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EXPLORING THE EFFICACY OF BETULIN IN THE DEVELOPMENT OF NOVEL LOCAL HEMOSTATIC AGENTS: A SCIENTIFIC INVESTIGATION

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This article aims to present a thorough and systematic analysis of the utilization of bioactive components obtained from diverse sources including minerals, synthetics, botanicals, and organics for the formulation of topical hemostatic agents. The study endeavors to assess the applicability of these agents, identify optimal combinations for clinical use, and scrutinize the underlying scientific principles governing their efficacy.

Hemostasis, the biological process of arresting blood flow, constitutes a crucial aspect of surgical and medical interventions. Local hemostatic agents are widely deployed to achieve hemostasis in a targeted and controlled manner, thereby mitigating the potential hazards associated with bleeding.

The use of biologically active constituents obtained from various sources, including minerals, synthetic materials, plants, and organic compounds, has an extensive history in the development of hemostatic agents. These constituents can act via multiple mechanisms, such as inducing platelet aggregation, activating the coagulation cascade, or stimulating the formation of blood clots.

Recent advancements in biotechnology and nanotechnology have led to the development of novel hemostatic agents that possess improved properties. Among these agents, a hemostatic powder named "PLANTOR" has been developed utilizing biologically active components. PLANTOR has been suggested as a supplementary method for revitalization and local coagulation during emergency medical care to stop external bleeding.

Various techniques and methods have been proposed for temporarily stopping critical external bleeding, including the utilization of a hemostatic tourniquet, compression bandage, wound tamponade, and contact hemostatic agents. However, none of these approaches are universally applicable (Rass, 2021; PGR Teixeira et al., 2018; Palmer, 2022; Sung et al., 2021).

Hemostatic powders and granules have gained popularity due to their ease of use and broad applicability in various emergency situations. These agents can quickly and effectively stop bleeding, preventing further blood loss and potentially saving lives.

Hemostatic powders and granules are a type of emergency medical treatment used to control bleeding in traumatic injuries such as gunshot wounds, blast injuries, and other severe hemorrhagic injuries. They work by promoting the rapid formation of blood clots at the site of injury, helping to stop the bleeding and prevent further blood loss.

Hemostatic powders and granules are typically made from natural or synthetic materials that are designed to be biocompatible and safe for use in humans. Some common materials used in hemostatic powders and granules include zeolite minerals, kaolin, and chitosan.

When applied to a bleeding wound, hemostatic powders and granules rapidly absorb water and other fluids from the blood, forming a clot that can help control bleeding. This process is known as hemostasis, and it is critical for saving lives in emergency situations where rapid bleeding control is essential.

Hemostatic powders and granules are often used by emergency responders in the field to control bleeding before transport to a medical facility. They can be applied directly to the wound or packed into the wound cavity to promote clotting and stop bleeding. These products are often used in conjunction with other interventions such as pressure bandages, tourniquets, and intravenous fluids to optimize patient outcomes.

Overall, hemostatic powders and granules have become an essential tool in the management of traumatic bleeding, helping emergency responders to save lives and improve outcomes for patients with severe hemorrhagic injuries.

Hemostatic biologically active components

After a comprehensive review of domestic and foreign literature, adsorbing materials derived from biologically active components of mineral, synthetic, phyto- and organic sources with a broad spectrum of action served as the basis for the hemostatic agent (Huang et al., 2020; Zhang et al., 2018; Xiang-Fei et al., 2023). However, the primary challenge in selecting the components for the tool was that most scientific studies lacked significant practical benefit for clinical implementation. While laboratory studies on rats demonstrated the mechanisms of action of the components, they did not account for regulatory guidelines on the production of the drug for clinical use. Additionally, most researchers did not address the issue of the sterility of hemostatic agents. Hence, our group of scientists conducted appropriate microbiological studies to ensure the sterility of the hemostatic agent, leading to the search for biologically active components of mineral, synthetic, phyto- and organic sources with a broad spectrum of action capable of achieving the required level of microbial contamination control for the drug.

Hemostatic powders and granules can be made from various ingredients, depending on the specific product and manufacturer. However, some of the most commonly used ingredients in hemostatic powders and granules include:

Zeolite minerals: Zeolite minerals are naturally occurring minerals that have a high surface area and can absorb water and other fluids quickly. When applied to a bleeding wound, zeolite-based hemostatic agents can promote clotting and help control bleeding.

Kaolin: Kaolin is a type of clay mineral that has been used for its hemostatic properties for centuries. When applied to a bleeding wound, kaolin-based hemostatic agents can activate clotting factors and help promote the formation of blood clots.

Chitosan: Chitosan is a biopolymer derived from chitin, a substance found in the exoskeletons of shellfish. Chitosan-based hemostatic agents work by promoting clotting and enhancing the stability of blood clots, helping to control bleeding and prevent further blood loss.

Calcium salts: Calcium salts are essential for blood clotting, and they can be used in hemostatic powders and granules to promote clotting and control bleeding.

Overall, these ingredients work by promoting the formation of blood clots and enhancing the stability of these clots, helping to control bleeding and prevent further blood loss in emergency situations. However, each of these substances has its own adverse side effects.

Zeolit.

The use of zeolite salts may be associated with certain side effects.

Local cytotoxicity of old generation hemostatic agents treated with smectite or zeolite has been observed in many studies (Wright et al. 2004, Gerlach et al. 2010, Kheirabadi et al. 2010, Li et al. 2013). H

One potential side effect of zeolite salts is the induction of local inflammation. Zeolite particles have been shown to elicit an inflammatory response in some individuals, leading to redness, swelling, and pain at the site of application. Additionally, zeolite particles may exacerbate pre-existing inflammation or cause an allergic reaction in some patients.

Another potential concern with zeolite-based hemostatic agents is the potential for systemic toxicity. Although zeolite particles are generally considered safe, excessive exposure to these particles may lead to the accumulation of toxic levels of heavy metals or other contaminants within the body. This may result in a range of adverse effects, including organ damage, metabolic disturbances, and neurological dysfunction. Erionite, a type of fibrous zeolite, is carcinogenic when inhaled (Carbone M et Al, 2011; Metintas M et Al, 2010) . Zeolites may also cause local irritation and may alter the ionic composition, pH and buffering capacity of the gastrointestinal tract under conditions of overexposure (Fruijtier-Polloth C.2009).

Chitosan

Chitosan is a natural biopolymer derived from chitin, a substance found in the exoskeleton of shellfish. It is commonly used as a dietary supplement and in various industrial and biomedical applications. Chitosan has been studied for its potential anti-inflammatory properties, but it can also induce inflammation in certain circumstances.

When chitosan is introduced into the body, it can activate immune cells and trigger an inflammatory response. This response can lead to the release of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). These cytokines can recruit and activate other immune cells, leading to the development of inflammation (Caires Hugo R. et al.,2016)

In some studies, chitosan-induced inflammation has been used to investigate the role of inflammation in the development and progression of various diseases. For example, chitosan-induced inflammation has been used to study the role of inflammation in obesity, diabetes, and cardiovascular disease. Additionally, chitosan has been shown to be effective in reducing inflammation in certain experimental models, suggesting its potential as a therapeutic agent for inflammatory diseases.

Kaolin

Kaolin is a naturally occurring clay mineral that has been used for many years in the management of various health conditions, including diarrhea, as well as a component of hemostatic powders to control bleeding. Kaolin has also been studied for its potential anti-inflammatory properties.

When kaolin is introduced into the body, it can activate immune cells and trigger an inflammatory response. This response can lead to the release of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). These cytokines can recruit and activate other immune cells, leading to the development of inflammation (Wiemann M., et al.,2020).

However, in some studies, kaolin has been shown to have anti-inflammatory effects. Kaolin can bind to and neutralize pro-inflammatory cytokines, such as IL-1β and IL-6, and reduce their levels in the body. Additionally, kaolin has been shown to inhibit the activation of certain immune cells, further reducing inflammation.

Calcium salts

Calcium salts are an essential component of the blood clotting cascade, and they are often used in hemostatic powders and granules to promote clotting and control bleeding in wounds. However, excessive or inappropriate use of calcium salts can lead to side effects and complications.

One potential side effect of calcium salts in wounds is the formation of an excessive blood clot, which can impede blood flow to surrounding tissues and cause tissue damage or death. This can occur if too much hemostatic agent is applied to the wound or if the agent is not properly removed once the bleeding has stopped.

Additionally, if calcium salts are not used appropriately, they may cause irritation and inflammation in the wound. This can lead to delayed wound healing, infection, and other complications. In some cases, the use of calcium salts may also cause allergic reactions, particularly in individuals with a history of hypersensitivity to these compounds.

Cellulose-based hemostatics

Cellulose-based hemostatics products have some limitations that should be considered prior to their use; due to their poor adhesion to the tissues in wet environments, their effective deployment may prove difficult in this setting. The application of adequate pressure at the site of hemorrhage is needed to provide the tamponade necessary to improve their effectiveness. The effect of these products depends on an intact coagulation mechanism, and their use in patients with coagulopathy is limited. Some of the complications associated with the use of local cellulose-based hemostatics products consist of local inflammatory reaction, ischemia due to compression, injury to the ureters, nerves and other specialized structures because of local desiccation and promotion of infection and abscess formation (Recinos G 2008; Khoshmohabat H 2019).

In light of Russian aggression against Ukraine, we conducted an analysis of medical examination data of the wounded and causes of death in hostilities, which revealed massive blood loss as one of the leading causes of fatalities (Mazuchowski et al., 2020; Kotwal et al., 2018; Howard et al., 2019).

The composition of the hemostatic agent had to be modified to meet European standards and requirements, and technical conditions for the production of the hemostatic agent - PLANTOR powder (TU U 20.4-44827581-001:2022) - had to be developed.The hemostatic powder "PLANTOR™ " operates through a special sorption effect from biologically active components of phyto- and organic origin, including carrageenan of a specific brand, and pharma grade Betulin. The product's well-designed composition results in the biophysical process of liquid absorption, causing "PLANTOR ™" to form a blood clot in the wound.

Kappa Carrageenan serves as the foundation for the composition of the hemostatic agent PLANTOR. The selection of Carrageenan was predicated on three key factors: 1) its ability to swiftly and effectively halt bleeding (Kumandas, A. et al,2022); 2) its capacity to facilitate rapid wound healing; and 3) its tendency to elicit fewer side effects (Binnetoğlu, K., 2021)

Kappa Carrageenan has demonstrated potent hemostatic properties, enabling it to rapidly staunch bleeding at the site of application. Moreover, it is known to promote wound healing by stimulating the proliferation of cells involved in tissue regeneration. Finally, Carrageenan has been shown to be generally well-tolerated, making it an attractive choice for use in hemostatic agents.

The utilization of kappa Carrageenan as a fundamental component of PLANTOR underscores its efficacy and safety in the management of bleeding wounds. By leveraging the hemostatic and wound-healing properties of Carrageenan, PLANTOR is well-positioned to serve as a valuable tool for healthcare professionals in the field of emergency medicine.

Carrageenan is a polysaccharide derived from red seaweed and is commonly used as a food additive and thickening agent. It is also used in laboratory studies to induce inflammation in animal models.

When carrageenan is introduced into the body, it triggers an immune response that leads to the release of inflammatory mediators such as cytokines, histamine, and prostaglandins. These mediators cause blood vessels to dilate and become more permeable, allowing immune cells to migrate to the site of injury or inflammation.

This process, known as acute inflammation, is a natural defense mechanism of the body to protect against harmful pathogens or tissue damage. However, when inflammation is prolonged or excessive, it can lead to chronic inflammation and tissue damage.

Studies have shown that carrageenan-induced inflammation can be used to study the mechanisms underlying acute and chronic inflammation, as well as the development and progression of inflammatory diseases such as arthritis, inflammatory bowel disease, and cancer.

Despite the benefits of kappa Carrageenan as a hemostatic agent, its use can be associated with the induction of local inflammatory processes. To address this concern, the interaction of various molecules with Carrageenan was explored in order to identify potential drawbacks of PLANTOR. In our investigations, we identified betulin, a safe and natural compound capable of mitigating the inflammatory responses triggered by Carrageenan.

By examining the available literature, we determined that betulin possesses anti-inflammatory properties that make it an ideal candidate for use in conjunction with Carrageenan. Betulin has been shown to modulate various cellular pathways that contribute to inflammation, thereby reducing the local inflammatory reactions associated with Carrageenan.

The identification of betulin as a potential additive to PLANTOR highlights our commitment to developing hemostatic agents that are both effective and safe. By supplementing Carrageenan with betulin, we are able to harness the hemostatic properties of Carrageenan while simultaneously minimizing its potential to elicit local inflammation. Wounds usually are infected and antibacterial activity is critically important. Overall, this approach represents a promising avenue for improving the efficacy and safety of hemostatic agents.

Betulinic acid (BA) is a natural triterpenoid compound found in a variety of plants, including birch trees, and it has been studied for its potential anti-inflammatory properties. Studies have investigated the effects of BA on Carrageenan-induced inflammation in animal models.

Studies have found that treatment with BA can reduce the severity of Carrageenan-induced inflammation, as evidenced by decreases in paw swelling, inflammatory cell infiltration, and levels of pro-inflammatory cytokines.

The mechanisms by which betulinic acid exerts its anti-inflammatory effects are not fully understood, but some studies suggest that it may modulate the activity of pro-inflammatory enzymes, such as cyclooxygenase-2 (COX-2), and reduce the levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).

Betulin is also used as plaster for sterilization of wounds.

Bernard et al, determined that Betulin and betulinic acid (BA) were found to inhibit phospholipaseA2 activity at 5 M concentrations by 30% and 40% respectively (Bernand et al., 2001). It has also been demonstrated to exhibit inhibitory effects on nitric oxide (NO) and prostaglandin E2 production in mouse macrophages [94]. According to the latest research done by Preet Amol Singh et al, stem bark extract of Dillenia indica f. elongata (Miq.) Miq. showed significant (P <0.01) anti-inflammatory activity in formalin and carrageen an induced inflammation models (Singh AP 2016). Moreover, betulinic acid was found to exihibit anti-ulcer action for the models in which the infection of mucus coat of stomach in rats is caused by indomethacin and aspirin with the dose of 50 mg/ kg (Das SK., 2006).

BA administered orally at 100 mg/kg, alsos promoted a reduction in paw edema induced by carrageenan (Recio et al., 1995). Further studies confirmed the anti-inflammatory activity of BA in carrageenan-induced edema, in doses ranging from 10 to 100 mg/kg, mainly by oral route (Mukherjee et al., 1997; Tsai et al., 2011; Oyebanji et al., 2014; Armah et al., 2015; Ou et al., 2019). Importantly, when administered by intraperitoneal route, BA also reduced carrageenan-induced paw edema in Wistar rats (Oyebanji et al., 2014). In addition, Oyebanji et al. (2014) observed a reduction in carrageenan-induced-pulmonary edema in Wistar rats treated intraperitoneally with BA at 10, 20 or 40 mg/kg. The main mechanism associated with carrageenan-induced inflammation is well characterized and involves the reduction of inflammatory mediators such as COX-2, Il-1β, NO, PGE2, and TNF (Salvemini et al., 1996; Tsai et al., 2011). In addition, the inhibition of antioxidant enzymes, lipid peroxidation, and production of free radicals, such as hydrogen peroxide, superoxide, and hydroxyl radical in the liver, are common features in carrageenan-induced inflammation related to cell injury (Cuzzocrea et al., 1999). Interestingly, treatment with BA decreased the production of the inflammatory mediators described above at the inflammation site and increased enzyme activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRd) in the liver (Tsai et al., 2011; Ou et al., 2019). Moreover, BA decreased malondialdehyde (MDA) levels, a key mediator of oxidative stress and widely used as a marker of free radical mediated lipid peroxidation injury, at the inflammation site (Tsai et al., 2011). Lastly, Ou et al. (2019) provided evidence that BA downregulates MAPK signaling pathways (ERK1/2, JNK, and p38) in the paw edema tissue, which, in part, explains the inhibition of cytokine production (IL-1β and TNF), COX-2 expression, and PGE2 production.

In accordance to Valterová et al, the antibacterial activity of C-3 substituted derivatives of betulin with respect to a number of bacteria (Staphylococcus aureus, Staphylococcus faecalis and Staphylococcus beta haemolyticus) was depicted (Prachayasittikul S et Al.,2010). Furthermore, Hess et al, concluded that Betulinic acid has been found to be inactive against Staphylococcus aureus, Escherichia coli, Bacillussubtilis and Micrococcus luteus (Yli Kauhaluoma J.,2007). Antimicrobial activity of betulin and its derivatives have been reported against Streptococcus pyogenes with a minimum inhibitory concentration (MIC) of 85 μg/mL, and considerable activity has also been observed against other bacteria, i.e. Escherichia coli, Staphylococcus aureus and Enterococcus faecalis (Steele JC et Al.,1999)

Betulinic acid proved to be a versatile molecule, able to modulate a number of key mediators in the inflammatory process, including COX-2, ICAM-1, IL-1β, IL-6, IL-12, MCP1, PGE2, and TNF, both in vitro and in vivo, in different disease models (Figures 2, 3). Most of these effects related to inhibition of NF-kB and MAPK pathways. Importantly, BA promotes the production of IL-10, a critical anti-inflammatory mediator able to modulate several immune cell types (Saraiva and O'Garra, 2010). Moreover, BA can be produced by synthetic routes and its structural changes have generated more potent and selective derivatives, making its use as a prototype for the generation of new classes of anti-inflammatory drugs promising. In order to develop BA-based treatments, there is a need for toxicological, as well as clinical studies that will demonstrate the safety and efficacy of this compound in inflammatory and immunemediated diseases.

The hemostatic agent-powder "PLANTOR" (TU U 20.4-44827581-001:2022) was evaluated for clinical efficacy in emergency medical care provision for victims with trauma in pre-hospital (emergency medical aid teams) and early hospital (emergency medical aid department) settings. A total of 78 victims with isolated trauma and polytrauma were treated for critical and non-critical bleeding according to the recommendations of the product owner LLC "Plantor" (Ukraine, Dnipro). The average age of the victims was 43.5 (27; 58) years, and the average body weight was 78.7 (64.3; 94.6) kg.

Upon contact with blood, "PLANTOR" powder absorbed plasma and formed a blood clot that swelled and stuck together into a single thick mass, which prevented further bleeding and significantly reduced blood loss. The microbiological purity and antiseptic component of the product effectively prevented the development of wound infections, and it did not delay regeneration or natural wound healing. The product was easily washed out of the wound during surgical treatment and did not cause anaphylaxis, inflammatory complications, or thermal or chemical burns. Furthermore, it showed efficacy in stopping bleeding under conditions of hypothermia and in the presence of antiaggregants and anticoagulants.

"PLANTOR" powder was found to be effective in treating critical and non-critical external bleeding, including all bleeding wounds, lacerations, sores, and cuts. The product can be used by pouring quantum satis into the victim's wound and immediately tamponing the wound for 2-3 minutes in cases of critical external bleeding. For non-critical external bleeding, the wound should be tightly bandaged after pouring the product into the wound.

Based on the above information, we recommend the use of the hemostatic agent-powder "PLANTOR," which is produced by "Medpro Nutraceuticals" (Latvia) on behalf of LLC "Plantor" (Ukraine, Dnipro), the owner and developer of the "PLANTOR" technology. This hemostatic agent-powder (TU U 20.4-44827581-001:2022) is recommended as an auxiliary revitalization tool for local coagulation and wound surface treatment during emergency medical care for external bleeding.

Currently, Ukraine is in urgent need of hemostatic agents, and the "PLANTOR" tool has the potential to save the lives of both military and civilian personnel in field conditions where evacuation to a hospital is difficult. These agents quickly stop bleeding in challenging situations, making their presence crucial.

The "PLANTOR" hemostatic agent-powder is a remarkable example of combining Ukrainian folk medicine traditions, the knowledge and expertise of folk healers, the Cossack characters of Zaporizhzhya Sich, and contemporary researchers, production organizers, and volunteers. In a short amount of time, they managed to develop and organize the production of such an essential tool.

The hemostatic agent "PLANTOR" was developed through volunteer efforts and is currently being provided to Ukrainian military brigades and TRO at no cost until they are fully supplied. We express our sincere gratitude to the Academy of Technological Sciences of Ukraine, the Official Representation of the International Nobel Information Center in Ukraine, the Charity Fund "Volunteers of the World," LLC "Sokolovsky - Fortuna," Dnipro Medical Institute of Traditional and Non-Traditional Medicine, Zaporizhzhia State Medical University, and specialists from the University of Pharmacy in Kharkiv for their scientific and organizational support and advice in the development of this important tool.

Keywords: hemostasis, local hemostatic agents, betulin, betulinic acid, biologically active components, PLANTOR, wound treatment.

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