DEVELOPMENT OF A NOVEL MINERAL BASED HAEMOSTATIC AGENT CONSISTING OF A COMBINATION OF BENTONITE AND ZEOLITE MINERALS

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Background: Haemorrhage remains the greatest threat to life on the battlefield, accounting for half of all deaths. Over the past decade the US army has widely studied new technologies for stopping sever haemorrhages and has introduced an effective zeolite based haemostatic agent. In this paper the bio-stimulatory effect of burned radioactive lantern mantles powder as well as two minerals; bentonite and zeolite are presented. **Methods:** In this experimental study, 50 male Wistar rats were divided randomly into 5 groups of 10 animals each. Following anaesthesia, animals' tails were cut off at a thickness of 5 mm by using a pair of surgical scissors. No intervention was made on the animals of the 1st group. The 2nd to 5th group received topical non-radioactive lantern mantle powder, radioactive lantern mantle powder, Bentonite mineral or a mixture of bentonite-zeolite minerals respectively. After treatment with above mentioned agents, the volume of blood loss was measured using a scaled test-tube. The bleeding time (BT) and clotting time (CT) were also measured using a chronometer. Analysis of variance (ANOVA) was used for comparing the means of each parameter in the 5 groups. **Results:** The volume of blood loss, bleeding and clotting time in control animals were 4.39±1.92 ml, 112.10±39.60 sec and 94.9±54.26 sec respectively. In the 2nd group, in which the animals were treated with a non-radioactive lantern mantle, the volume of blood loss, bleeding and clotting time were 2.34±0.35 ml, 54.50±14.77 sec and 22.9±6.54 sec, respectively. In the 3rd group, in which the animals were treated with a radioactive lantern mantle, the volume of blood loss, bleeding and clotting time were 1.50±0.58 ml, 37.10±7.81 sec and 33.5±15.76 sec respectively. In the 4th group, in which the animals were treated with bentonite mineral, the volume of blood loss, bleeding and clotting time were 1.81±0.62 ml, 55.70±16.73 sec and 45.9±32.17 sec, respectively. In the 5th group, in which the animals were treated with a mixture of bentonite-zeolite minerals, the volume of blood loss, bleeding and clotting time were 1.31±0.60 ml, 34.50±4.65 sec and 24.2±4.61 sec, respectively. **Conclusion:** To our knowledge, this is the 1st study to investigate the alterations of bleeding and clotting time following the use of lantern mantle powder as well as bentonite or the mixture of bentonite-zeolite minerals. The results obtained in this study clearly show the significant alterations in the volume of blood loss as well as the bleeding or clotting time following the topical use of the mixture of bentonite-zeolite minerals. Controlling the generation of heat was a great achievement in development of the novel haemostatic agent produced in this study.

Keywords: Bleeding Time, Clotting Time, Radioactive Lantern Mantle, Minerals, Bentonite, Zeolite

INTRODUCTION

It has been widely reported that haemorrhage is still the greatest threat to life on the battlefield, accounting for half of all deaths.[1] Furthermore, it has recently been indicated that today there is an increase in the pattern of extremity vascular injury compared with previous wars.[2,3] On the other hand, bleeding represents the second leading cause of civilian trauma deaths, including the vast majority of patients who die in the operating room following major trauma.[4] Achieving rapid haemorrhage control and initiating a haemostatic resuscitation plan for correcting metabolic imbalances as well as preventing the onset or progression of a traumatic coagulopathy are among the standard damage control principles.[5,6]

Over the past decade the US army has widely studied new technologies for stopping sever haemorrhages. The Food and Drug Administration (FDA) has given clearance on QuikClot (Z-Medica Corp., Wallingford, CT); a haemorrhage control agent that is being used by American soldiers wounded on the battlefield. The US army has confirmed the efficacy of QuikClot that is a zeolite based haemostatic agent.[7] QuikClot is composed of porous, aluminosilicate beads, offering a large surface area. Zeolites are the aluminosilicate members of the family of microporous solids known as 'molecular sieves'. The term molecular sieve refers to a particular property of these materials, i.e., the ability to selectively sort molecules based primarily on a size exclusion process. This is due to a very regular pore structure of molecular dimensions. When zeolite comes into contact with blood, it rapidly adsorbs water from the blood and holds the water molecules in the pores by hydrogen bonds. The manufacturers of QuikClot believe that the effect of locally concentrating the proteins and cellular elements can catalyse the clot formation. Additionally, the nano-engineered negative charge surface beads provide key surface chemistry, rapidly activating the coagulation process.[8–14]

In an attempt to find an economical radon-resistant construction technique for developing efficient national radon-reduction programs, Mortazavi and his colleagues used bentonite and zeolite minerals.[15] Bentonite can be defined as an aluminium phyllosilicate absorbent that generally contains impure clay consisting mostly of montmorillonite, Na, Ca) 0.33 (Al, Mg) $2Si_4O_{10}(OH)_2.(H_2O)_n$. Two types of bentonite are available: swelling bentonite, which is also called sodium bentonite, and non-swelling bentonite or calcium bentonite. Bentonite is formed from the weathering of volcanic ash, most often in the presence of water. After combination with water (hydration), bentonite has an enormous surface area. In other words bentonite absorption leads to a swelling phase, a phase in which it stretches open like a highly porous sponge. Bentonite attracts toxins like heavy metals, free radicals, and pesticides to its extensive surface area.

Bentonites are widely used for waste repository systems because of their hydrodynamic, surface and chemical-retention properties.[16] Highly compacted bentonite is usually used as the buffer material around the waste package. Water-saturated highly compacted bentonite is an ideal barrier material because of its physical and chemical properties.[17]

Zeolite is a naturally occurring mineral group consisting of over 50 different minerals. It is made of a special crystalline structure that is porous[18–19] but remains rigid in the presence of water. Zeolites can be adapted for a variety of uses, including water filtration, heavy metal removal[20] and gas separations. Zeolite can also be used in radioactive waste management for site remediation/decontamination[21]. There is a wide variety of naturally occurring and synthetic zeolites, each with a unique structure.

Zeolites have an ‘open’ structure that can accommodate a wide variety of cations, such as Na^+, K^+, Ca^{2+}, Mg^{2+} and others. These positive ions are rather loosely held and can readily be exchanged for others in a contact solution. Zeolites have a rigid, three-dimensional crystalline structure (similar to a honeycomb) consisting of a network of interconnected tunnels and cages. Water moves freely in and out of these pores but the zeolite framework remains rigid. The porous zeolite is host to water molecules and ions of potassium and calcium, as well as a variety of other positively charged ions, but only those of appropriate molecular size to fit into the pores are admitted, creating a ‘sieving’ property. One important property of zeolite is the ability to exchange cations. This is the trading of one charged ion for another on the crystal.

On the other hand, Mortazavi and his colleagues recently studied the bio-stimulatory effects of the topical application of radioactive lantern mantle powder on wound healing.[22] Furthermore, they have shown significant changes in some histological parameters concerning healing.[23] In some parts of Iran, poorly educated people use lantern mantle powder as a therapeutic agent for enhancing wound healing unaware of its radioactivity and possible dangers. Some lantern mantles which are commonly used for camping contain different levels of thorium compounds.[24] Thorium is used in mantles to produce incandescence when lantern fuel is burned in the mantle. Although only thorium is initially used in the mantles, the daughters of thorium build up, and when the mantle is used significant quantities of these daughters exist. Some of these daughters are released when the lantern fuel is burned in the mantle.[25] Recently, in some developed countries the use of thorium-free mantles has become popular due to the risks associated with the use of a radioactive heavy metal.[26] Thorium oxide is a known human carcinogen. In Australia, in November 1992, the National Health and Medical Research Council (NHMRC) recommended that lantern mantles containing thorium should be withdrawn from sale over time and that in the meantime, packets containing these lantern mantles should carry a warning.[27] In this paper the bio-stimulatory effects of burned non-radioactive and radioactive lantern mantles powder as well as two minerals; bentonite and zeolite are presented.

MATERIALS AND METHODS

Intact male Wistar rats (200–250 g body weight) were kept on standard laboratory diet with free access to water, under 12-h light-dark cycles. In this experimental study, 50 male rats were divided randomly into 5 groups of 10 animals each. Before cutting off the animals’ tails, the animals were anesthetized with ether until the eyelid closure reflex is lost. All animals were positioned in a glass chamber with a wire mesh at the bottom to avoid direct contact of the ether with the skin of the rat. Ether is poured into the closed chamber immediately before the animal is put into it. Following anaesthesia, animals’ tails were cut off at a thickness of 5 mm using a pair of surgical scissors. No intervention was made on the animals of the 1st group. The 2nd to 5th group received topical non-radioactive lantern mantle powder, radioactive lantern mantle powder, bentonite mineral or a mixture of bentonite-zeolite minerals respectively (Figure-1). After treatment with above mentioned agents, the volume of blood loss was measured using a scaled test-tube. The bleeding time was measured using a chronometer. To measure the clotting time, cardiac puncture was used for blood withdrawal. In this paper, what is referred to as clotting time is the time required from the instant the blood taken into a blood collecting tube is started on its coagulation process to the time when the blood no longer flows when the tube is placed in an inverted position.[28] Analysis of variance (ANOVA) was used for comparing the means of each parameter in the 5 groups.

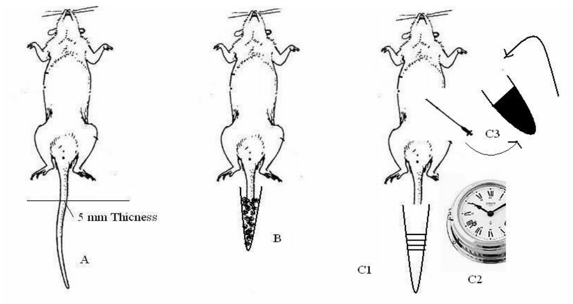

Figure-1. Three different stages of the experiment. A: Following anaesthesia, animals' tails were cut off at a thickness of 5 mm by using a pair of surgical scissor. B: Treatment with different minerals by inserting the animal tail in a powder containing tube. C: Measuring the volume of blood loss by using a scaled tube C1. and measurement of bleeding time by a chronometer C2. and measuring the clotting time by tilting the test tube and measuring the time when the blood no longer flows when the tube is placed in a horizontal position. (C3).

RESULTS

The volume of blood loss, bleeding and clotting time in control animals were 4.39±1.92 ml, 112.10±39.60 sec and 94.9±54.26 sec respectively (Table-1, Figures-2–4). In the 2nd group, in which the animals were treated with a non-radioactive lantern mantle, the volume of blood loss, bleeding and clotting time were 2.34±0.35 ml, 54.50±14.77 sec and 22.9±6.54 sec respectively. In the 3rd group, in which the animals were treated with a radioactive lantern mantle, the volume of blood loss, bleeding and clotting time were 1.50±0.58 ml, 37.10±7.81 sec and 33.5±15.76 sec respectively. In the 4th group, in which the animals were treated with bentonite mineral, the volume of blood loss, bleeding and clotting time were 1.81±0.62 ml, 55.70±16.73 sec and 45.9±32.17 sec respectively. In the 5th group, in which the animals were treated with a mixture of bentonite-zeolite minerals, the volume of blood loss, bleeding and clotting time were 1.31±0.60 ml, 34.50±4.65 sec and 24.2±4.61 sec, respectively.

Table-1: The mean volume of blood loss, bleeding and clotting time in control animals compared to those of the animals treated with non-radioactive lantern mantle, radioactive lantern mantle, bentonite and the mixture of bentonite-zeolite minerals.

Groups \ Parameters Tested	Blood Loss* (ml)	Bleeding Time* (sec)	Clotting Time* (sec)
Control	4.39 ±1.92†	112.10±39.60†	94.9 ±54.26†
Non-radioactive lantern mantle	2.34±0.35	54.50±14.77	22.9 ±6.54+
Radioactive lantern mantle	1.50±0.58	37.10±7.81	33.5±15.76
Bentonite	1.81±0.62	55.70±16.73	45.9±32.17
Bentonite-Zeolite	1.31±0.60+	34.50±4.65+	24.2±4.61
p-Value (ANOVA)	$p<0.0001$	$p<0.00001$	$p<0.01$

*Mean±SD, +Lowest Value in Column; †Highest Value in Column

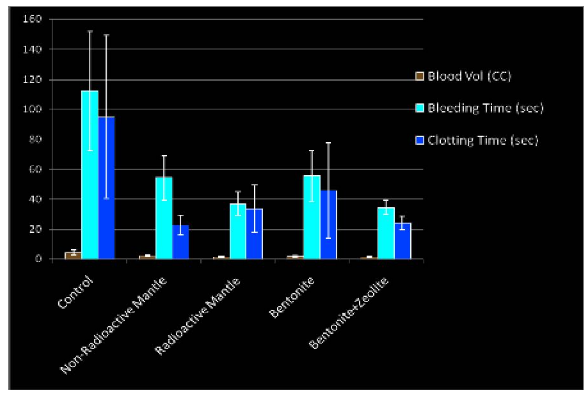

Figure-2: Comparison of the volume of the blood loss, bleeding and clotting time in control animals with those treated with non-radioactive lantern mantle, radioactive lantern mantle, bentonite and the mixture of bentonite-zeolite minerals.

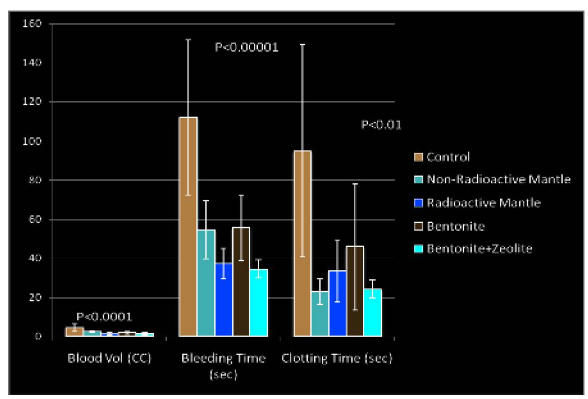

Figure-3: Alterations in the volume of the blood loss, bleeding and clotting time in control animals with those treated with non-radioactive lantern mantle, radioactive lantern mantle, bentonite and the mixture of bentonite-zeolite minerals.

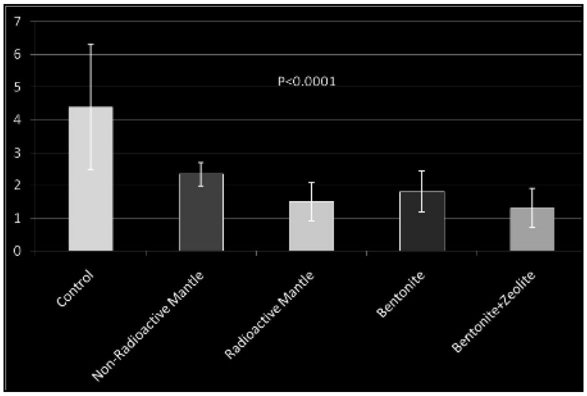

Figure-4: Volume of the blood loss in control animals with those treated with non-radioactive lantern mantle, radioactive lantern mantle, bentonite and the mixture of bentonite-zeolite minerals.

DISCUSSION

This is the 1st study to look at the alterations of BT and CT following the use of lantern mantle powder as well as bentonite or the mixture of bentonite-zeolite minerals. The results show significant alterations in volume of blood loss as well as BT or CT following the topical use of the mixture of bentonite-zeolite minerals. The radioactive and non-radioactive lantern mantle and the bentonite mineral alone had considerable effects but altogether the magnitude of effect was less than that of mixture of bentonite-zeolite minerals. The rationale in our study for using bentonite was the extraordinary effectiveness of bentonite in water absorption. Once hydrated or combined with water, bentonite has an enormous surface area. Due to its superfine particles, it has been reported that many bentonite clays are known to possess a surface area of several hundred meters squared per gram.[29]

The use of topical agents to gain haemostatic control of vascular injuries sustained in combat remains a challenge.[30] It has been reported that haemorrhage in trauma accounts for 30–40% of all fatalities, second only to central nervous system injury as a cause of death. However, hemorrhagic death is the leading preventable cause of mortality in combat casualties.[31] It has been also reported that uncontrolled haemorrhage is a leading cause of traumatic death.[32] Zeloite and Chitosan[§] are the most important new generation of local hemostatics.[33]

The efficacy of granular QuikClot (QC) in Clinical use on the battlefield has been investigated and follow up of 103 cases, has shown an efficacy rate of 92%. The failure of the dressings was attributed to second bleeding, or ‘re-bleeding’, leading to death.[34]

As a great achievement, in this study we controlled the heat generation that is among the great concerns associated with the use of zeolite. Despite the significant efficacy of zeolite in haemostasis, when water is absorbed by the zeolite and trapped by hydrogen bond formation, heat is generated. In this light, the induction of exothermic reactions generated from water absorption by inert inorganic elements of zeolite (aluminium/silicate), potential burns are major concerns. The heat generated by zeolite in contact with aqueous components of the blood in wounds has been reported to reach up to 70 ºC.[10,12] Potential burns induced by application of QuikClot has been a concern of users and is a reported negative aspect of these zeolite products.[8,10,34–36] The issue of heat generation has been addressed by ‘cooler’ non-exothermic QuikClot (ACS+) a product in which the zeolite is preloaded with some water (hydration) to significantly reduce heat generations while maintaining the clotting efficacy.[14] In an attempt to compare the elevation of temperature of these formulations upon contact with blood, Arnaud et al found that temperature recorded at the dressing/tissue interface was significantly lower with ACS+ vs ACS (40.3±1.8 vs 61.4±10.7 ºC, respectively, $p<0.01$).[7] In our study the maximum temperature rise in the site of the wound was 5 ºC which can be tolerated by the human tissues.

CONCLUSION

This study clearly showed significant alterations in the volume of blood loss as well as the bleeding or clotting time following the topical use of the mixture of bentonite-zeolite minerals. The haemostatic agents developed so far can produce undesirable side effects such as burns induced by the exothermic reactions. Controlling the generation of heat was a great achievement in development of the novel haemostatic agent produced in our study. The radioactive and non-radioactive lantern mantle and the bentonite mineral alone had considerable effects but altogether the magnitude of the effect was less than that of the mixture of bentonite-zeolite minerals.

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[§]*Chitosan is produced commercially by deacetylation of chitin , which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.). Chitosan's properties allow it to rapidly clot blood, and has recently gained approval in the USA for use in bandages and other hemostatic agents. Chitosan purified from shrimp shells is used in a granular hemostatic product, Celox, made by Medtrade Biopolymers Inc. of Crewe, England and in the chitosan dressings made by HemCon Medical Technologies Inc. of Portland, OR, USA.*

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