A PILOT STUDY ON THE EFFECT OF A HOMOEOPATHIC REMEDY *ARNICA MONTA.NA* 12 *CN* ON BLOOD COAGULATION

By

VICKY AYESHAMOTALA
STIJDENT NUMBER: 9416118

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SPECIALIST SUPERVISOR:
DR. JENNIE L. CALVERT-EVERS

SUPERVISOR:
DR. BRIAN R. VAN OLDEN

DECLARATION

I declare that this dissertation is my own unaided work. It is being submitted for the Degree of Master in Technology: Homoeopathy at the Technikon Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to this or any other Technikon or University.

In the Dame of Allah, most Gracious, most Merciful.

ABSTRACT

The purpose of this study was to determine the effect of the Homoeopathic remedy *Arnica montana* 12 CH on blood coagulation. For years *Arnica montana* has been used to treat injuries where there is bleeding and bruising. The arrest of bleeding is accomplished by a number of mechanisms, one of which is blood coagulation.

The study was conducted using 21 volunteers with each person acting as his or her own control Two blood samples were collected from each patient over a three day period. After preparing fresh whole blood samples and platelet-poor plasma, full blood count and coagulation assays were run using the Cell-Dyn 1700 system and the Automated Coagulation Laboratory analyser respectively. The average from the two days were calculated and statistically analysed using the Two way ANDVA method of analysis.

Results showed that the differences between the experimental group and the control group were too small to be statistically significant. This showed that *Arnica montana* 12 CH had no significant effect on blood coagulation. It is interesting to note that the alcohol group had almost the same results as the experimental group.

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1.0 INTRODUCTION

1.1 The Purpose of the Study

Blood is an essential element to life. Today's modern medicine often relies on it as a tool to determine the cause of disease. Acting as a life-sustaining transport fluid, up to 8% of our body weight is made up of blood (Marieb, 1992). Blood usually flows through the intact circulatory system. If a blood vessel wall breaks, a number of reactions take place to accomplish haemostasis. Haemostasis requires the combined activity of vascular, platelet and coagulation factors counterbalanced by mechanisms to limit the accumulation of platelets and fibrin to the area of trauma. Haemostatic abnormalities can lead to excessive bleeding or thrombosis (Berkow, 1992).

Homoeopathically prepared *Arnica montana* is known to be useful in treating injuries when there has been extravasation of blood. For this reason it is especially beneficial in treating contusions, sprains and after surgical trauma (Morrison, 1993).

This research aimed at identifying the effect of *Arnica montana* 12 CH on blood coagulation in particular. Scientifically valid research is needed to determine its specific action and prove its efficacy in treating certain conditions as well as to establish safety measures.

Screening tests can be carried out to assess the efficacy of the clotting cascade and may be used to detect cloning factor deficiencies. The tests used in this study include:

- Prothrombin Time (PT) test: measures the overall efficiency of the external pathway (Dacie and Lewis, 1995);

- Partial Thromboplastin Time (pTI) test: this measures the intrinsic and common pathway;
- Thrombin Time (TT) test: measures the following sequence: Fibrinogen. fibrin monomer - initial clot;
- Fibrinogen assay: fibrinogen conversion to fibrin (Babior and Stossel, 1994).

These tests are described in greater detail in Chapter 2.

1.2 Blood Coagulation

1.2.1 Introduction

Blood is a connective tissue - the only fluid tissue in the body. Living red blood cells are suspended in a non-living matrix or plasma. When a blood sample is mixed with an anti-coagulant and spun in a centrifuge, erythrocytes settle at the bottom while the less dense plasma rises to the top. A thin white layer (huffy coat) containing platelets and leucocytes separates the plasma and erythrocytes (Marieb, 1992).

Blood has a number of functions that include distribution (of oxygen, carbon dioxide and hormones), regulation (maintenance of appropriate body temperature, body tissue pH and adequate fluid volume) and protection (prevention of infection, blood loss) [Marieb, 1992].

These functions rely on the maintenance of haemostasis, i.e. maintaining normal blood flow. Blood remains in a fluid state while circulating in the vascular system. Bleeding at the site of injury is arrested by formation of a haemostatic plug that must be removed once healing is achieved (Dacie and Lewis, 1995).

1.2.2 Historical background

Throughout the centuries, people have formulated theories on blood coagulation. More than 2000 years ago, Ancient Greeks had knowledge of the components of blood, recognised its liquid intravascular state and observed clot formation. In the 17th century, Malpighi observed that strands of fibres remained after washing a blood clot. In 1845 Buchanan noted that fibrin was formed when plasma and serum were mixed. Hammerstein isolated fibrinogen in 1877 and Arthus and Pagis showed the importance of calcium in the coagulation process (Cronje, 1985).

Morawitz proposed the classic theory of blood coagulation in 1905. He explained that tissue extract (thromboplastin) accelerated blood coagulation in the presence of calcium. According to his theory, in Stage I prothrombin requires calcium + thromboplastin to convert into thrombin. In Stage II fibrinogen is converted by thrombin to fibrin (Cronje, 1985).

Although this theory is outdated, it does provide a basis as it divides the clotting process into three major stages:

- Firstly, an activator is generated that activates prothrombin.
- A second step, in which the prothrombin activator converts prothrombin to thrombin.
- Thirdly, thrombin converts fibrinogen to fibrin (Rapaport, 1971).

Except for factor XIII all clotting factors discovered since Morawitz's theory are suggested to participate in the generation of a prothrombin activator (Rapaport, 1971).

1.2.3 Coagulation pathways

Coagulation occurs due to activation of either the extrinsic or the intrinsic pathway that ultimately leads to the formation of a fibrin clot (common pathway).

(i) Extrinsic Pathway

Tissue factor is a co-factor for the extrinsic pathway. It is a lipoprotein, which is membrane bound and present in many tissues. When cells are damaged, their tissue factor is exposed to factor VII, a vitamin K-dependant protein in the blood, and a tissue factor - VII complex forms in the presence of calcium. This complex can activate both factor X and factor IX, thus stimulating two routes to thrombin production. The tissue factor-VII complex is a feeble protease that slowly activates factor X to factor Xa. Factor Xa in turn transforms the tissue factor - VII complex into a more potent complex that can now convert factor X to Xa much faster than it did before its activation. By activating factor X, Factor VII accelerates its own activation and vice versa indicating that this is a positive feedback system (Babior and Stossel, 1994; Daeie and Lewis, 1995). This is a very rapid action and occurs in 10 to 14 seconds. In this pathway platelets or platelet factors are not required for clot formation (Cronje, 1985).

Defects in any of the above mentioned factors lead to a prolonged one stage prothrombin time (Cronje, 1985). Common causes of this include the administration of oral anticoagulant drugs, liver disease, vitamin K deficiency, disseminated intravascular coagulation and previously undiagnosed factor VII, X or prothrombin deficiency (Daeie and Lewis, 1995).

(ii) The Intrinsic Pathway

The contact phase begins when blood binds to a negatively charged surface *(in vivo,* an area of blood vessel injury; *in vitro* glass/clay surface). The negatively charged surface attracts four proteins: factor XII (Hageman factor) and high molecular weight kininogen (HMWK) which directly attach to the negatively charged surface and factor XI and prekallikrein, which are bound to HMWK and travel with it to the surface. Factor XII on the negatively charged surface has a limited but specific proteolytic activity acting on its two substrates, converting XI to XIa and prekallikrein to kallikrein. Reinforcement results from the prekallikrein ➡ kallikrein conversion since kallikrein is a very specific protease. In this positive feedback system it converts circulating factor XII to factor XIIa to in tum then generate more kallikrein from prekallikrein (Babior and Stossel, 1994). Kallikrein is also involved in the fibrinolytic system, inflammation and has additional physiologic roles (Thompson and Harper, 1983).

Factor XIa and VIIa are both able to activate factor IX. Factor VIIa requires calcium, phospholipid and tissue factor. The complex of factor IXa, VIII, calcium and phospholipid is called tenase and is a strong activator of factor X. While factor IXa acts as a protease, factor VIII serves as an accelerator of the process. Factor VIII, the largest protein involved in clotting, is activated by traces of thrombin to function effectively. Once thrombin-modified, factor VIII becomes less stable and its activity is easily destroyed. Factor VIII is sensitive to degradation by plasmin and activated protein C. The major portion of this protein may be regarded as the "von Willebrand factor". It is important to note that the most severe coagulation disorders are due to factor VIII deficiency (Babior and Stossel, 1994; Rapaport, 1971).

This process lasts between 25-40 seconds. Defects in any of the abovementioned factors lead to prolonged clotting time and prolonged activated partial thromboplastin time (APTI) [Cronje, 1985). Common causes include disseminated intravascular

coagulation, liver disease, massive transfusions with stored blood, administration or contamination of heparin, circulating anticoagulant, deficiency of a coagulation factor other than factor VII (Dacie and Lewis, 1995).

The importance of these pathways *in vivo* can be noted by the severity of bleeding seen in patients with very low levels of one of the initiating factors. In general, a deficiency in factor VII causes severe bleeding. Low levels factor XI results in a mild bleeding disorder without serious tissue bleeding but post operative oozing. With deficient contact factor no bleeding occurs. Patients who lack factor XII and animal species without factor XII (ducks, horses, porpoises) have no bleeding abnormalities. This difference between factor XII and XI deficiency suggests that a mechanism exist *ill vivo* for activating factor XI independent of factor XII. Patients with factor IX deficiency (haemophilia B) have a much more severe bleeding problem compared to patients with factor XI deficiency (Rapaport, 1971).

(iii) The Common Pathway

The two pathways converge at the point of activation of factor X. Factor X can be activated by the above mentioned tenase (intrinsic pathway) or by factor Vlla (external pathway) in the presence of calcium, phospholipid and tissue factor. Factor Xa then combines with factor V plus calcium and phospholipid to form prothrombinase that catalyzes the activation of prothrombin to thrombin. Factor Xa is the enzyme that carries out this reaction and factor V the accelerator (Babior and Stossel, 1994). No other activated clotting factor of the clotting factor complex is able to carry out this process.

Thrombin is the last protease produced in the coagulation cascade. It has a number of functions; most importantly, it converts fibrinogen to fibrin. It activates factor XI and converts factors V and VIII into more potent forms thereby promoting its own

production (another example of a positive feedback system). It is also one of the principle activators of platelets (Babior and Stossel, 1994).

Clot formation begins with the thrombin catalyzed conversion of fibrinogen to fibrin. Fibrinogen is a long, thin molecule consisting of three polypeptides named A (α), B (β) and G *(y)* held together at their ends by disulfide bonds. Thrombin splits pieces called fibrinopeptides off the A and B chains. The altered fibrinogen molecule/fibrin monomer polymerizes spontaneously to form the initial clot (Rapaport, 1971; Babior and Stossel, 1994). Thrombin also activates factor XIII in the presence of calcium to factor XIIIa. The activated factor XIII catalyzes cross-linking between fibrin molecules thus adding to the stability of the clot. Patients with hereditary factor XIII deficiency bleed seriously and show poor wound healing (Rapaport, 1971).

Common causes of prolonged thrombin time include hypofibrinoginaemia (as in disseminated intravascular coagulation), raised concentrations of fibrinogen degradation products (in cases of liver disease or disseminated intravascular coagulation), dysfibrinogenaemia (found congenitally or acquired in liver disease). Extreme prolongation of thrombin time is usually due to the presence of heparin, which interferes with the fibrinogen-thrombin reaction (Dacie and Lewis, 1995).

The fourth test in this study measures fibrinogen quantitatively as a thrombin clottable protein. Enough thrombin is added to convert all the fibrinogen to fibrin, and the amount of protein in the resulting fibrin clot is measured. If a congenital fibrinogen abnormality is suspected disseminated intravascular coagulation and hyperfibrinolysis must first be excluded (Babior and Stossel, 1994).

. The coagulation cascade is summarized in the following diagram:

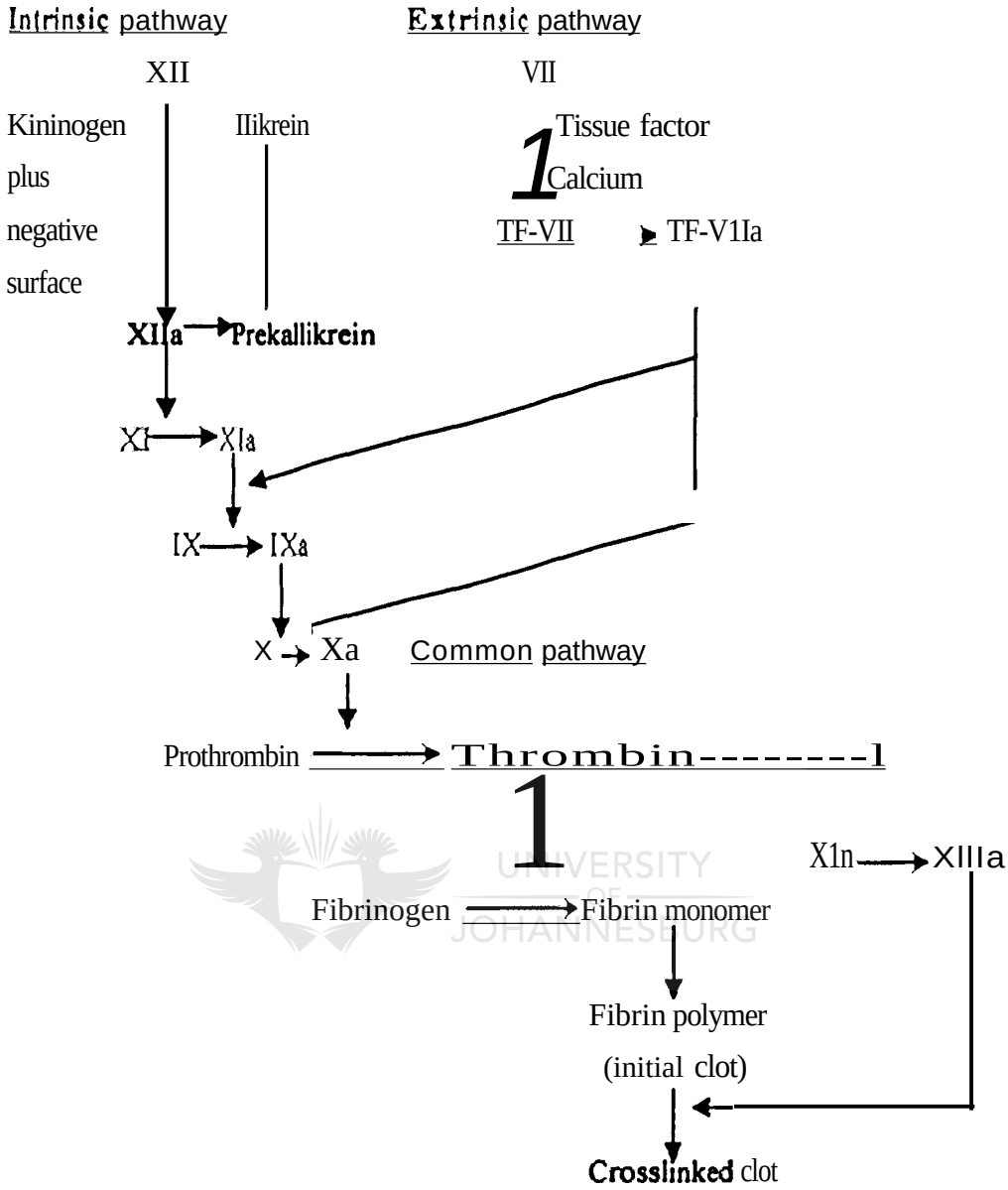

Fig. 1.1 Diagram of Blood Coagulation Pathway (adapted from Babior and Stossel, 1994; Brown, 1993).

Calcium and phospholipid are necessary for all steps except during the activation of factor IX by factor XIa, which requires only calcium, and the reactions of the contact phase, which require neither.

1.3 Regulation Of Coagulation

The coagulation process is regulated by two general mechanisms: the elimination of activated clotting factors and destruction of the fibrin clot (Babior and Stossel, 1994). Unlimited blood coagulation would lead to occlusion of the blood vessels (thrombosis) or disseminated intravascular coagulation (Hoflbrand and Pettit. 1993). Firstly, activated clotting factors are cleared from circulation by the liver. Factors are also eliminated by three circulating anticoagulant systems namely the antithrombin lIIIproteoglycan system, the protein C/protein S system and the tissue factor pathway inhibitor/phospholipid system (Babior and Stossel, 1994). Secondly, the fibrinolytic system is activated by fibrin deposition. Plasmin is a protease and acts by splitting fibrin. It also acts on other proteins such as fibrinogen and factors V and VIII. Fibrin-split products are potent inhibitors of coagulation (Babior and Stossel, 1994).

1.4 Homoeopathy

1.4.1 Introduction

Homoeopathy is a combination of science, an and medical philosophy and experience. It aims at preventing and treating disease and is based on certain principles. The first, as stated by Hahnemann in the Organon of Medicine, is the Law of Similan. It states that illness can be treated and cured by using a stronger stimulus that closely resembles the illness (Eizayaga, 1991).

1.4.2 Origin of Homoeopathy

Samuel Hahnemann founded Homoeopathy in the late 18th century. Years of experimenting led him to believe that if a substance can produce symptoms in a

healthy individual, that same substance can cure similar symptoms in a sick individual. Thus the therapeutic principle "like cures like" (Vlthoulkas, 1980).

Five centuries BC, the philosopher Empedocles of Agrigentum was the first to observe that similars are attracted by similars. The father of medicine, Hippocrates, then formulated the principle "similia similibus curentur" (like cures like). The Law of contraries "contraria contrariis curentur" was also formulated by him, and at that time most medical treatment was based on this principle. It was only in the 16th century that physicians such as Paracelsus, Von Helmont and others viewed the similitude principle with some enthusiasm (Eizayaga, 1991).

1.4.3 Principles of Homoeopathy

0) Law of Simi/ars

Hahnemann, although qualified as a physician, supplemented his income by writing and translating articles and books on chemistry and medicine. He could also read Greek and Latin and was familiar with, and had great respect for Hippocrate's work. He was thus aware of the similitude principle as described by Hippocrates as well as from his own experience (Eizayaga, 1991).

Practically, this principle implies that:

- Every biologically active substance produces characteristic symptoms in healthy bodies that are susceptible to being in some way disturbed by the substance.
- Every sick body expresses a series of characteristic symptoms, which are typical of the pathological alteration of that particular subject.
- The process of healing a sick body, characterized by the progressive disappearance of all symptoms, may be obtained by targeted administration of the drug that produces a similar symptom picture in healthy bodies (Bellavite and Signorini, 1995).

(ii) Pravings

To determine the full action of medicines, Hahnemann conducted certain experiments. He recognized the importance of using healthy individuals (opposed to sick individuals) to elicit the probable symptoms of the substance tested and using attenuated doses of the medicine. The detailed recording of all symptoms produced is called a proving or pathogenesis. After conducting provings on several medicines over a period of time, Hahnemann wrote the "Homoeopathic Materia Medica" (Eizayaga, 1991).

(ill) Potentization

Hahnemann administered small, dilute doses of medication to his patients. After some experimentation, he devised a process of serial dilution and succussion called potentization. He discovered that higher potentized remedies were more efficacious and penetrating in their action and that the quantity of drug required is inversely proportional to its similarity with the symptoms of the patient. From this the Arndt/Schulz law or rule was formulated: "Small excitements provoke vital activity; medium ones increase them; strong excitements inhibit them; exaggerated excitements abolish them; but excitement is proportioned, degree by degree, to the excitability of the individual" (Eizayaga, 1991).

Even though there is no limit to potentisation, substances potentised beyond Avogadro's number i.e. 24 X or 12 CH are unlikely to contain molecules of the base substance. Avogadro's number is defined as the number of molecules contained in one mole of a substance i.e. $N_A = 6{,}022 \times 10^{23}$ molecules/mole. If the concentration of a substance in a dilution is less than $(N_A)^{-1}$, there is no molecule of the substance in the dilution. The effectiveness of homoeopathic remedies can still be seen after this point when correctly selected. For example, if given in its tincture form the remedy will cause a response in the patient. Higher potencies may however elicit a response of

complete disappearance of symptoms over a shorter period with no relapse (Vithoulkas, 1980).

A possible explanatory hypothesis for the mode of action of high potencies. is the Information Medicine Hypothesis, which proposes that the actions of Homoeopathic medicines should be understood in terms of physically stored information rather than in chemical terms. The Information Medicine Hypothesis states: "Under certain circumstances, water (and perhaps other polar solvents) are capable of receiving and storing information about substances with which they have previously been in contact and of transmitting this information to presented biosystems". If verified, this hypothesis would represent an important scientific advance (Schulte and Endler, 1998).

(iv) Single and combined remedies

A minimum dose is used as Homoeopathic remedies act qualitatively rather than quantitatively. Their action can be compared to the action of hormones and enzymes that are present in minute quantities but even so greatly effect changes in the body. These remedies do not have to be present in the organism continuously to create changes since they only acts to change the organism, which then takes over the process of curing (Roy, 1994).

Homoeopathic remedies are considered to stimulate the body into action, as it is the organism that has the ability to maintain health. The medicine is thus only required to create change; indicating the use of a single dose. There are cases though where multiple doses of remedies are indicated i.e. in chronically ill patients using low potencies (Roy, 1994).

In classical Homoeopathy, the patient is treated using a single remedy. There is usually only one medicine that covers the totality of symptoms in the person and only

the most similar should be used. When symptoms change, a new remedy, selected as described previously, may be indicated. As stated by Hahneman in Aphorism 273: "In no case is it necessary to employ more than one single medicine at a time" (Eizayaga, 1991). Furthermore, most remedies are tested singly and the effect of combined remedies is not known (Roy, 1994).

1.4.4 The Homoeopathic concept of disease

In Aphorism 9 of the Organon of Medicine, Hahnemann describes the vital force as "the dynamis that animates the material body (organism), rules with unbounded sway, and retains all parts of the organism in admirable, harmonious, vital operation... ". One of the expressions of the vital force is its function as defence mechanism that responds specifically to disease. It functions holistically on all planes (physical, emotional and mental) and systematically defends the mental/spiritual plane as best as it can. The physiological and chemical processes in our bodies are facilitators of this defence. It is of utmost importance for the health worker to observe this activity as it reflects the degree of disease and the progress of the patient (Vithoulkas, 1980).

Constantine Hering observed a natural mechanism which takes place in the diseased patient as cure occurs, whether spontaneous or due to medicinal action. Referred to as Hering's Law, it states the following:

- Healing takes place from above downwards,
- Disease improves from within outwards,
- Symptoms disappear in the same order in which they appeared, the most important or vital organs being relieved first, then the less important ones, and mucous membranes and skin at the end (Eizayaga, 1991).

1.4.5 Preparation of Homoeopathic remedies

Homoeopathic remedies are prepared under very strict conditions as required by the different Pharmacopoeias. A mother tincture is made from the raw substance. In the case of fresh vegetable substances a process of lixiviation is used and for dry ones maceration. These products are diluted in alcohol (Eizayaga, 1991).

According to the German Pharmacopoeia (1990), 10 parts mother tincture (0) is then diluted to 90 parts of the same concentration ethanol to make a 1 CH (Centesimal Hahnemannian) potency. This dilution is succussed vigorously 100 times. From the succussed solution one part is taken and added to 99 parts of 20 % ethanol in a separate jar and succussed 100 times to produce a 2 CH potency. The process increases the therapeutic properties while nullifying the toxic properties. This method of dilution and succussion is repeated until the desired potency is reached (Vithoulkas, 1980).

It is of vital importance that remedies are manufactured according to certain requirements. Only extremely pure solvents should be used and preparation must be carried out in a sterile atmosphere if possible (Jouanny, 1994a).

1.5 Arnica montana

1.5.1 Description

Arnica montana, also known as Leopard's bane or Fallkraut, is a member of the Compositae family. It is a 20-30 cm tall perennial herb. It has 1-3 (or rarely five) terminal, bright yellow, daisy-like flower heads. Receptacles are 5·8 em broad, with 15·25 Iigulate florets. Characteristic greyish white bristly pappus hairs form a wreath at the upper end of the long, slender, brown ovary of the ligulale and tubular florets

and are responsible for the greyish white appearance of the herbal preparation. The stem has a rosette of dark green leaves nearer the base; the smaller and fewer stem leaves are ovate to lanceolate, opposite and sessile (Czygan *et al.,* 1994). The generic name *Arnica* is thought to be derived from the Greek word amikos, meaning lamb's skin, an allusion to the texture of the leaves (Stodola and Volak, 1992). The root is woody, with a small number of radicles. The plant is faintly aromatic with a slightly bitter, somewhat pungent taste. The mother tincture is prepared using the flowers, leaves and root after careful removal of the larvae of the Arnica fly which are commonly found on the plant (Gibson, 1994). See figure 1.1 for a colour depiction of *Arnica montana.*

1.5.2 Chemical composition and effects

The following table shows a breakdown of *Arnica montana's* chemical constituents.

Table 1.1 The chemical constituents of *Arnica montana* (adapted from Newall *et al.*, 1996).

Amines	Betaine, choline, trimethylamine
Carbohydrates	Mucilage, polysaccharides including Inulin
Coumarins	Scopoletin, umbelliferone
Flavanoids	Isoquercitrin, astragalin, etc.
Terpenoids	Sesquiterpenes including helenalin and 11,13-dihydrohelenalin. arnifolin,
Volatile oils	Thymol and thymol derivatives
Other censtituenn	Amicin (bitter principle), caffeic acid, carotenoids, phytosterols, resin, tannin

Fig. 12 *Arnica montuna* {SIt Ill. and ohik, I

The essential active principles of *Arnica montana* are the sesquiterpene lactones (helenalin and dihydrohelenalin esters). These constituents have been shown to have strong antimicrobial, antiphlogistic, antirheumatic, anti-arthritic, antihyperlipidaemic, and respiratory analeptic properties. They also affect the heart and circulatory system. In addition, cholagogic and diuretic effects (due to sesquiterpene lactones, flavanoids, chlorogenic acid, cynarin, caffeic acid) as well as reflex modulating effects on the central nervous system have been described. Due to the toxicity of sesquiterpene lactones, oral use of *Arnica montana* as herbal medicine should be avoided. Side effects may include gastroenteritis with dyspnoea, cardiac arrest and death if too high doses are ingested. The helenolides are known to have damaging effects on the heart (Czygan *et al., 1994).*

Allergic contact dermatitis due to a number of species of the *Compositae* family has long been recognised. Family members include Chrysanthemums, Chamomile, *Arnica montana* and Yarrow. Sesquiterpene lactones, found abundantly in the *Compositae* family, have been identified as the sensitizers. Helenalin acetate and amifolin are two of eight different sesquiterpene lactones from *Arnica montana* that may be potential allergens (Hausen *et aJ.*, 1978). Due to this sensitizing effect, *Arnica montana* should never be applied to broken skin. For external use on intact skin, it is suggested that five to ten drops of *Arnica montana* mother tincture, or preferably *Arnica montana* I CH, be diluted in 500 ml distilled water or weak alcohol. Even then contact with the skin should not be prolonged beyond 24 hours (Gibson, 1994).

Research by Schroder *et al.* (1990), demonstrated that helenalin and Ila13-dihydrohelenalin inhibit platelet function via interaction with platelet sulfhydryl groups. It was suggested that further research be conducted on the antithrombotic potential of *Arnica momana's* constituents and to define a therapeutic application.

Coumarin is known to be the nucleus of the widely prescribed anti-coagulant, Warfarin. Currently all other vitamin K antagonists are also derived from hydroxycoumarin. Its action leads to a deficiency of reduced vitamin K. This in tum then results in a state of impaired coagulation by an acquired deficiency of factors VII, VIII, IX and X. The anticoagulated state is achieved once all four mentioned factors have reached their final levels. Prothrombin levels take up to 3 or 4 days to fall (Babior and Stossel, 1994).

The combination of coumarin (a product containing a C=O carbonyl group) and polyene derivatives (with several C-triple bond-C acetylene groups) is found in a number of plants. The polyene derivatives are found in two groups of plants:

a) Plants with a homoeopathic action in the convulsive syndromes include: *Cicuta virosa* (spasms in hypertension, epilepsy), *Artemisia vulgaris* (menstrual or peripuberal epilepsy), *Chamomilla* (in certain convulsions).

b) Plants with a homoeopathic action on the inflammatory process and haemostasis include:
Chamomilla, Arnica montana (traumatisms, ecchymoses, haematomas), *Bellis perennis* (traumas, ecchymoses, haematomas), *Echinacea angustlfolia* (suppuration, abscesses) [Bellavite and Signorini, 1995].

1.5.3 Indications of *Arnica montana*

As far back as the 16th century its healing properties have been known. It has been used in orthodox medicine to treat gout, rheumatism, malaria and dysentry (Lockie and Geddes, 1995). Mountain dwellers in the Andes have been reported to use *Arnica montana* as a herbal infusion when injured (Gibson, 1994). Whenever there is trauma (generalized or local) *Arnica montana* should be considered (Jouanny, 1994b).

Homoeopathically prepared *Arnica montana* used as first aid remedy not only arrests bleeding and promotes healing, but is of great value in treating the associated shock and emotional trauma as well (Gibson, 1994). Hahnemann included in the benefits its use in treating even severe wounds (as caused by bullets and blunt objects), after dental and surgical procedures, after childbirth and relieving pains of dislocated joints (Tyler, 1952).

Arnica montana affects the blood vessels, in particular the capillaries, by dilating them in order that extravasation of blood becomes possible. *Arnica montana* is of special value in treating concussion and other acute injuries where there is bruising and simple ecchymoses. When a chronic disease can be traced back to its traumatic origin, *Arnica montana* may be used with great success (Farrington, 1908).

The mental state of the *Arnica montana* patient can be one of morosity, intolerable pain and forgetfulness. There might even be unconsciousness. When speaking to the patient he will respond correctly but then relapse immediately. Another characteristic is that even in this serious condition he will send the doctor away denying his illness. The person is easily startled, wants to be left alone and can not bear being touched. Physical characteristics include a hot head and cold face and nose, or hot upper body and cold lower body as well as restless moving and twisting because the bed feels hard (Gibson, 1994).

The importance of prescribing *Arnica montana* according to the described symptom picture may be supported by the following finding of Scofield (1984). He analysed results of a clinical trial in which the effect of *Arnica montana* 30 CH and *Arnica montana* I M were tested in cases of a stroke. The researchers found no significant benefit from the treatment. However, Scofield showed that of the 40 patients entered into the study, only three had a typical *Arnica montana* symptom picture, and these three showed good progress during homoeopathic therapy.

2.0 MATERIALS AND METHODS

2.1 Study Sample

A total of 21 volunteers was used in the study. They were recruited by means of an advertising campaign. Posters containing information on the research were placed at the Technikon Witwatersrand Doornfontein campus and surrounding areas. Persons fulfilling the necessary requirements were informed about the research project and the role that they would play in the study. An information sheet explaining the research procedures was given to each of them. Volunteers had to sign a consent form. Details of the patient consent form and patient information sheet are included in Appendix A. Ethical clearance was granted by the Technikon Witwatersrand Committee for Research on Human Subjects (protocol number: EC 17/99/076). Participants had to be between the ages of 18 and 60 years, not using any anti-coagulant therapy (for example Warfarin) and free of any haematological disorder or family history thereof. For the duration of the study. it was also necessary to keep alcohol. coffee. cigarette and garlic consumption to a minimum.

2.2 Methods

2.2.1 Collection of blood

In this trial panicipantsactedas their own control. An independent person assigned an identity number to each volunteer. Thus. the researcher only knew the volunteer by number.

It was decided to take two blood samples over a three day period in order to eliminate random error and tQ.minimize the etreetofphysiologicaJ and environmental factors.

A qualified phlebotomist drew blood by means of veni-puncture. After inspection and cleaning the area with 70% alcohol solution, blood was drawn into four 5ml trisodium citrate tubes and one vacutainer containing ethylenediaminetetraacetic acid (EDTA). EDTA binds the calcium in the blood to prevent clotting. The blood was thoroughly mixed with the anticoagulant by inverting the tube several times.

2.2.2 Preparation of platelet-poor plasma

Platelet-poor plasma was prepared from the blood in the trisodium citrate tubes by centrifugation at 3000 rpm for 10 minutes. Daeie and Lewis (1995) suggests that plasma should be kept at room temperature if it is to be used for prothrombin time tests, factor VII assays or platelet function testing, and at 4°C for other assays. They also recommend that testing should be done within two hours of collecting the sample. Samples may be frozen at -40°C for several weeks without any significant loss of activity of most haemostatic components to be measured.

2.2.3 Preparation of reagents

Reagents were prepared according to the ILEX package insert in order to carry out the screening tests. Details of reagent preparation are described in Appendix B.

2.2.4 Coagulation analysis

All samples were handled with extreme care because of the risk of transmission of hepatitis and human immunodeficiency virus (HIV). The platelet-poor plasma of each subject was transferred into three 0.5 ml sample cups. The first sample cup would act as control, one drop (equal to 16µl) of 20% alcohol was added to the second CUp. and one drop *Arnica montana* 12 CH to the third cup. The sample cups were loaded onto the sample tray and directly placed into the Automated Coagulation Laboratory (ACt) machine supplied by ILEX. The robotic sampling arm pipettes both sample

and reagents automatically into the rotor cuvettes. After dispensing each sample. automatic rinsing occurs. Although coagulation tests can be carried out manually. the use of the ACL machine saves time and provides high accuracy. The machine's parallel processing ensures that all reactions start and stop simultaneously. so reaction timing is uniform. Light is scattered by the fibrin strands as they form and is measured at right angles to each optical cuvette. Up to 1100 consecutive readings per reaction defines the clot curve to its completion. This makes differentiating abnormal clotting times much more accurate.

2.2.5 Analysis of whole blood samples

It is recommended that fresh whole blood samples be used when doing a Full blood count. The International Committee defines this for Standardization in Haematology as a sample processed within four hours of collection. Well-mixed whole blood samples, collected in EDTA anticoagulant and run within eight hours after collection, provides the most accurate results for all parameters. The Cell-Dyn 1700 system uses the Electrical Impedance Method for determining white blood cell, red blood cell and platelet data and the Modified Cyanmethemoglobin Method for determining haemoglobin. During each count cycle, the sample is aspirated, diluted and mixed before each parameter is measured.

2.2.6 The Homoeopathic remedy

The medication, *Arnica montana* 12 CH, was donated by Natura Homoeopathic Laboratory. It was prepared according to the methods as set by the German Pharmacopoeia. described under *1.4.5.*

2.3 Statistics

From the original 25 volunteers that fulfilled the criteria, 21 completed participation in the study. Blood samples were collected over a two day period in order to eliminate physiological factors. Tests were run using the ACL coagulation analyser. Prothrombin, Activated Prothrombin, Thrombin and Fibrinogen times were measured. The average of the two readings was used for further statistical analysis. Full blood cell counts were done in order to compare results before and after medication was added to the samples. These results would only be considered if the coagulation tests showed any abnormal results.

All the data was statistically analysed (Appendix C) under the supervision of Prof. Boyd, the appointed statistician at Technikon Witwatersrand. Initially data was analysed using a One Way Analysis of Variance test (one way ANDVA). No statistically significant results were obtained. Due to the nature of the data, the statistician then recommended the use of a Two Way Analysis of Variance (two way ANDVA) method. This data was then analysed with data from two very similar studies. In these studies *Arnica montana* mother tincture and *Arnica monana* 12 CH were used in order to obtain a more accurate estimate of the within, between and residual variances

To examine whether or not there are statistical significances between the means of the populations from which the samples are selected, the two way ANDVA method of analysis employs estimates and compares the mean square errors for the within and between variances with residual variance.

The efficacy of the medication, *Arnica montana* 12 CH, was evaluated by determining whether a significant difference existed between the unmedicated

(control) blood sample, the sample medicated with alcohol, and the sample medicated with *Arnica montana* 12 CH. These tests were conducted *in vitro*.

3.0 RESULTS

3.1 Prothrombin Time

The two-way ANOVA method of analysis showed that the differences in the mean values among the different patients are greater than would be expected by chance after allowing the effects of differences in medication. There is a statistically significant difference (p= 0, 00000000029). Patients were tested by using a multiple comparison procedure and most pairs (203 out of 21O) were found to be statistically significant at P= .05.

Analysis using the All Pairwise Comparison Procedures (Student-Newman-Keuls Method) showed that the control is significantly different from each of the four treatments but that no two of the three treatments (*Amica montana* Mother Tincture,. *Arnica montana* 12 CH and *Arnica montana* 30 CH) were significantly different. The difference between *Arnica montana* 12 CH and alcohol are not significant enough to exclude the possibility that the results for *Arnica montana* 12 CH were partly caused by the alcohol used in its preparation.

Note that in the case of Prothrombin Time the normality test failed and so a parametric test is not totally reliable. In the case of Activated Partial Prothrombin Time, Thrombin Time and Fibrinogen assay the normality test was passed, so for comparison purposes the Prothrombin Time was analysed in the same way as the others. Although a non-parametric test could have been used, it would have been weaker than the parametric test.

3.1 Activated Partial Prothrombin Time

The two-way ANOVA method of analysis showed that the differences in the mean values among the different patients are greater than would be expected by chance after allowing the effects of differences in medication. There is a statistically significant difference (p= 1.43E-066). Patients were tested by a multiple comparison procedure and most pairs (203 out of 210) were found to be statistically significant at P=.OS.procedure.

Analysis using the All Pairwise Comparison Procedures (Student-Newman-Keuls Method) showed that the control is significantly different from each of the four treatments. There was no significant difference between any pair of the treatments (*Arnica montana* Mother Tincture, *Arnica montara* 12 ClI, *Amtca montana* 30 eli). The differences between *Amico montana* 12 CII and alcohol are not significant enough to exclude the possibility that the results for *Arnica montana* 12 CH were caused by the alcohol used in the preparation.

3.3 Thrombin Time

The two-way ANDVA method of analysis showed that the differences in the mean value among the different patients are greater than would be expected by chance after allowing the effects of differences in medication. There is a statistically significant difference (p-0.OOOOOOOOOI41). Patients were tested by a multiple comparison procedure and most pairs (203 out of 210) were found to be statistically significant at PROS.

Analysis using the All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method) showed that there was no significant difference between any pair of

the five treatments (control. alcohol, *Arnica montana* mother tincture. *Arnica montana* 12 CH and *Arnica montana* 30 ClI).

3.4 Fibrinogen Assay

The two-way ANOVA method of analysis showed that the differences in the mean values among the different patients are greater than would be expected by chance after allowing the effects of differences in medication. There is a statistically significant difference (p=1.44E-052). Patients were tested by a multiple comparison procedure and most pairs (203 out of 210) were found to be statistically significant at P=,OS

Analysis using the All Pairwise Comparison Procedures (Student-Newman-Keuls Method) showed that the control is significantly different from each of the four treatments but that no two of the treatments were significantly different.

4.0 DISCUSSION AND CONCLUSION

The importance of an intact circulatory system is well known. Haemostasls, of which coagulation is one part, is a process aimed at maintaining this. The purpose of this study was to determine the effect of *Arnica montana* 12 CH on blood coagulation *in vitro.*

Tests most often used to-assess the efficacy of the coagulation pathway include the Prothrombin Time. Activated Partial Prothrombin Time. Thrombin time and Fibrinogen assay. These were also used in this study. After medicating the blood samples as described previously, coagulation tests were conducted using the ACL machine.

After statistical analysis, the results showed however that the difference between the experimental groups and the control group were too small to be significantly different. It is interesting to note that the alcohol group had almost the same results as the experimental group. This was obtained between students and is illustrated graphically by comparing the 95% confidence interval for the treatments. It may be noted that all the treatment effects were small in comparison to the between patient effects. Since Coumarin is one of the constituents of *Arnica montana,* it was expected that the results would show a prolonged Prothrombin Time. The two way ANDVA test showed however that *Arnica montana* 12 CH had no effect on the Prothrombin Time, Activated Partial Prothrombin Time, Thrombin time or Fibrinogen assay and thus no effect on blood coagulation.

Although this study has not produced conclusive evidence to validate the effect of *Arnica montana* 12 CH on coagulation it should be considered to investigate other parts of haemostasis. These include its effect on blood vessels, platelets and the fibrinolytic system:

The measurements obtained in this research reflected changes purely on a physical level, it leaves room for further more complex experimentation. To conduct a true Homoeopathic study, *in vivo* experimentation has to be conducted administering *Arnica montana* 12 CH orally over a period of a few days. This way a more accurate reflection of the remedy's action on all three levels of the body may be observed.

It is also important to note that Prothrombin levels in the body take up to three or four days to fall, emphasizing the importance of observing *Arnica montana's* action over a period of time.

4.1 Recommendations

The effect of *Arnica montana* 12 CH needs to be examined in a large homogeneous group of patients who are carefully chosen so as to reduce, if possible, the between patient differences found in the above study.

It is also recommended that when research is conducted, it should be considered that individualising treatment according to the totality of symptoms might yield more favourable results.

Considering that the alcohol and experimental group results were very similar, it would be interesting to compare the results of alcohol with that of *Arnica montana*- prepared and stored in pure, distilled water- in order to ascertain the true effect of the remedy.

A.I THE CONSENT FORM

The research you will be participating in is being done to determine the effect of *Arnica montana* 12 CH on blood coagulation. This study intends to demonstrate the effects of *Arnica montana* on the coagulation pathway.

Arnica montana is a popular self-prescribed prophylactic remedy and treatment for trauma, injury and surgery. No research has yet been done to determine *Arnica montana's* mode of action on the blood.

Volunteers will only be considered for this research study if they:

- Are between 18 and 60 years of age;
- Are free of any bleeding and clotting disorders;
- Have no previous history or family history of these disorders;
- Are not on any anticoagulant medication e.g, Aspirin, Heparin, Warfarin.

Procedure

As a volunteer you will be required to undertake two blood tests over a three day period, i.e. Monday and Wednesday. The maximum amount of time spent at the Technikon to draw the blood samples will be twenty minutes. You will not be required to take any medication, as the medicine will be added to the blood sample in the laboratory. Tests will be run on the blood, testing blood coagulation factors.

Volunteers are requested to refrain from using alcohol for three days before the first test and also during the week of testing. Smoking, caffeine and garlic consumption should be minimal. The reason for this is that test results will be influenced by the use of these substances, and will not give a true reflection of the effect of *Arnica montana.*

If at any time a volunteer feels uncomfortable participating in the study, please feel free to contact me as you are under no obligation to continue the study.

All information gathered from volunteers is confidential, although individual information can be made available to that specific volunteer on request. The Technikon Witwatersand will not be held responsible for any damage or injury caused to the volunteer during the course of the trial.

I understand and agree to abide to the above mentioned procedures.

Name of volunteer:	Name of researcher: _____
Signature and date:	Signature and date: _____
Contact number: _____	Contact number: _____

A. 2 PATIENT INFORMATION SHEET

(FOR RESEARCHER'S USE)

Age: ..

Please state how many cups of coffee you drink on average per day .
Please state the brand name of the coffee you drink ..
Please state how many cups of tea you drink on average per day .
Please state the brand name of the tea you drink ..

Please state how many Coca Cola and / or similar caffeine containing soft drinks you consume on average per day:
Name of drink: Quantity of each drink:

Please state how many energy drinks such as Red Bull, Sobe and the likes you consume per day:
Name of drink: Quantity of each drink:

Do you take any slimming and / or meal supplements?
Examples of these are Formula 2001, Thinz, Be-Thin and Nobese.
Please specify: .
State whether you take garlic capsules on a daily basis: Yes / No
State which brand of garlic capsules you take and how many:

Please state whether you use garlic for cooking: Yes / No
If yes, answer the following:
State how much garlic you use: .
State in what form the garlic is in when you use it: ..

Please state whether you take Aspirin on a regular basis: Yes / No
State how many Aspirin tablets you would take at anyone time: .
State how often you would repeat that dose: , .
State for what ailments you usually take Aspirin: .
State whether you smoke: Yes / No
[fyes, please answer the following questions:
State whether you smoke cigarettes, cigars, or pipe tobacco: .
State how many cigarettes, cigars and / or pipes you smoke on average per day:

State brand of cigarette, cigar or pipe tobacco you smoke:

Gender: .

- If female, state last date of menstrual period: " .
- If female, state whether you are using any form of hormonal contraception: Yes/No
- State name of contraceptive: "., , " .

Please state whether you have been diagnosed with a liver disease/s or disorders: Yes/ No
If yes, please state condition: " .

Have you ever had three or more of the following symptoms at the same time?
If yes, please tick the symptoms:
Jaundice " .
Pale stool .
Dark urine .
Pruritis .
Headache .
Malaise .
Chills and fever ..
Nausea, vomiting and diarhoea

Please state any other illness: ..

I confirm that the above information is correct

Volunteer name and signature: .

BLOOD COAGULATION REAGENTS

Preparation of reagents according to ILEX package inserts:

B.1 Normal control plasma

- Intended use: For quality control (precision and accuracy) of coagulation assays in the normal range.
- Principle: Prepared from human citrated plasma from healthy donors.
- Preparation: Contents of vial were dissolved with I ml of distilled water. It was left at room temperature for 30 minutes. Before use it was gently inverted.

B.2 PI-Fibrinogen Recombinant

- Intended use: A high sensitivity reagent based on recombinant rabbit tissue factor for the determination of prothrombin time (PT) and fibrinogen for the evaluation of the extrinsic pathway.
- Principle: Prepared as a lyophilized rabbit brain extract with an optimal concentration of calcium. Manufactured to show high sensitivity to factors II, V, VII and X.
- Preparation: The contents of each vial of thromboplastin were dissolved by pouring the contents of one vial of buffer (CaCll) into the reagent vial. The vial was stoppered and swirled gently. It was left at room temperature for 30 minutes and gently inverted to mix before use.

B.3 APTT-SP

- Intended use: For the *in vitro* determination of activated partial thromboplastin time (APTT) in citrated plasma for the evaluation of the intrinsic coagulation pathway.
- Principle: This test used a contact activator to stimulate the production of factor XIIa by providing a surface for the function of high molecular weight kininogen, kallikrein and factor XIIa. Contact activation was allowed to proceed at 37°c for a specific period of time. Calcium was then added to trigger further reactions and the time required for clot formation is measured. Phospholipids are required to form complexes that activate factor X and prothrombin.
- Preparation: APTI reagents were vials of silica dispersion with synthetic phospholipids that were shaken vigorously for 15 seconds. Calcium chloride was ready for use.

B.4 Thrombin time

- Intended use: For the quantitative determination of thrombin.
- Principles: A purified bovine thrombin is used to evaluate DIC, heparin therapy, qualitative and quantitative fibrinogen abnormalities and increased fibrinolysis.
- Preparation: A dilution of 1:4 diluent and distilled water were made. Five ml diluent was used to dissolve 3 ml bovine thrombin. It was left at room temperature for 30 minutes and gently inverted to mix before use.

8.5 Fibrinogen-C

- Intended use: For the quantitative determination of fibrinogen.
- Principles: An excess of thrombin is used to convert fibrinogen into fibrin in diluted plasma.
- Preparation: The bovine thrombin was prepared by dissolving the contents of the vials with 2 ml distilled water. The abnormal control plasma was made by dissolving the contents of the vials with 1 ml distilled water. Both of these preparations were left at room temperature for 30 minutes and then inverted to mix before use.

TWO-WAY ANALYSIS RESULTS

The following abbreviations apply under Appendix C:

C = Control

A = Alcohol

MT = *Arnica montana* Mother Tincture

12CH *Arnica montana* 12CH

30CH *Arnica montana* 30CH

SEM Standard Error of Mean

OF ::: Degrees of Freedom

SS ::: Sum of Squares

MS = Mean Squares

F::: Fisher's Variance ratio

P ::: Probability

PROTHROMBIN TIME

Two Way Analysis of Variance

General Linear Model (no interactions).

Dependent Variable: Activated PartiaJ Prothrombin Time Value

Least Square means of treatment:

Group	Mean	SEM
C	12.6	0.0353
A	12.8	0.0353
MT	12.9	0.0353
12CII	13.0	0.0353
JOCH	13.0	0.0353

Source of Variance	DF	SS	MS	F	P
Patient	20	1.84	0.4590	17.5	
Treatment	4	86.27	4.3135	164.7	
Residual	80	2.09	0.0262		
Total	104	90.2	0.8673		

All Pairwise Multiple Comparison Procedures (Student-Newman-Keels Method):

Comparison	DifT or Meaos	P	Q
30C8 vs C	0.3333	4	9.440
30CII vs MT	0.0238	2	0.674
30CII vs A	0.1381	3	3.911
12CH vs 30 en	0.0262	2	0.742
12CH vs C	0.3595	5	10.181
12CH vs MT	0.0500	3	1.416
12CH vs A	0.1643	4	4.652
A vs C	0.1952	2	5.529
AvsMT	0.1143	2	3.236
MTvsC	0.3095	3	8.765

Comparison	P<O.05
30CBvs C	Yes
30COvs MT	Do not test
30CO vs A	Yes
12CH vs 30 CD	Do not test
12CH vs C	Yes
12CBvs MT	No
12CBvs A	Yes
AvsC	Yes
AvsMT	Yes
MTvsC	Yes

There is no significant difference between any pair of the three treatments (*Arnica montana* Mother Tincture, *Arnica montana* 12 CH, *Arnica montana* 30 CH).

ACTIVATEO PARTIAL PROTHROMBIN l'IME

Two Way Analysis of Variance

General Linear Model (no interactions)

Dependent Variable: Prothrombin Time Value

Normality Test: Passed (P=0.0701)

Equal Variance Test: Passed (p=1.0000)

Least square means for treatment:

Group	Mean	SEM
C	32.6	0.0927
A	33.2	0.0927
MT	33.0	0.0927
11C8	33.2	0.0927
3OC8	33.4	0.0927

Source Of Variance	OF	55	MS	F	P
Patient	20	1074.19	53.710	297.81	<0.0001
Treatment	4	6.55	1.639	9.09	<0.0001
Residual	80	14.13	0.180		
Total	104	1095.18	10.531		

All Pairwise Multiple Comparison Procedures (Student-Newman-Keels Method):

Comparison	DilT of Means	P	Q
JOCDvs C	0.7167	5	7.733
JOC" vs MT	0.3262	4	3.520
JOCII vs A	0.1548	3	1.670
JOCHvs 12CH	0.1167	2	1.259
12CII vs C	0.6000	4	6.474
12CH vs MT	0.2095	3	2.261
12CH vs A	0.0381	2	0.411
AvsC	0.5619	3	6.063
AvsMT	0.1714	2	1.850
MTvsC	0.3905	2	4.214

Comparison	P<O.05
JOCD vs C	Yes
JOCRvs MT	No
JOCR vs A	Do not test
JOCR VI 12CH	Do not test
12CH vs C	Yes
12CH vs MT	Do not test
12CH vs A	Do not test
AvsC	Yes
AvsMT	Do not test
MTvsC	Yes

There is no significant difference between any pair of the three treatments (*Arnica montana* Mother Tincture, *Arnica montana* 12 CH, *Amica montana* 30 CH).

THROMBIN TIME

Two Way Analysis of Variance

General Linear Model (no interactions)

Dependent Variable: Thrombin Time Value

Normality Test: Passed (paO.OOOI)

Equal Variance Test: Passed (P=1.0000)

Least square means for treatment:

Group	Mean	SEM
C	10.5	0.272
A	11.1	0.272
MT	11.3	0.272
UCR	11.2	0.272
JOCR	11.2	0.272

Source of Variance	DF	S8	MS	F	P
Patient	20	214.66	10.73	6.91	<0.0001
Treatment	4	9.77	2.44	1.57	0.1894
Residual	80	124.17	1.55		
Total	104	348.60	3.35		

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparison	DifTorMean	P	Q
MTvsC	0.85714	5	3.1528
MTvsA	0.188104	4	0.6919
MTvs 12CH	0.13095	3	0.4817
MTvsJOCH	0.312143	2	0.466
30CII vs C	0.73571	4	2.7062
30CUvs A	0.06667	3	0.2452
JOCHvs 12CH	0.00952	2	0.0350
12CH vs C	0.72619	3	2.6711
12CH vs A	0.05714	2	0.2102
AvsC	0.66905	2	2.4609

Comparison	P<O.05
MTvsC	No
MTvsA	Do not test
MTvs 12CH	Do not test
MTvsJOCH	Do not test
JOCHvs C	Do not test
JOCHvsA	Do not test
30CH vs 12CH	Do not test
12CH vs C	Do not test
12CH vs A	Do not test
AvsC	Do not test

There was no significant difference between any pair of the three treatments (*Arnica montana* mother tincture, *Arnica montana* 12 CH and *Arnica montara* 30 CH).

FIBRINOGEN ASSAY

Two Way Analysis of Variance

General Linear Model (no interactions)

Dependent Variable: Fibrinogen Assay Value

Normality Test: Passed (p=0.0 ISS)

Equal Variance Test: Passed (P=1.0000)

Least square means for treatment:

Group	Mean	SEM
C	3.21	Do not test
A	3.14	Do not test
MT	3.17	Do not test
nCB	3.16	Do not test
JOCB	3.19	Do not test

Source of Variance	DF	55	MS	F	P
Patient	20	9.9087	0.49543	131.07	<0.0001
Treatment	4	0.0548	0.01369	3.62	0.0091
Residual	80	0.3024	0.00378		
Total	104	10.2658	0.09871		

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparison	DilT or Means	P	Q
C vs A	0.0648	5	4.83
Cvs 12CH	0.0490	4	3.66
CvsMT	0.0343	3	2.56
CvsJOCH	0.0171	2	1.28
30CII vs A	0.0476	4	3.55
JOCH vs 12CH	0.0319	3	2.38
30CHvsMT	0.0171	2	1.28
MTvsA	0.0305	3	2.27
MTvs 12CH	0.0148	2	1.10
12CH vs A	0.0157	2	1.17

Comparison	P<0.05
CvsA	Yes
Cvs 12CH	No
CvsMT	Do not test
Cvs30CH	Do not test
30CHvsA	No
30CHvs 12CH	Do not test
30CHvs MT	Do not test
MTvsA	Do not test
MTvs 12CH	Do not test
12C8 vs A	Do not test

Control is significantly different from each of the four treatments; no two of the treatments differed significantly from each other.

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