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Clinical Trial: Management of Post-Haemorrhoidectomy Wound Healing by Bergamot Flavonoid-Based Gel and Sodium Hyaluronate: An Observational, Multicentric Trial

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Abstract:

Objective: Haemorrhoidal disease (HD) is a very diffuse anorectal condition that involves a large part of the population, both male and female of every age. Among the procedures proposed to treat HD, conventional excisional surgery remains one of the most performed. Milligan-Morgan (MM) technique is one of the most used haemorrhoidectomy techniques. In this technique, the wounds are left open and re-epithelialization requires almost 3-5 weeks, in which patients generally experience pain and intense discomfort improving over the weeks.

Methods: The aim of this study was to evaluate the effect of topic administration of Benebeo Gel®, mainly composed by bergamot-derived flavonoids and hyaluronic acid, on post-operative wound healing after open MM haemorrhoidectomy. An observational prospective study was carried out, involving 205 patients aged between 18 and 75.

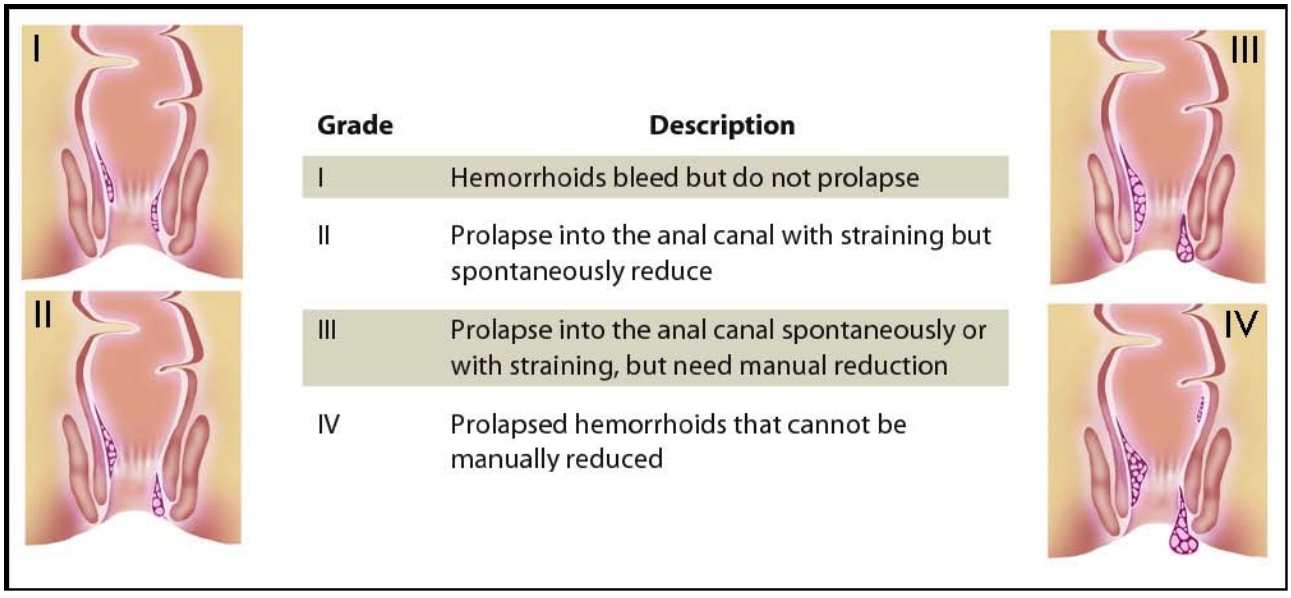
Results and Conclusion: The results after 2 weeks of treatment seem to be promising with a very good clinical outcome and patient satisfaction within 1 month.

1. INTRODUCTION

Haemorrhoidal Disease (HD) is a very diffuse anorectal condition that involves a large part of the population, both male and female of every age [1,2]. The age mainly involved ranges between 45 and 65 years old with a peak at 45 and 55 years [3]. HD affects millions of people around the world revealing to be a major medical and socioeconomic problem [4,5]. The system of classification currently used to categorize this pathology is that of Goligher (1975, Table **1**) [6]. Several attempts in literature have been made to refine this classification, but no single system has been universally accepted.

There is considerable heterogeneity in outcomes in studies reporting on the treatment of HD. The recently developed European Core Outcome Set (COS) for HD identified patient-reported symptoms, as the primary core outcome for clinical HD studies [7]. Several mechanisms are involved in HD. For a long time the degradation of the extracellular matrix has been involved in the etiopathogenesis of HD [8]. A recent paper by Serra *et al*. [9] showed that high-graded HD is characterized by high levels of matrix metalloproteinases (MMPs) which regulate extracellular structural proteins and tissue remodeling, and neutrophil gelatinase-associated lipocalin (NGAL) which is involved in the regulation of MMP activity. NGAL is commonly known as marker of neutrophil activation which can be induced by several cytokines and growth factors. Treatment options for HD vary based on the degree and severity of symptoms. Management of haemorrhoids may be medical (prescribing high fiber diet, antimotility agents, topical analgesics and corticosteroid creams for symptomatic relief, alternative/traditional medicine like oral flavonoids), nonoperative (sclerotherapy, cryotherapy, rubber band ligation, infrared photocoagulation, etc.) [10], or surgical (open, closed, or stapled haemorrhoidectomy). Multiple drugs, such as metronidazole (10 percent) [11], glyceryl trinitrate ointment [12], sucralfate [13], atorvastatin (2%) [14], folinic acid [15], are applied directly to the wound area to relieve pain in the perianal area and promote wound recovery. Traditional healthcare systems are known to have different treatment regimens for management of haemorrhoids. Ayurveda is one of the traditional health care systems prevalent and practiced widely in India and other countries. Indian Regulations permit use of traditional medicine under proprietary ayurvedic medicine licenses [16]. In traditional Persian medicine several medicinal plants are used for the treatment of haemorrhoids [17]. Among these, *Terminalia chebula* Retz. (Fam. Combretaceae), which is called the “King of Medicine” in Tibet and always listed at the top of the list of “Ayurvedic Materia Medica”, is one of the best choices for the treatment of haemorrhoids [18]. In the traditional Chinese medicine, compound *Sophora flavescens* has shown to enhance wound healing in a rat model of perianal ulceration [19]. *Solanum melongena* L. (eggplant) is used for treatment of rheumatism, beriberi, itching, toothache, bleeding, asthma, bronchitis, cholera, neuralgia, and haemorrhoids in traditional medicine (Turkish, Chinese,

Table 1: Classification of Haemorrhoidal Disease



Grade	Description
I	Hemorrhoids bleed but do not prolapse
II	Prolapse into the anal canal with straining but spontaneously reduce
III	Prolapse into the anal canal spontaneously or with straining, but need manual reduction
IV	Prolapsed hemorrhoids that cannot be manually reduced

and Indian). *S. melongena* L. extract has demonstrated significant anti-haemorrhoidal activity [20]. *Vitis vinifera* L. and *Aesculus hippocastanum* L. extracts have been also used for the treatment of pain and inflammation associated with haemorrhoid disease [21]. A polyherbal formulation, Anoac-H, seems to suppress the expression of regulated upon activation, normal T cell expressed and presumably secreted (RANTES) and vascular endothelial growth factor (VEGF) for the management of bleeding haemorrhoids [22]. The polyherbal formulation AnoSpray/PiloSpray®, an Ayurvedic polyherbal formulation in the form of a spray, consisting of lodhara (*Symplocos racemosa*), daruharidra (*Berberis aristata*), mocharas (*Bombax ceiba*), kapur (*Cinnamomum camphora*), pudinah (*Mentha piperita*), til (*Sesamum indicum*) and kokam (*Garcinia indica*) oils in aerosol form, has recently demonstrated its utility in the management of haemorrhoids, acute anal fissures and perineal wounds, by suppressing the expression of pro-inflammatory cytokines COX‑2 and RANTES [23]. The use of flavonoids in the treatment of haemorrhoids is known [24,25]. Among these, the flavonoid rutin, has been widely used in the treatment of chronic venous insufficiency, due to its anti-inflammatory properties, and has been suggested for the treatment of haemorrhoids, varicose veins, poor circulation [26]. The oral micronized, purified flavonoid fraction (MPFF) (Daflon® 500 mg) is one of the most commonly used treatment option for Grade 1 and Grade II haemorrhoids. Catechins and epicatechins have also demonstrated interesting activity in the treatment of haemorroids. A herbal preparation, namely Roidosanal® (a mixture of four herbs, that is, gum-resin from *Commiphora molmol* (50%), gum-resin from *Gardenia* spp. (16.6%) and inflorescence from *Tagates erecta* (16.7%), and *Mesua ferrea* (16.7%), as capsules, standardized to contain not less than 7% of total catechins and epicatechins was studied. The efficacy of Roidosanal® was compared to Daflon® 500 mg: they were equally effective in resolving signs and symptoms of haemorrhoids [27]. *Aloe vera* has demonstrated activity in the treatment of haemorrhoids and been suggested as an important resource for producing novel drugs for this disease [28–30]. We recently demonstrated that the treatment with Proctosoll Allevia® gel, consisting of *Aloe vera*, Olio di Jojoba and hyaluronic acid, was effective against I–II degree symptomatic haemorrhoids with a good profile of tolerability and safety [31]. Hyaluronic acid (HA) is a biopolymer present in most of human tissues and it is a component of the extracellular matrix (ECM) [32]; it is a type of glycosaminoglycan that is extensively distributed across the skin, muscle, cartilage, and blood vessels [33,34]. It contributes to maintain hydration, turgidity and viscosity. HA has shown several pharmacological functions, such as antioxidant, vasoprotective, anti-inflammatory, stimulating angiogenesis, moistening wounds, reducing exudation, is vasoprotective [35,36]. Its use in the treatment of haemorrhoids has been described [37]., Topical and systemic medical treatment are recommended as first line treatment to treat Goligher Grades I and II haemorrhoids, but in case of failure or highly symptomatic high-graded prolapse, surgery remains the main option [38]. Among the several procedures proposed to treat HD, conventional excisional surgery remains one of the most performed, especially for the most advanced stages, Grade III or IV, and bleeding [39]. Haemorrhoidectomy includes open Milligan-Morgan (MM), sub mucous resection (Park), closed (Hill-Ferguson) or Stapled techniques [40,41]. In the MM technique, the wounds are left open for healing by a secondary mechanism and, until it is completely re-epithelialized requiring almost 3-5 weeks, patients usually experience pain and intense discomfort that improve over the weeks [42]. However, although haemorrhoidectomy may be performed using energy devices, such as thunderbeat-assisted haemorrhoidectomy trying to reduce post-operative bleeding and pain [43], post-operative pain still remains one of the main problem which may affect patients' quality of life (QoL) and frequently induce the patients to postpone the procedure [44,45]. The pain is mainly related to the anal wounds and the healing speed surely influences the post-operative course. Moreover, pain in association with bleeding and anal stricture represent the most frequent complications after MM, which may require re-intervention in the short or long term [46]. The actual aim is to mitigate post-operative symptoms in patients whose preoperative anatomo-clinical condition required an excisional haemorrhoidectomy according to MM procedure [47]. Over the years, proctologists have considered many medical and surgical options to reduce the post-operative pain to perform an internal lateral sphincterotomy [48]. In this context, plant extracts and oils have gain their position in the current clinical practice. Among the wide group of natural products, this work focused on bioactive compounds derived from bergamot, particularly Benebeo Gel®.

Benebeo Gel® is an anal ointment made of bergamot polyphenolic fraction, pantenol, sodium hyaluronate

and gomma xantana indicated for anal and perianal inflammation. A recent study suggested that the anti-inflammatory function of Benebeo Gel® may be due to reduction of the intracellular free radicals, in particular of the COX-2, and to the inhibition of prostaglandins and leukotrienes production [49]. It has demonstrated to stimulate tissues repairing and vasoprotective effect thus is also being suggested in the treatment of anusitis-proctitis [50]. The aim of this study was to evaluate the effect of Benebeo Gel® in patients for post-operative wound healing after open haemorrhoidectomy.

2. BENEBEO GEL®

Benebeo Gel® is a bergamot-derived gel consisting of bergamot-derived flavonoids (hesperidin, naringin, apigenine, eriocitrin, hesperetin, neoeriocitrin, neohesperidin), pantenol, sodium hyaluronate and gomma xantana.

2.1. Bergamot

Bergamot is the common name for *Citrus bergamia* Risso et Poiteau, a plant belonging to the Rutaceae family (subfamily Esperidea), defined as a hybrid of bitter orange (*C. aurantium* L.) and lemon (*C. limon* L. Burm. f.) or lime (*C. aurantifolia* [Christm. and Panzer] Swingle). It may be native plant of the Calabria region (Italy), as a result of mutations from other species. Alternatively, it may originate from Antilles, Greece, and the Canary Islands, from where Christopher Columbus imported it [51]. Bergamot essential oil (BEO) is used in perfumes, cosmetics, for stress reduction and in chronic psoriasis. Juice from *C. bergamia* has been used for hyperlipidemia improving lipoprotein profile in moderate hyperlipidemia [52]. Bergamot flavonoids have also demonstrated anti-inflammatory, analgesic and anti-proliferative properties, as well as antimicrobial activity against both gram-positive and gram-negative bacteria [53]. The characterization of Bergamot flavonoids have been described in the literature [54].

2.1.1. Bergamot Essential Oil (BEO) and its Flavonoids

BEO comes from the pressing of the peel of the ripe fruit. It has been used in Italian folk medicine for centuries, especially against fevers and gastro-intestinal problems. Several other activities have also been suggested for BEO such as antioxidant and anti-inflammatory [55]. Its importance in the field of psychiatry as anxiolytic [56] has been demonstrated, as well as its *in vitro* antiproliferative effects and anticancer activities [57]. BEO also seems to be useful in the treatment of vascular disorders in which proliferation of smooth muscle cell and LD-related endothelial cell dysfunction are involved [49]. Its beneficial effects may derive from its chemical composition, consisting of amino acids, mineral salts, vitamin C and above all from the high concentration of flavonoid polyphenols. The radical scavenging and antioxidant action is determined by the inhibition of cellular myeloperoxidase (MPO) activity and by the inhibition of the adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and P-selectin involved in inflammatory processes. Furthermore, in our study we observed its ability to reduce the nuclear transcription factor-kappa B (NF-kB) translocation and the release of proinflammatory cytokines in the colitis induced in laboratory mice. NF-kB is the main regulator of the inflammatory response that drives the activation of genes associated with the transcription of inflammatory mediators, such as interleukins (ILs), tumor necrosis factor α (TNF-α) and prostaglandins (PGs), as well as inflammatory enzymes such as nitric oxide and cyclooxygenases, particularly COX2. Their inhibition is the basis for the reduction of the inflammatory process [58]. The most important flavonoids found in bergamot are briefly described. Hesperidin is one of the characteristic flavonoids of bergamot and it is mainly concentrated in the skin of the fruit. Its benefits on cutaneous functions have been attributed to its antioxidant properties, including inhibition of microtubule associated protein kinase (MAPK)-dependent signaling pathways, and stimulation of epidermal proliferation, differentiation, and lipid production [59]. As anti-inflammatory, it inhibits both gene expression and secretion of lipopolysaccharide (LPS)-induced pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) by a mechanism involving the inhibition of NF-kB activation [60]. A recent meta-analysis study suggest that hesperidin supplementation significantly improves vascular cell adhesion molecule-1 (VCAM-1) levels [61]. Naringin is a glycoside flavonoid which confers the typical bitter sour taste of bergamot and display strong anti-inflammatory and antioxidant activities [62]. These effects may be due to the reduction of the nuclear translocation of NF-kB responsible for the activation of the pro-inflammatory cytokines and to the inhibition of MPO [63]. Moreover, several studies suggest that naringin supplementation is beneficial for the treatment of obesity, diabetes, hypertension, and metabolic syndrome, by influencing AMP-activated protein kinase (AMPK)-, peroxisome proliferator-activated receptor-

alpha (PPARα)–, and carnitine palmitoyl transferase-1 (CPT-1)–mediated fat utilization and preserving mitochondrial function. It also prevents the TNFα–mediated inflammatory process and tissue damage in liver and vasculature [64]. Apigenin (4',5,7-trihydroxyflavone) is a polyphenol present in food and studied for its anti-tumor effects [65]. Like most flavonoids, apigenin has shown anti-inflammatory, antispasmodic and antioxidant properties. It may inhibit the expression and activation of inflammation mediators at the cellular level, especially ILs and NF-kB [66]. Specifically, apigenin reduces the expression of MMP-1, pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, and TNF-α) and AP-1 proteins (c-Jun, c-Fos, and JunB). It also inhibits the LPS-induced expression of iNOS and COX-2 [67]. It also has vascular properties, strengthening the dermal matrix that supports the microvascular network. This activity may be related to the inhibition of the activator protein-1 (AP-1), leading to the degradation of the collagen of the dermal matrix. This leads to greater resistance and elasticity of the vessel wall [68,69]. Naringenin is the aglycone of naringin [70]. It possesses excellent antioxidant, anti-inflammatory, and antimicrobial activities. These activities promote wound healing activity. Naringenin (0.5% and 1%), incorporated in a carbopol gel base formulation, showed a significant increase in the percentage of wound contraction, good healing index and shorter epithelization period [71]. Eriocitrin (eriodictyol 7-*O*-rutinoside) is a flavonoid with different pharmacological properties, such as antioxidant, anti-inflammatory and immunomodulatory [72,73]. It has also shown nephroprotective against cisplatin-induced renal toxicity by alleviating oxidative stress, preventing apoptosis and DNA damage [74] and has been suggested as a novel chemotherapeutic agent to treat breast cancer [75]. It acts also as an epithelizing, anti-infective, emollient, anti-edematous, local anesthetic agent and lipid-lowering agent [50,76,77]. Interestingly, eriocitrin has shown the ability to stimulate the processes of tissue regeneration in the treatment of cutaneous wounds along with a decrease in capillary permeability [78]. A diet supplemented with eriocitrin has given good results in the prevention of periodontitis [79]. Recently, eriocitrin has demonstrated a potent antinociceptive effect in postoperative pain conditions, which seems to be mediated through opioid and $GABA_A$ receptors [80].

2.2. Hyaluronic Acid

Hyaluronic acid (HA) is a natural polysaccharide that is consisted of glucuronic acid and *N*-acetylglucosamine repeats via β-1,4 linkage. Repetitive disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid linked by β (1,4) and β (1,3) glycosidic linkages, respectively, constitute the linear structure of HA [81]. HA is an essential constituent of ECMs in most mature tissues of vertebrates. It stimulates angiogenesis, moistens wounds, reduces exudation, is vasoprotective in venous leg ulcers and exerts fibrogenic action in inflamed and impaired healing tissues [82–84]. Recent studies have also shown that HA has double functionality as anticoagulant and promoter of endothelialization [85]. Several reports have demonstrated the antioxidant properties of HA [86], exerted by ROS scavenging activity thus protecting cells from oxidative stress-induced damages [87]. In a rat model of carbon tetrachloride-induced liver injury, injection of HA reduced serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) rise and lipid peroxidation, and increased superoxide dismutase (SOD) and glutathione peroxidase activities [88]. High molecular weight HA (HMWHA) with average molecular weight of 5400 kDa and 2000 kDa attenuated DNA damage in leukocytes during oxidative burst via reducing intracellular level of reactive oxidants [89]. The antoxidant ability of low molecular weight HA (LMWHA, 200–230 kDa) seems to be related to their ability to penetrate the skin preventing ROS damage in granulation tissue and promoting incisional wound healing in a rat experimental model [90,91]. The administration of LMWHA increased the activity of SOD, catalase, glutathione peroxidase, and total antioxidant capacity in cyclophosphamide-induced immunosuppressed mice [92]. The effect of oligosaccharides of HA (400–2000 Da) on epidermal stem cells was also studied. The results indicated that oligosaccharides of HA promote differentiation as well as proliferation of epidermal keratinocytes, probably by altering the expression of ECM proteins such as integrins α6 and β1 [93]. HA supports tissue regeneration and wound healing by promoting cell proliferation and migration during wound healing [94,95]. It has been observed that local administration is effective helping wounds healing due to the barrier effect protecting the injured epithelium. The effects of HA on perianal wound healing in a rat model were investigated. Topical application of HA film to perianal wounds was shown to improve the wound healing rate in a rat model, thus suggesting a potential benefit of HA film application in promoting wound healing after anal surgery in humans [96]. However, a difference was found between HMWHA and short HA oligosaccharides. The former promotes anti-inflammatory responses, whereas the latter seem to

produce inflammatory reactions. HMWHA was shown to suppress pro-inflammatory cyotokines and proteoglycans. Moreover, HMWHA suppress prostaglandin E_2 (PGE_2) production via cluster determinant 44 (CD44) through the downregulation of nuclear factor NF-κB. HMWHA also decrease MMP levels by increasing levels of tissue inhibitor of MMP-1 (TIMP-1) [97]. HA also significantly reduces levels of MPO as observed in the synovial fluid of patients who underwent arthrocentesis for the treatment of internal derangement of temporomandibular joint [98].

3. MATERIALS AND METHODS

An observational prospective multicentric study was conducted in:

- Proctological and Pelvic Floor Clinical Centre (PPFCC) of the University Hospital of Pisa
- Department of General, Oncological and Laparoscopic Surgery, Civil Hospital A.G.P. Piedimonte Matese (CE)
- U.O.S.D. of General Surgery, P.O. Basilotta (EN).

From April 2019 to January 2021 205 patients (117 males and 88 females) were enrolled for our study. The mean age was 49 years (range 18–75). All enrolled patients, underwent open haemorrhoidectomy according to the MM procedure. The exclusion criteria for the participstion in the study were: concomitant anal disease or previous proctologic surgery which could influence the study, inflammatory bowel disease (IBD) [62], previous local radiotherapy, neoplastic disease, pregnancy or breast feeding, oral anticoagulant therapy for any reason and poor general health which could make the patient unable to understand the purpose and the aim of the study. All the patients were preoperatively screened according to the current guidelines to evaluate the bowel function. Benebeo Gel® was used in the days following haemorrhoidectomy. All the patients were instructed to topically apply the gel upon injured area twice a day by using the cannula (provided with the product) and patient's finger. The administration of the product began from the 4^{th} post-operative day and. one application in the morning after defecation and one at night before sleeping was recommended for 25 days. During this period digital exploration and anoscop was routinely performed to assess the healing process while, transanal 3D 360° ultrasound and colonoscopy were performed when required. The procedures were performed with electric scalpel and energy devices as the combination of ultrasound and bipolar energy. The post-operative course was managed with a combination of paracetamol 1 gr and ketorolac 30 mg for 5 days and after that in association with oral administration of diosmine 500 mg 3 times per day for 30 days as rescue therapy to prevent post-operative haemorrhoidal acute complications. The post-operative follow-up was scheduled as follows: 7, 15, 22 and 30 days after surgery. The primary end point of this study was the time taken to get complete wound healing with a re-epithelized tissue. Secondary endpoints were evaluating post-operative pain using visual analogue scale (VAS) scale, bleeding, discharge and overall patients' satisfaction about the procedure and the topical gel.

4. RESULTS AND DISCUSSION

Study Patients' characteristics are reported in Table **2**. The mean post-operative pain at 7 days was 6±2, at 15 days 4±1, at 22 days 3 and at 30 days 2±1. The mean time to get complete wound healing was 23±4 days. There were 3 cases of post-operative bleeding (1,46%), respectively at day 7, 11, 13 post operatively, treated conservatively. All the patients referred a discharge resolution within 30 days with a significant reduction perceived within 15 days. No difference was found in safety and efficacy of gel between patients

Table 2: Patients Involved in the Study

Patients	
Age mean	47
Range	18–75
Gender	205
Male	117
Female	88
Bowel	
Regular	105
Constipation	55
Diarrhea	45
Goligher grade	
II	7
III	79
IV	119
Piles treated	
3 piles haemorrhoidectomy	85
2 piles haemorrhoidectomy	57
2 piles haemorrhoidectomy +1 mucopexy	43
3 piles haemorrhoidectomy +2 mucopexy	20

undergoing two or three piles' excision; or between the two groups treated with or without energy devices. All the patients were satisfied about the topical gel with a mean rate of 9/10 while the mean satisfaction about the procedure and healing was 7/10. Benebeo Gel®, by reducing the pro-inflammatory cytokines activation and lowering the intracellular free radicals, prevents the extracellular matrix degradation. It could help in reducing the post-operative edema and pain, with a faster post-operative recovery. The results after 2 weeks of treatment seems to be promising with a very good clinical outcome and patient satisfaction within 1 month. The investigational product showed a good profile of tolerability, safety and efficacy without any major adverse events associated with the use of the new product. Hence, the treatment of post-haemorrhoidectomy wound with bergamot-derived gel should be recommended to get a quick healing with subsequent pain reduction and faster return to normal activity.

5. CONCLUSIONS

HD is a growing health condition affecting millions of people around the world. The actual aim is to mitigate post-operative symptoms in those patients in which the preoperative anatomo-clinical condition required to perform an excisional haemorrhoidectomy according to MM procedure. In the MM technique, the wounds are left open for healing by a secondary mechanism and, until it is completely re-epithelialized requiring almost 3-5 weeks, patients usually experience pain and intense discomfort that improve over the weeks. However, post-operative pain still remains one of the main problems affecting patients QoL and frequently inducing patients to postpone the procedure. Moreover, pain in association with bleeding and anal stricture represents the most frequent complications after MM, which may require re-intervention in the short or long term. Over the years, proctologists have considered many medical and surgical options to reduce the post-operative pain. In this context, plant extracts and oils are taken in consideration in the current medical clinical practice. Among the wide group of natural products, it was shown that bioactive compounds derived from bergamot have relevant anti-inflammatory and vasoprotective properties, helping and supporting haemorrhoidal tissue. Moreover, HA contributes to maintain hydration, turgidity and viscosity. It has been involved in several processes such as tissue regeneration and matrix organization and its local administration has demonstrated effectiveness in helping wounds healing due to the barrier effect protecting the injured epithelium. Benebeo Gel®, which is an anal ointment mainly composed of bergamot polyphenolic fraction and sodium hyaluronate (the sodium salt of HA) seems to prevent the extracellular matrix degradation by reducing the pro-inflammatory cytokines activation and lowering the intracellular free radicals. It could help in reducing the post-operative pain and edema, thus leading to a faster post-operative recovery. The present study was aimed to evaluate the efficacy of this topical in patients treated with excisional haemorrhoidectomy. The results after 2 weeks of treatment seems to be promising with a very good clinical outcome and patient satisfaction within 1 month. The investigational product showed a good profile of tolerability, safety and efficacy without any major adverse events associated with the use of this product. Hence, the treatment of post-haemorrhoidectomy wound with bergamot-derived gel should be recommended to get a quick healing with subsequent pain reduction and faster return to normal activity.

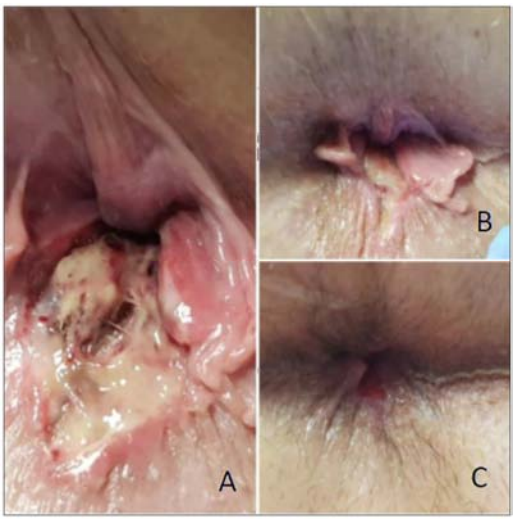


Figure 1: A) Wound after 7 days; **B**) Wound after 15 days; **C**) Wound after 22 days.

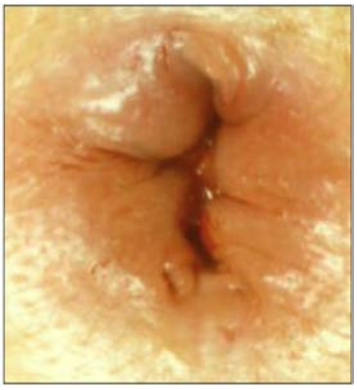


Figure 2: Wound after 30 days.

STROBE GUIDELINES

AZIENDA OSPEDALIERA-UNIVERSITARIA PISANA

Comitato Etico Regionale per la sperimentazione clinica Regione Toscana Prot. N° 222207 del 15/04/2019

ABBREVIATIONS

ALT = Alanine aminotransferase

AMPK = AMP-activated protein kinase

AST = Aspartate aminotransferase

BEO = Bergamot essential oil

CD44 = Cluster determinant 44

COS = Core Outcome Set

CPT-1 = Carnitine palmitoyl transferase-1

ECM = Extracellular matrix

HA = Hyaluronic acid

HD = Haemorrhoidal Disease

HIV = Human immune deficiency virus

HMWHA = High molecular weight hyaluronic acid

IBD = Inflammatory bowel disease

ICAM-1 = Intercellular adhesion molecule-1

IL = Interleukin

LMWHA = Low molecular weight hyaluronic acid

LPS = Lipopolysaccharide

MAPK = Microtubule associated protein kinase

MM = Milligan-Morgan

MMPs = Matrix metalloproteinases

MPFF = Micronized purified flavonoid fraction

MPO = Myeloperoxidase

NF-kB = nuclear transcription factor-kappa B

NGAL = Neutrophil gelatinase-associated lipocalin

PG = Prostaglandin

PPARα = Peroxisome proliferator-activated receptor-alpha

PPFCC = Proctological and Pelvic Floor Clinical Centre

QoL = Quality of life

RANTES = Regulated on activation normal T cell expressed and secreted

SOD = Superoxide dismutase

TIMP-1 = Tissue inhibitor of MMP-1

TNF-α = Tumor necrosis factor α

VAS = Visual analogue scale

VCAM-1 = Vascular cell adhesion molecule-1

VEGF = Vascular endothelial growth factor

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