

THE IMPACT OF ANTICOAGULANTS OF THE COELOMIC FLUID OF AN EARTHWORM AND THEIR SUBSEQUENT EFFECT ON ANGIOGENESIS DURING REGENERATION

PROJECT WORK

Submitted in partial fulfillment of the requirements for the
award of Bachelor of Science in Biotechnology

BY

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Yazhini K (41770039)** who have done the Project work as a team who carried out the project "**IMPACT OF ANTICOAGULANTS ON THE COELOMIC FLUID OF AN EARTHWORM AND THEIR SUBSEQUENT EFFECT ON ANGIOGENESIS DURING REGENERATION**" under our supervision from September 2023 to April 2024.

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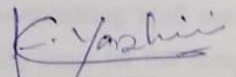
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DECLARATION

I YAZHINI K (41770039) hereby declare that the Project Report entitled "IMPACT OF ANTICOAGULANTS ON THE COELOMIC FLUID OF AN EARTHWORM AND THEIR SUBSEQUENT EFFECT ON ANGIOGENESIS DURING REGENERATION" done by me under the guidance of Dr. L. Inbathamizh (Internal) and Dr. S. Jackson Durairaj (External, IRC) at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology.

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ABSTRACT

The primary circulatory system of the human body is composed of blood. The primary circulatory system of earthworm is composed of coelomic fluid. Earthworm has an open circulatory system. Coelomic fluid is used as an alternative for Fetal Bovine Serum (FBS) in animal cell culture. Regeneration is associated with angiogenesis. The earthworms used for experiment is *Eudrilus eugeniae*. The earthworm, *E. eugeniae* were maintained under suitable conditions. The coelomic fluid of an earthworm is extracted through electric shock to observe the sticky nature. Earthworms were treated with water for control and then with anticoagulants (aspirin, heparin, warfarin). Following anti-coagulant treatment, the sticky nature of the coelomic fluid is reduced, which implies the anti-coagulant has a mild effect on the coelomic fluid. Anti-coagulant has a different effect on angiogenesis process in order to understand that aspirin was injected into the worm and observed for their regeneration ability on 4th day. The data clearly confirms aspirin augments the regeneration and angiogenesis process in the earthworm. The results conclude, aspirin has a better effect on anticoagulant property on coelomic fluid and it enhance regeneration and angiogenic process in the earthworm.

Key words: Aspirin, Coelomic fluid, Angiogenesis, Anticoagulant, Earthworm.

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
AP	Acute Pancreatitis
A β PP	Amyloid- β Protein Precursor
BBB	Blood-Brain Barrier
CAA	Cerebral Amyloid Angiopathy
COX	Cyclooxygenase
DC	Direct Current
DOACs	Direct Oral Anticoagulants
DVT	Deep Vein Thrombosis
FDP	Fibrinogen Degradation Products
LMWH	Low Molecular Weight Heparin
OAC	Oral Anticoagulant
RCA	Regional Citrate Anticoagulation
SAP	Secere Acute Pancretitis
TBI	Traumatic Brain Injury
TM	Thrombomodulin
VEGF	Vascular Endothelial Growth Factor
VKA	Vitamin K Antagonist
VTE	Venous Thromboembolism

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CHAPTER 1

INTRODUCTION

Anticoagulants play a very important role in the Coelomic fluid of earthworms and their impact on angiogenesis during regeneration. Coelomic fluid is the circulatory fluid of the earthworm's body cavity. Coelomic fluid contains different essential elements for wound healing and regeneration including growth factors and immune cells. The main role of the anticoagulants is to prevent the clotting of the coelomic fluid (Subbiahanadar Chelladurai *et al.*, 2021). It allows researchers to study its composition and effects on regeneration. Angiogenesis is the formation of new blood vessels. During regeneration angiogenesis is a very critical process for supplying oxygen and nutrients to regenerating tissues. Anticoagulants can influence angiogenesis by maintaining the fluidity of coelomic fluid which facilitates the transport of growth factors for angiogenesis process (Costa *et al.*, 2002). Coelomic fluid plays a very important role in earthworm physiology including wound healing and regeneration. By understanding how anticoagulants affect angiogenesis during earthworm regeneration could provide understanding into building regenerative abilities in different organisms. Anticoagulants can change the composition, appearance, structure and function of coelomic fluid which influence the regeneration process (Banik and Chaudhuri, 2017). Studying the impacts of anticoagulants on coelomic fluid and angiogenesis in earthworm regeneration provides understanding into the mechanisms underlying in repair and regeneration in these organisms. Studying these processes could develop the understandings applicable to human wound healing and regenerative medicine (Gates, 1949). This study brings knowledge in pharmacology, physiology and regenerative biology in biological fields.

1.1 COELOMIC FLUID OF EARTHWORMS

Earthworms have coelomic fluid filled in coelomic cavity. Coelomic fluid is the circulating fluid of an earthworm. As coelomic fluid is the circulatory fluid, it transports nutrients, exchange of gases, removal of wastes and immune defense (Fiołka *et al.*, 2019).

The composition of coelomic fluid is enzymes, proteins, ions, hormones and cells together contribute to regenerative process and wound healing. Coelomic fluid plays a very important role in supplying the needy factors for the repair of tissue, growth factors, cytokines during regeneration (Haque *et al.*, 2023).

1.2 EFFECT OF ANTICOAGULANTS

Anticoagulants inhibit blood clotting by interfering with the coagulation. When Anticoagulants are injected to earthworms it can change the composition and function of coelomic fluid (Mousa, 2010). This is because of affecting the clotting and dynamics of molecular mechanisms. The anticoagulants which may have various effects on coelomic fluid and function are such as heparin, aspirin and warfarin which involved in wound healing.

1.3 ANGIOGENESIS DURING REGENERATION

Angiogenesis is the process of formation of new blood vessels from pre-existing vessels. This is helpful in supplying nutrients and oxygen for regeneration of tissues and for removing the waste. It is also necessary for establishing support to growth and differentiation of new tissues. Angiogenesis influencing factors such as Vascular Endothelial Growth Factor (VEGF), cytokines (Das *et al.*, 2022).

1.4 INTERDISCIPLINARY IMPLICATIONS

Studying the effects of anticoagulants involves understanding knowledge from physiology, pharmacology and regenerative biology. Understanding the effects of anticoagulants may also have environmental applications such as in assessing the impacts of pollutants or toxins on earthworm health and ecosystem functioning (Mousa, 2010).

1.5 COAGULATION

Coagulation is a changing process and the understanding of the blood coagulation system has evolved over the recent years (Monroe, 2017). Although the classification of the coagulation system into extrinsic and intrinsic pathway is still valid, the newer insights into coagulation provide more authentic description of the same (Palta, Saroa and Palta, 2014). Normal coagulation pathway represents a balance between the pro coagulant pathway that is responsible for clot formation and the mechanisms that inhibit beyond the injury site. Imbalance of the coagulation system may occur in the preoperative period or during critical illness, which may be secondary to numerous factors leading to a tendency of either thrombosis or bleeding (Palta, Saroa and Palta, 2014). A systematic search of literature on Pub Med with MeSH terms 'coagulation system, haemostasis and anaesthesia revealed twenty eight related clinical trials and review articles in last 10 years. Since the balance of the coagulation system may tilt towards bleeding and thrombosis in many situations, it is mandatory for the clinicians to understand physiologic basis of haemostasis in order to diagnose and manage the abnormalities of the coagulation process and to interpret the diagnostic tests done for the same. The concept of blood coagulation dates back to 1960's when Davie, Ratnoff and Macfarlane described the "waterfall" and "cascade" theories outlining the fundamental principle of cascade of pro enzymes leading to activation of downstream enzymes. Haemostasis, defined as arrest of bleeding, comes from Greek, haeme meaning blood and stasis meaning to stop. This thrombo haemorrhagic balance is maintained in the body by complicated interactions between coagulation and the fibrinolytic system as well as platelets and vessel wall (Morgan, 1901). Usually, the coagulation process is under the inhibitory control of several inhibitors that limit the clot formation, thus avoiding the thrombus propagation. In summary checking the intricate interplay between anticoagulants, coelomic fluid composition and angiogenesis during earthworm regeneration offers valuable understanding into biological processes.

1.5.1 COAGULATION FACTOR

Clotting factor transfusions are vital for people with diseases such as haemophilia. In the

1970s and 1980s, transfusions with pooled plasma led to a high number of recipients becoming infected with blood-borne pathogens such as HIV and hepatitis. This situation triggered the development of the virus-free factor which concentrates through a combination of improved donor selection and screening. Effective virucidal technologies, and recombinant protein expression biotechnology (Wadsworth *et al.*, 2021). There is now a wide range of recombinant factor concentrates, and an impressive safety record with respect to pathogen transmission. However, remaining therapeutic challenges include the potential threat of transmission of prions and other pathogens, the formation of inhibitory alloantibodies, and the international disparity that exists in product availability due to differences in licensure status as well as prohibitively high costs. In the future, it is likely that bioengineered recombinant proteins that have been modified to enhance pharmacokinetic properties or reduce immunogenicity, or both, will be used increasingly in clinical practice (Monroe, 2017).

1.5.2 COAGULATION THERAPY:

Acute pancreatitis (AP) poses a significant global health burden, with a pooled incidence of 34 cases per 100,000 individuals annually, and a mortality rate of 1.16%. While historically viewed as autodigestion due to pancreatic enzyme release, recent research has illuminated additional mechanisms, such as ischemia exacerbating pancreatic edema, leading to acute necrotizing pancreatitis (ANP) (Boutros *et al.*, 2010). This condition not only affects pancreatic function but also impairs microcirculation in extrapancreatic organs, potentially via intravascular thrombosis. Clinical studies have revealed elevated levels of plasma fibrinogen degradation products (FDP) in AP patients, with severity correlating with higher FDP levels. Portosplenomesenteric venous thrombosis occurs in approximately 17.86% of early-stage severe acute pancreatitis (SAP) cases. Anticoagulant therapy, particularly with low molecular weight heparin (LMWH) and thrombomodulin (TM), has shown promise in improving outcomes by addressing microcirculatory disturbances, even when pancreatic necrosis is established. However, the molecular mechanisms underlying coagulation disorders in SAP and optimal anticoagulant usage remain unclear.

Beyond immediate health implications, AP is associated with long-term complications such

as diabetes mellitus, pancreatic exocrine insufficiency, and chronic pancreatitis, further underscoring the need for effective therapeutic interventions. Thus, there's an urgent call for safe and efficacious treatments targeting microcirculatory disturbances to prevent AP progression. In this context, understanding coagulation system dysregulation in SAP, exploring potential therapeutic targets, and developing safe and effective interventions are critical for advancing both clinical and basic research in this field.

1.6 ANTICOAGULATION

Calcium plays a crucial role in blood clotting, and various inorganic molecules that bind to calcium have been identified to mitigate clot formation. Citrate, a calcium chelator, serves as an inhibitor of the coagulation cascade in blood transfusions and is utilized as an anticoagulant during dialysis to maintain the extracorporeal circuit's patency, a method known as regional citrate anticoagulation (RCA). To achieve this, citrate levels must be carefully adjusted to reduce ionized calcium concentrations in the extracorporeal circuit, thereby minimizing coagulation cascade propagation.

In RCA, citrate is infused into the afferent tubing, while calcium is infused into the efferent tubing. The dialysate typically lacks calcium. This setup has demonstrated high efficacy with minimal clotting risk. Rigorous monitoring of blood electrolytes is imperative. Alternatively, utilizing a high-calcium dialysate leads to calcium loading, eliminating the need for a separate calcium infusion pump. This approach simplifies RCA delivery and stabilizes systemic calcium levels. Currently, citric acid is sometimes incorporated into the acid concentrate as a substitute for acetic acid.

Comparisons between RCA and citrate-containing dialysate highlight their differences and similarities. RCA presents as an excellent alternative to heparin for patients at high risk of bleeding, offering effective anticoagulation while maintaining circuit patency during dialysis treatments.

1.6.1 ANTICOAGULANT THERAPY

Clotting within the extracorporeal circuit during hemodialysis presents a significant challenge, leading to missed or shortened dialysis sessions, increased nursing workload, and elevated treatment costs. Furthermore, the repercussions of inadequate dialysis, such

as blood loss, fluid overload, mineral accumulation, and higher hospitalization rates, greatly impact patients' well-being and quality of life, contributing to heightened distress. Effective anticoagulation therapy is essential for preventing circuit clotting and ensuring dialysis adequacy. However, despite the severe consequences patients face due to circuit clotting, their perspectives on anticoagulation therapy decision-making remain poorly understood. This article explores patients' viewpoints and priorities concerning clotting and anticoagulation therapy, emphasizing the importance of shared decision-making in supporting their treatment. Understanding patients' perspectives on managing thrombotic complications in the extracorporeal circuit can guide strategies to enhance care and outcomes for individuals undergoing hemodialysis. Clotting within extracorporeal circuits presents a significant challenge for patients undergoing hemodialysis. With a lack of standardized recommendations on anticoagulation therapy, providing patients with accessible information and education, training clinicians in effective communication, and fostering shared decision-making are essential for delivering patient-centered care.

1.6.2 ANTICOAGULANT EFFECTS

Traumatic Brain Injury (TBI) represents a critical neurological event often leading to complications like ischemia, edema, and inflammatory responses, significantly impacting morbidity and mortality rates globally. With an estimated 10 million individuals affected annually, TBI poses a substantial socioeconomic burden (Hasan, *et al.*, 2023).

Managing TBI patients is inherently complex, particularly in cases where patients are concurrently receiving anticoagulant therapy for conditions such as atrial fibrillation, deep vein thrombosis, pulmonary embolism, and certain cardiac diseases. As the population ages and cardiovascular diseases become more prevalent, the intersection of anticoagulation therapy and TBI is increasingly encountered in clinical practice. Anticoagulants primarily function by interrupting the coagulation cascade to prevent clot formation. However, in the context of trauma, especially TBI, this anticoagulated state can exacerbate intracranial hemorrhage (ICH) and potentially worsen injury severity. Post-TBI expanding hematomas are particularly worrisome, as they can elevate intracranial pressure and compromise cerebral perfusion. Thus, managing TBI outcomes in patients on anticoagulation therapy necessitates careful consideration of anticoagulant reversal,

timely surgical interventions, and vigilance for hemorrhagic progression complications. While several studies have explored the impact of prior anticoagulation on TBI outcomes, findings remain inconclusive. Some studies suggest an elevated risk of adverse outcomes, including increased ICH, prolonged hospitalizations, and higher mortality rates in anticoagulated patients with TBI. Conversely, other research indicates that with meticulous management, outcomes in these patients can be comparable to those not receiving anticoagulation therapy.

Nursing care assumes a crucial role in the management of TBI patients, particularly those on anticoagulant therapy. Meticulous monitoring, timely interventions, and patient education provided by nurses significantly influence patient outcomes, potentially mitigating complications and facilitating recovery. Understanding the dynamic interplay between anticoagulation and TBI is thus paramount for optimizing nursing care for this patient population.

This study critically evaluates the impact of prior anticoagulation therapy on post-TBI outcomes, offering evidence-based guidance for clinicians and nurses. Distinguishing itself from prior analyses, this study incorporates a comprehensive, up-to-date dataset that includes recent studies previously not assessed.

1.7 TYPES OF ANTICOAGULANTS

- Aspirin
- Heparin
- Warfarin

1.7.1 Aspirin

Aspirin, the oldest non-steroidal anti-inflammatory drug, continues to hold significance in the management of severe inflammatory disorders linked to elevated cardiovascular risk. While its role in chronic inflammatory joint diseases has somewhat diminished over time, its potent anti-inflammatory properties remain invaluable in conditions such as Kawasaki disease, adult-onset Still's disease, rheumatic fever, or recurrent pericarditis. Dosages ranging from 650 mg to 4 g/day have been employed in these contexts, resulting in notable reductions in inflammation-related symptoms and plasma inflammatory marker

concentrations.

1.7.2 HEPARIN

Aspirin, or acetylsalicylic acid, achieves its pharmacological effects through several mechanisms, primarily by irreversibly inhibiting the enzyme cyclooxygenase (COX). Here's how it works: Inhibition of Cyclooxygenase (COX) Enzymes: Aspirin acetylates and inhibits both isoforms of the cyclooxygenase enzyme, COX-1 and COX-2. These enzymes play a crucial role in converting arachidonic acid into prostaglandins, which are lipid mediators involved in inflammation, pain, and fever. By blocking COX, aspirin reduces prostaglandin production, leading to its anti-inflammatory, analgesic, and antipyretic effects.

Antiplatelet Activity: Aspirin's inhibition of COX-1 in platelets prevents the synthesis of thromboxane A₂, a potent platelet aggregator and vasoconstrictor. This action inhibits platelet aggregation and reduces the risk of blood clot formation, making aspirin a vital medication for preventing cardiovascular events like heart attacks and strokes.

Antipyretic Effects: By reducing prostaglandin synthesis in the hypothalamus, aspirin lowers the body's set-point temperature, resulting in decreased fever.

Analgesic Effects: Aspirin's anti-inflammatory properties contribute to its analgesic effects by reducing inflammation and inhibiting the sensitization of pain receptors.

Cardioprotective Effects: Aspirin's ability to inhibit platelet aggregation helps prevent clot formation in coronary arteries, reducing the risk of cardiovascular events.

Other Effects: Aspirin has been associated with various other effects, including modulation of immune responses, inhibition of cancer cell proliferation, and potential neuroprotective effects. However, these mechanisms are less well understood and require further research. While aspirin is generally considered safe, it can cause side effects such as gastrointestinal irritation and bleeding due to its impact on prostaglandin synthesis. Therefore, it should be used cautiously and under medical supervision, especially in individuals with a history of gastrointestinal bleeding or ulcers.

1.7.3 WARFARIN

Warfarin, a vitamin K antagonist (VKA) oral anticoagulant (OAC), is frequently prescribed for preventing stroke, venous thromboembolism (VTE), and other thromboembolic complications in patients with atrial fibrillation and mechanical heart valves (Mirleau, *et al.*, 2024). While the introduction of direct oral anticoagulants (DOACs) has led to a decline in warfarin use in recent years, it remains the preferred anticoagulant for many patients. This preference may stem from patient choice, specific medical conditions necessitating warfarin, or financial considerations where the additional cost of DOACs is not feasible.

CHAPTER 2

LITERATURE SURVEY

In a studies by Ronald LG Flumignan *et al.*,2022. The predominant manifestation of coronavirus disease 2019 (COVID-19) is respiratory insufficiency, often linked to diffuse pulmonary microthrombosis and thromboembolic events like pulmonary embolism, deep vein thrombosis, or arterial thrombosis. Patients with COVID-19 who experience thromboembolism tend to have a poorer prognosis. Anticoagulants, including heparinoids (heparins or pentasaccharides), vitamin K antagonists, and direct anticoagulants, are utilized for both the prevention and treatment of venous or arterial thromboembolism. Beyond their anticoagulant effects, heparinoids possess an additional anti-inflammatory potential. However, the efficacy and optimal use of anticoagulants in COVID-19 patients remain subjects of ongoing debate. The efficacy and safety of higher-dose anticoagulants compared to lower-dose regimens in COVID-19 patients have been extensively studied. According to current evidence, higher-dose anticoagulants do not significantly affect all-cause mortality (risk ratio (RR) 1.03, 95% CI 0.92 to 1.16, 4489 participants; 4 RCTs) and may increase the risk of minor bleeding (RR 3.28, 95% CI 1.75 to 6.14, 1196 participants; 3 RCTs). However, they likely reduce the incidence of pulmonary embolism (RR 0.46, 95% CI 0.31 to 0.70, 4360 participants; 4 RCTs) while slightly increasing the risk of major bleeding (RR 1.78, 95% CI 1.13 to 2.80, 4400 participants; 4 RCTs). The impact on deep vein thrombosis, stroke, major adverse limb events, myocardial infarction, atrial fibrillation, and thrombocytopenia is uncertain. Moreover, the effect of higher-dose anticoagulants on additional respiratory support, COVID-19-related mortality, and quality of life remains unclear. In hospitalized individuals with COVID-19, higher-dose anticoagulants show little to no difference in all-cause mortality compared to lower-dose regimens but increase the risk of minor bleeding within 30 days. They may possibly reduce pulmonary embolism incidence but also slightly elevate the risk of major bleeding. However, their impact on hospitalization duration, deep vein thrombosis, stroke, major adverse limb events, myocardial infarction, atrial fibrillation, and thrombocytopenia remains uncertain. When compared with no treatment, anticoagulants may reduce all-cause mortality, although this evidence is derived from non-randomized studies and is highly uncertain. The effects of anticoagulants on other

outcomes compared to no anticoagulants are also uncertain due to very low-certainty evidence or lack of data. While we have strong confidence that new randomized controlled trials (RCTs) will not alter the effects of different anticoagulant doses on mortality and minor bleeding, high-quality RCTs are still imperative. These trials should primarily address additional respiratory support necessity, compare anticoagulation types, and explore the effects of prolonged anticoagulant therapy.

Similarly Renske H. Olie *et al.*, 2023, Vitamin K antagonists (VKAs), primarily warfarin, have been the cornerstone of oral anticoagulant (OAC) therapy for decades. However, in the early 21st century, a new class of OACs emerged. Initially labeled as new OACs, they were later termed nonvitamin K OACs, and more recently, based on their pharmacodynamics (**PD**), reclassified as direct oral anticoagulants (DOACs). While VKAs inhibit the hepatic synthesis of mature vitamin K–dependent coagulation enzymes, particularly factors II (prothrombin), VII, IX, and X, DOACs directly target either thrombin (dabigatran) or activated factor X (FXa; rivaroxaban, apixaban, and edoxaban) to inhibit procoagulant enzymatic activity. Both VKAs and DOACs inhibit thrombin and fibrin formation, albeit through different mechanisms. In addition to pharmacodynamic differences, DOACs exhibit distinct pharmacokinetic (PK) profiles, leading to variations in OAC management. VKAs necessitate meticulous monitoring of prothrombin clotting time, standardized as international normalized ratio (INR), with dose adjustments aimed at maintaining an INR-based therapeutic range of 2–3 in most cases. In contrast, DOACs are administered in fixed doses, adjusted according to age, body weight, and/or renal function, without routine drug monitoring requirements. Large Phase III randomized clinical trials (RCTs) have firmly established the efficacy and safety of direct oral anticoagulants (DOACs) compared to vitamin K antagonists (VKAs) in preventing and treating venous thromboembolism (VTE), including pulmonary embolism, as well as in preventing cardioembolic stroke in nonvalvular atrial fibrillation (AF). Notably, nonvalvular AF represents the majority of oral anticoagulant (OAC) use worldwide, accounting for approximately 65% of cases. Due to their favorable practical management and lack of monitoring requirements, DOACs have largely supplanted VKAs in many major indications. However, VKAs are still preferred in certain high-thrombogenic conditions such as end-stage renal failure and hemodialysis (HD), mechanical heart valves, triple-positive antiphospholipid syndrome (APS),

and valvular AF. The most significant adverse effect of all anticoagulants is major bleeding, with rates ranging between 1.6% and 3.4% per year for both VKAs and DOACs, depending on patient characteristics and comorbidities. DOACs offer an advantage over VKAs with a lower risk of intracranial bleeding, reported at 0.1% annually compared to 0.3% for VKAs. Beyond their primary anticoagulant effects, both VKAs and DOACs may exert off-target effects of clinical relevance. VKAs have been associated with increased vascular calcification due to inhibition of vitamin K–dependent proteins in vascular tissue, although the long-term clinical implications remain uncertain. Conversely, the impact of DOACs on vascular calcification is not well understood. DOACs may also interfere with protease-activated receptor (PAR) signaling in various tissues, including the vasculature, potentially affecting the progression of atherosclerosis. However, the long-term clinical implications of these effects require further investigation, necessitating postmarketing surveillance of DOACs to elucidate any additional clinical outcomes related to both on- and off-target effects. In this review, we focus on the current indications for DOACs, particularly in challenging management scenarios, uncertain conditions, or complex patient populations. We also discuss drug-drug interactions (DDIs), indications for drug monitoring or measuring, and provide insights into new developments in oral anticoagulation therapy.

According to the study by Rozina Ghulam Mustafa *et al.*, 2022, Infection occurs when disease-causing agents, such as bacteria, fungi, parasites, or viruses, are transmitted from one individual to another, either through direct skin contact or indirectly via contaminated water or food. Food-borne bacterial diseases, for example, can be caused by organisms like *S. aureus*, *E. coli*, *Salmonella* species, *K. pneumoniae*, *Listeria monocytogenes*, and *Campylobacter* species. Recent research in antimicrobial studies has underscored the threat posed by bacteria, which can lead to both nosocomial and community-acquired infections, posing a significant clinical challenge. Antibiotics serve as crucial tools in combating bacterial infections and have been instrumental in promoting health. However, their efficacy is increasingly compromised by the rapid emergence of antibiotic-resistant bacteria worldwide. This resistance is attributed to various factors, including the misuse of antibiotics and a scarcity of new drug development by the pharmaceutical industry. Traditional methods of antibiotic discovery have also contributed to the problem by fostering the emergence of multidrug-resistant bacteria. Mechanisms of antibiotic resistance include modifications of drug

target sites, enzymatic inactivation of antibiotics, alterations in cell wall proteins leading to decreased antibiotic penetration, presence of antibiotic-resistant genes carried by plasmids, activation of efflux pump mechanisms, modification of metabolic pathways, and the presence of antibiotic-degrading enzymes. To address the challenge of antibiotic resistance, researchers are exploring novel strategies for developing antibacterial drugs. These include targeting bacterial proteins, modulating host response pathways, using bacteriophages to treat bacterial infections, employing enzymes in combination with antibiotics, utilizing bioenhancers, producing antimicrobial peptides from various sources, developing hybrid antibacterial drugs, utilizing liposome-mediated drug delivery, and exploring herbal derivatives. Furthermore, antioxidants such as flavonoids, tannins, anthocyanins, and phenolic acids have garnered attention for their potential as antioxidant, antibacterial, anti-inflammatory, antiviral, and anticancer agents. Phenolic compounds derived from lignocellulosic waste, for instance, have demonstrated antimicrobial properties against food-borne pathogens. Additionally, earthworms have been found to contain antimicrobial and anticancer molecules, highlighting the potential of natural sources in drug discovery. Screening of earthworm species has revealed their antimicrobial activities, further expanding the repertoire of potential therapeutic agents. Carmine Siniscalchi *et al.*, 2023, Venous thromboembolism (VTE) presents a significant global burden in terms of morbidity and mortality, posing substantial challenges to healthcare systems worldwide. Standard treatments for VTE, including low molecular weight heparin (LMWH), unfractionated heparin (UFH), vitamin K antagonists (VKA), and direct oral anticoagulants (DOACs), are effective but carry inherent risks of bleeding complications. In patients with unprovoked VTE, discontinuing anticoagulation increases the risk of VTE recurrence, while the recurrence rate during anticoagulation remains notable within the initial months. An ideal therapeutic approach for VTE would entail reducing the risk of recurrence without exacerbating bleeding risk or mortality. Researchers have explored the potential of statins, potent lipid-lowering medications that inhibit cholesterol biosynthesis by targeting hydroxymethylglutaryl coenzyme-A reductase. Statins are widely prescribed for individuals with or at risk of cardiovascular disease, given their demonstrated efficacy in reducing the incidence of myocardial infarction, stroke, and cardiovascular mortality. Beyond their cholesterol-lowering effects, statins exhibit various pleiotropic effects that may contribute to their beneficial

outcomes in cardiovascular disease. These effects, independent of cholesterol reduction, have been proposed as potential mechanisms underlying the efficacy of statins in cardiovascular protection.

According to the study by M. Kopytek *et al.*, 2020, The thromboelastograph (TEG) is a diagnostic tool designed to assess and quantitatively analyze the coagulation status of whole blood samples. This dynamic global test provides valuable information on clot formation kinetics, clot strength, and fibrinolysis rate. Widely used in clinical practice, particularly in trauma and surgical settings, the TEG helps evaluate bleeding risk and guide hemostatic therapy. Additionally, it finds applications in various research fields. Emerging evidence suggests that TEG parameters can effectively evaluate the effects of oral anticoagulants on hemostasis. Studies have shown that TEG parameters, particularly in the kaolin test, are sensitive to different concentrations of direct oral anticoagulants (DOACs) both in healthy controls and patients on DOAC therapy. Certain TEG parameters have also demonstrated utility in assessing DOAC-induced coagulopathy, particularly in patients with atrial fibrillation (AF), where differences in TEG indices were observed depending on the type of DOAC used. Previous research has linked both venous and arterial thrombosis to a prothrombotic fibrin clot phenotype characterized by reduced clot porosity and impaired susceptibility to lysis. Treatment with vitamin K antagonists (VKA) or rivaroxaban has been shown to improve these clot characteristics. Dabigatran has been found to enhance plasma clot susceptibility to tissue-type plasminogen activator-induced lysis in a concentration-dependent manner while reducing thrombin generation. Conversely, rivaroxaban and apixaban have been reported to alter factor Xa cleavage, increase factor X-dependent fibrinolysis, and enhance plasmin generation in plasma samples from AF patients. These findings highlight the complex interplay between different anticoagulants and their effects on clot formation and fibrinolysis, underscoring the importance of personalized therapy in managing thrombotic disorders.

In a study by Marco Heestermans *et al.*, 2022, Thrombosis stands as a foremost cause of global mortality and is prioritized by the WHO for incidence reduction among non-communicable diseases. It manifests primarily through vascular occlusion, notably resulting in myocardial infarction, stroke, or venous thromboembolism

(VTE). Antiplatelet drugs are pivotal in treating arterial thrombotic events like myocardial infarction and stroke, while anticoagulants are key in VTE prevention and treatment, counteracting coagulation, particularly secondary hemostasis. In hemostasis, clot formation halts bleeding from vascular disruptions, but in pathology, coagulation fosters unwanted intravascular clot formation, termed thrombi. The earliest anticoagulants for VTE prevention, unfractionated heparin, and warfarin, were serendipitously discovered in the early 20th century. Over time, these drugs were refined for more efficient VTE prevention or treatment. Recently, a new generation of anticoagulants targeting coagulation factor IIa (thrombin) and factor Xa (FXa) was introduced, offering more precise therapeutic options. Presently, physicians can select from various anticoagulant drugs tailored to patient needs. Despite their efficacy in VTE prevention, anticoagulants still pose maneuverability constraints and carry hemorrhagic risks. This review aims to explore the history and pharmacology of current anticoagulants and briefly address the challenges in improving these therapies in the thrombosis and hemostasis community.

As per the view of Grossmann *et al.*, 2020, The available drugs for Alzheimer's disease (AD), such as cholinesterase inhibitors (e.g., donepezil, galantamine, rivastigmine) and glutamate antagonists (e.g., memantine), offer only a temporary delay in the onset of dementia symptoms. Consequently, there is an urgent need in pharmaceutical research for novel and more effective treatments. Recent research highlights the significance of accumulations of misfolded, toxic proteins of amyloid- β ($A\beta$) in brain tissue as pivotal in triggering AD pathogenesis. $A\beta$ is enzymatically released by secretases from the amyloid- β protein precursor ($A\beta$ PP). While $A\beta$ PP contributes essential synaptic function, toxic $A\beta$ accumulates as soluble dimers and oligomers, as well as insoluble fibrils ($A\beta$ plaques). These plaques and oligomers are found between neurons in specific brain tissue areas, especially the neocortex and hippocampus, and around cerebral blood vessels. $A\beta$ exists in a delicate balance between soluble and insoluble forms, actively transported across the blood-brain barrier (BBB). Studies suggest that soluble $A\beta$ aggregates contribute to neuronal and synaptic damage, with gene modifications linked to increased AD risk associated with $A\beta$ plaque formation. Early treatment with anti- $A\beta$ antibody aducanumab shows promise in improving cognitive performance by reducing $A\beta$ deposition. Additionally, AD progression involves tau-protein fibril deposition, defects in the BBB, neural inflammation, oxidative stress, and

synaptic and neuronal loss. Cerebral amyloid angiopathy (CAA), characterized by A β accumulation in cerebral blood vessel walls, is a lesser-studied aspect of early AD development. In CAA, A β aggregates accumulate around and within cerebral arteries and capillaries, disrupting blood flow and nutrient supply to brain tissues. Impaired A β clearance from the brain into the blood exacerbates brain A β accumulation, furthering AD pathogenesis. Studies in AD patients and animal models indicate that A β deposits in cerebral blood vessels contribute to CAA and subsequent cerebral hypoperfusion, leading to hypoxia, enhanced A β production, inflammation, neurodegeneration, and cognitive decline. Pericyte contraction induced by A β deposits around cortical blood vessels reduces capillary diameter, leading to decreased cerebral blood flow, chronic hypoperfusion, and hypoxia. CAA is detected in the majority of AD patients, and cortical blood flow reductions are observed early in AD development, suggesting hypoperfusion's significant role in promoting AD pathophysiology.

According to the research by Nipa Banik *et al.*, 2021, The longstanding clinical use of heparin and its derivatives is facing new challenges with the emergence of competitive drugs, particularly orally administered anticoagulants. While heparin remains an FDA-approved safe drug and a standard treatment for conditions like deep vein thrombosis (DVT), pulmonary embolism, and stroke prevention, newer anticoagulants are gaining traction in the pharmaceutical market due to their oral administration capability. Heparins, including unfractionated heparin (UFH) and low molecular weight heparins (LMWHs), are challenging to absorb orally due to their large molecular weights and strong negative charges. In contrast, newer direct oral anticoagulants (DOACs) with lower molecular weights are more easily absorbed, making them increasingly preferred for conditions requiring daily preventive anticoagulation, such as venous thromboembolism after surgery or arthroplasty. Despite their primary use as anticoagulants, heparin and its derivatives have demonstrated diverse therapeutic functions. Their molecular structure, similar to that of heparan sulfate (HS) found in the extracellular matrix (ECM), allows them to interact with various proteins and cytokines, offering potential therapeutic benefits beyond anticoagulation. For instance, heparin derivatives have shown promise in impacting cancer progression through anti-angiogenic and anti-metastatic effects, improving survival rates in cancer patients independent of their anticoagulant properties. Additionally, heparins have been implicated in wound healing acceleration, antiviral activity against human immunodeficiency virus, and regulation of

inflammatory arthritis. Several non-anticoagulant applications of heparin derivatives have been explored, although eliminating their anticoagulant effect for such purposes remains a challenge. Chemical conjugation of heparins with other molecules presents a potential avenue to enhance biological activity or therapeutic effect while mitigating the anticoagulant properties of heparin. Thus, the development and study of appropriate heparin derivatives or conjugates suitable for clinical use are crucial for expanding their therapeutic applications.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM:

- ❖ To understand the impact of anticoagulants on the coelomic fluid of an earthworm and their subsequent effect on angiogenesis during regeneration.

3.2 OBJECTIVE:

- ❖ Collection of Earthworm coelomic fluid through electric shock method and analyzing its anti-coagulant properties through warfarin and aspirin.
- ❖ To evaluate the angiogenesis process during regeneration.
- ❖ To analyse the effect of anti-coagulant on angiogenesis process during regeneration.

CHAPTER 4

MATERIALS AND METHODS

MATERIALS

- 1.5V Battery (Eveready)
- Red wire – Positive
- Black wire – Negative



Fig 4.1 Electric shock is given by the carbon zinc battery, Heavy duty, D size, 1.5V.

4.1 EARTHWORM MAINTENANCE

The earthworm, *Eudrilus eugeniae* are obtained from the stock culture that are maintained in the confined environment of Molecular Biology and Stem Cell Research Laboratory, International Research Centre, Sathyabama Institute of Science and Technology, Chennai as described earlier.

Worms are cultures in the plastic tub that contain soil, leaf litters and cow dung. The moisture condition of the worm was maintained in between 22-25°C with good ventilation. Every week, jaggery filtrate was sprayed over the earthworm bed for effective culturing of the worm and once in a month, the vermicompost that formed in the culture bed is replaced with fresh feed. The day and dark rhythm were maintained 12 hrs throughout the experiments.

4.1.1 Worm selection

The mature worms which are evident with the buffy clitellum with the average weight of around 900-950 mg were chosen for the present experiments. Worms are divided into 4 groups namely as control, electric shock treated, 2 mM thymidine treated and 2 mM thymidine + electric shock treated.

4.1.2 Shock Treatment

For performing electric shock, initially the setup is made by connecting the cathode and anode of Eveready carbon zinc battery (Heavy duty, D size) of 1.5 V to a circuit using an electric wire. group of worms are picked separately from the culture bed and washed thoroughly in a distilled water, dried using filter paper and kept inside the glass beaker. The cathode and anode end of the electric wire that are connected to the 1.5 V battery is placed simultaneously over the worms for approximately 40 times in a minute to evenly distribute the electric shock to the individual worms in a group. Following electric shock, worms are carefully placed in the culture bed and monitored every 24 hrs.



Fig 4.2 Coelomic fluid of aspirin injected worms

Anticoagulants like Aspirin, Warfarin and heparin were treated or injected to the live *Eudrilus eugeniae* earthworms.

➤ ASPIRIN

Aspirin is treated to Set 1 earthworms

➤ WARFARIN

Warfarin is treated to Set 2 earthworms

➤ CONTROL

Water is treated to Set 3 earthworms

4.2 COELOMIC FLUID EXTRACTION USING ELECTRIC SHOCK

Live earthworms were used for performing experiment for the extraction of coelomic fluid.

POWER SUPPLY

power supply is set to deliver a low-voltage, direct current (DC) electricity. The voltage should be within a safe range for the earthworms (typically around 1-5volts) Now the two electrode were kept at the same time in the earthworm's body cavity for the extraction of the coelomic fluid. After a few minutes coelomic fluid were extracted in the beaker.



Fig 4.3 Water injected worms

Ensure the Electric shock is treated with Aspirin injected worms. Now the two electrode were kept at the same time in the earthworm's body cavity for the extraction of the coelomic fluid. After a few minutes coelomic fluid were extracted in the beaker. Electric shock is treated with Aspirin injected worms. Electric shock is treated with Water injected worms Connect the electrodes to the power source (variable power supply or battery).

4.2.1 ANTICOAGULANT

TREATMENT Two sets of experiment were

made Experiment 1:

1. 75mg of Aspirin pill is crushed & mixed with 400 μ l of water
2. Then take 40 μ l of mixture from it & then dilute with 160ul of water
3. And make it to 200ul solution mixture. Then take 2 μ l from this mixture & add it to 8ulwater
4. Now it is 10 μ l of Aspirin mixture is ready for injection
5. Inject 10 μ l of Aspirin liquid into a set of two worms for observation.
6. After injecting, transfer these worms back into the soil for observation.



Fig 4.4 Coelomic fluid on water

Experiment 2:

1. Take 75 mg Aspirin & dilute in 400 μ l of water.
2. Now, its 475 μ l of Aspirin solution mixture.
3. Now, take this 475 μ l of Aspirin solution mixture into a test tube. A containing 5 mlof water.
4. Now take only 5ml of water in the test tube B.

4.2.2 ANGIOGENESIS ANALYSIS

After the treatment of anticoagulants and water to earthworms, it is now necessary to observe for the angiogenesis. Head part (before clitellum) of the earthworms were dissected in the each set of theearthworms (Set 1, 2, 3) and Observed after 3 days.

CHAPTER 5

RESULT AND DISCUSSION

5.1 RESULTS

5.1.1 EARTHWORM MORPHOLOGY

Earthworms exhibit bilateral symmetry and are characterized by external segmentation, which corresponds to internal segmentation. They lack a skeleton and possess a thinly pigmented cuticle, adorned with setae on all segments except the first two. Their body structure includes an outer layer of circular muscles and an inner layer of longitudinal muscles. The body of the Earthworm is divided into 4 parts — Head, Clitulum, Abdomen and Tail. Head constitutes from

1 to 12th segments, Clitulum constitutes from 13 to 18th segments, Abdomen and Tail constitutes the rest.



Fig 5.1 Earthworm morphology

Earthworms typically exhibit a reddish-brown hue, with their dorsal surface darker than the ventral side. This dorsal darkness is attributed to the presence of a dark median mid-dorsal line, a result of the dorsal blood vessel running along the longitudinal axis of the body. Additionally, their dark brown coloration is influenced by the presence of porphyrin pigment.

5.1.2 ANTICOAGULANTS EFFECT ON COELOMIC FLUID

Coelomic fluid of an earthworm is extracted by Electric shock method (6 V) for about 2 minutes. The coelomic fluid of an earthworm is sticky.



Fig 5.2 Electric shock treatment

Due to the sticky nature of the Coelomic fluid, we initiated a research to test what will happen. Anticoagulants were added to the Coelomic fluid. As it is already sticky in nature when it comes out from earthworm, there is no result when Anticoagulants was added.

So we injected the Anticoagulants to the earthworm to test see the appearance of the Coelomic fluid. The coelomic fluid (CF) found in earthworms is a rich reservoir of cells and various molecular components crucial for innate immunity. Among these components, glycoproteins with lectin properties play a significant role in identifying foreign substances by selectively binding to carbohydrates. This binding action initiates processes such as agglutination and lysis, effectively immobilizing and destroying the foreign material. In essence, these lectin glycoproteins act as key players in the earthworm's defense mechanism against potential threats.

5.1.3 INJECTION OF ANTICOAGULANTS

We injected two types of anticoagulants and water (control) to see the results.

The anticoagulants we injected was – Aspirin and Warfarin



Fig 5.3 Aspirin injected to Worm

Control – Water is injected to worm for Control observation.

Aspirin and Warfarin reacts well as a anticoagulants in Closed Circulatory System (Human Body) like in Arteries and Veins.

So we did this research to test how it reacts on Open Circulatory System.

Effect is not more as Expected. It is very less effective

5.1.4 EFFECT OF ANTICOAGULANT ON ANGIOGENESIS

One group of scientists tell that Anticoagulants induces angiogenesis. The other group says Anticoagulants suppress Angiogenesis. So we did study on this to know the actual behavior. We dissected the Worm in 10th segment after the injection of Anticoagulants to the Earthworm.



Fig 5.4 Dissection of head

Earthworm is maintained for about 4 to 5 days for the Angiogenesis analysis.

DAY 5



Fig 5.5 Aspirin injected bud

Aspirin injected earthworm's bud formation (comparatively more blood vessel formation)

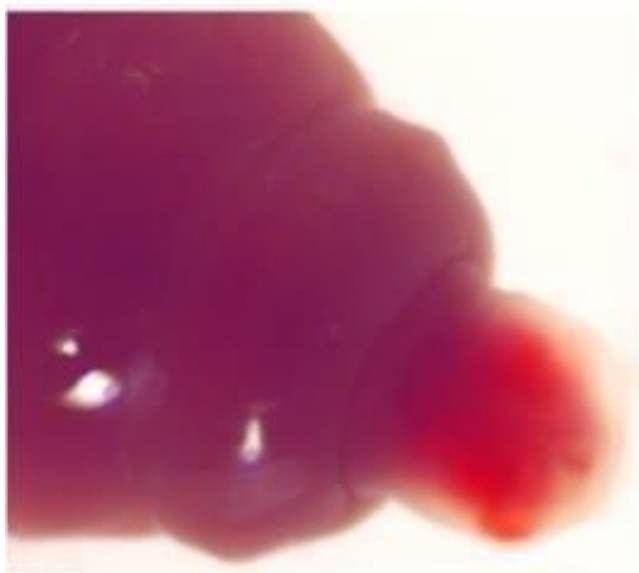


Fig 5.6 Warfarin injected bud

Warfarin injected worm's bud formation which is comparatively less as compared to Aspirin injected earthworm's bud formation.

From this we clearly says Aspirin induces more angiogenesis as compared to Warfarin

CHAPTER 6

SUMMARY AND CONCLUSION

The human body's primary circulatory system consists of blood. The main circulatory system of an earthworm consists of coelomic fluid. The earthworm possesses an open circulatory system. Coelomic fluid can be used as a substitute for Fetal Bovine Serum (FBS) in animal cell culture. Regeneration is closely linked to the process of angiogenesis. The earthworms utilized for the experiment are of the species *Eudrilus eugeniae*. The earthworm, *E. eugeniae* were kept in optimal conditions. An interesting experiment involves extracting the coelomic fluid of an earthworm using electric shock to observe its sticky nature. Water was used to treat the earthworms for control purposes, followed by the application of anticoagulants such as aspirin, heparin, and warfarin. Ultimately, after undergoing anti-coagulant treatment, the viscosity of the coelomic fluid decreases, suggesting that the anti-coagulant has a slight impact on the coelomic fluid. An anti-coagulant was used to study the impact on the process of angiogenesis. To investigate this, aspirin was administered to the worm and their ability to regenerate was observed on the fourth day. The data provides clear evidence that aspirin enhances the process of regeneration and angiogenesis in the earthworm. The findings indicate that aspirin has a more pronounced impact on the anticoagulant property of coelomic fluid, as well as promoting regeneration and the angiogenic process in the earthworm.

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