrated that the application of croton oil to the recto-anal area of rats causes inflammation, which results in hemorrhoids, and we suggest that it is a reliable method that can be used as an experimental model of hemorrhoids. Many parameters were investigated in this study, including histological, immunohistochemical, and biochemical analyses. Histological results showed that croton oil application induces severe inflammation that is improved with the use of herbal hemorrhoid cream. In parallel, inflammatory, and oxidative damage were also determined by measuring MPO, MDA, nitrate/nitrite, and nitrotyrosine levels and SOD activity. All results were in accordance with each other and demonstrated that the application of croton oil induced inflammation and tissue damage, increased all inflammatory markers measured, and was associated with a decrease in superoxide radical scavengers. In this model, the herbal hemorrhoid cream was found to exhibit antioxidant effects on the croton oil-induced hemorrhoids. This was the first study to use a combination of fig leaves, artichoke leaves, walnut husks, and horse chestnut fruit for the treatment of hemorrhoids based on their anti-oxidant and anti-inflammatory effects.

Consequently, we recommend that this preparation can be used safely for the treatment of hemorrhoids.
in the large population of patients who do not want to undergo surgery and do not want to use glucocorticoids due to their many side effects. Our natural and herbal hemorrhoid cream can improve the health and quality of life of people who suffer from hemorrhoid disease.

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