“EVALUATION OF ANGIOGENIC EFFECTS OF BELLIS PERENNIS 12X, 6CH, 30CH USING THE CHORIOALLANTOIC MEMBRANE ASSAY”

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IN

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SUBMITTED TO

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This is to certify that the Dissertation entitled, **"EVALUATION OF ANGIOGENIC EFFECTS OF BELLIS PERENNIS 12X, 6CH, 30CH USING THE CHORIOALLANTOIC MEMBRANRE ASSAY"** is a bonafide work carried out by **Dr. JAYANTHDEVAN. V,** a student of **M.D. (Hom)** in **DEPARTMENT OF HOMOEOPATHIC MATERIA MEDICA** (2020 to 2023) in **SARADA KRISHNA HOMOEOPATHIC MEDICAL COLLEGE, KULASEKHARAM, TAMILNADU**, under the supervision and guidance of **Dr. WINSTON VARGHEESE. V, M.D.(Hom), PROFESSOR, DEPARTMENT OF MATERIA MEDICA**, in partial fulfilment of regulations for the award of the degree of **DOCTOR OF MEDICINE (HOMOEOPATHY)** in **HOMOEOPATHIC MATERIA MEDICA**. This work confirms to the standards prescribed by **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI.**

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This is to certify that this dissertation work titled ***"Evaluation of Angiogenic Effects of Bellis Perennis 12X, 6CH, 30CH using the Chorioallantoic Membrane Assay"*** of the candidate **Dr. Jayanthdevan. V** with registration number **442220505504** for the award of **Doctor of Medicine** in the branch of **Homoeopathic Materia Medica**. I personally verified the Turnitin plagiarism detection report for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **14** percentage of plagiarism in the dissertation.

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ABSTRACT

Background: Bellis perennis homoeopathy medicine acts upon the muscle fibres of the blood vasculature. Many literatures shows that many tumour cases are treated with Bellis perennis and clinically Bellis perennis have strong action on tumours, Psoriasis and Deep Traumatic Injuries. So, Bellis perennis has Anti-angiogenic effect on both Tumours & Psoriasis and same Bellis perennis has Angiogenic promoting effect on Deep Traumatic Injuries. Angiogenesis is the process of which new blood vessels are developed from existing ones, it is vital to many physiological and pathological events. The formation of new blood vessels is a factor in many immune, ischemic, inflammatory, infectious, and malignant diseases when angiogenesis is out of control. It is theoretically highly important to understand the effect of bellis perennis on angiogenesis to use as therapeutics in various angiogenesis related pathological conditions. Chicken chorioallantoic membrane (CAM) on 9th day of incubation was incubated with 12X, 6CH and 30CH potency of Bellis Perennis for 24h, 48h, and 72 h using Whatman filter paper disc. CAM were exposed and photographed at 24h, 48h and 72h in various groups of eggs. The number of vessels was counted for three different time periods after the CAM was removed from eggs (24, 48 and 72 hour). Under a microscope, the number of vessels that were radially convergent towards the centre was counted and *Image J software* was used to analyse the photographs. The treated area was observed under microscope and histological evaluation was performed for the same. Tissue thickness was calculated morphometrically from haematoxylin and eosin-stained cross sections. Each group done in duplicate.

Results: Bellis Perennis has the ability to both induce neovascularization through sprouting and to inhibit angiogenesis in the treated area and growth of the newly formed blood vessels and cellular morphological changes occur in a potency dependent

manner. Increase in the tissue thickness at the treated area is suggestive of initiation of new capillary like structures.

Conclusion: *Bellis perennis* 6CH and 30CH showed significant angiogenic-effects at 72hours of incubation, while *Bellis perennis* 12X showed significant anti-angiogenic effects, indicating that the angiogenic potential of *Bellis perennis* depends on length of incubation period. Bellis perennis 30CH appears to have a greater ability than 6CH concentration to induce the sprouting of new blood vessels from the existing ones, according to an examination of the vessel growth pattern under a microscope. Bellis perennis can thereby induce neo-vascularization in a potency-dependent way; the 30CH potency may therefore be more suited, resulting in an earlier and more reliable angiogenesis. Overall, our findings suggest that Bellis perennis may be useful in angiogenic therapeutics and may be more effectively used in the treatment of wound healing, which requires the formation of new vessels, as well as tumours, which require anti-angiogenic activity.

Keywords: Angiogenesis, Bellis Perennis, Blood vessels, Chorioallantoic Membrane, CAM, Tumours, Wound Healing.

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1. INTRODUCTION

The use of homoeopathy in around the world has increased in popularity. The use of dynamized homoeopathic drugs in the management and treatment of various diseases has generated a great deal of interest as well as controversy. The scientific community is still skeptical despite its alleged efficacy and potential as a therapy.

In accordance with homoeopathy, medication preparations are created through gradual dilutions that preserve their original properties and lessen toxicity by initiating a gentle healing process.

Even though homoeopathic medicines have been shown to be effective in treating cancer, tumours, wounds, psoriasis, and other conditions, there is still a need for a reliable study on preclinical models (In Ovo and Ex Ovo) to identify their biological effects.

Angiogenesis is the process by which new blood vessels grow from existing ones. It appears in both disease & health throughout one's life, starting in the embryo & lasting till death.[1]. During this process, endothelial cells, which line the inside of blood vessels, migrate, grow, and differentiate.[2]

Chemical signals sent throughout the body regulate angiogenesis. Some of these signals bind to receptors on the surface of healthy endothelial cells, including vascular endothelial growth factor (VEGF). Signals are started inside endothelial cells when VEGF and other endothelial growth regulators bind to their receptor sites on endothelial cells, promoting the growth and survival of new blood vessels. Angiogenesis inhibitors are chemical signals that prevent blood vessel formation.

The angiogenesis stimulating & angiogenesis inhibiting effects of this chemical signals are normally balanced, so that blood capillaries form only when and where they

are needed, such as during growth and healing. However, for some reasons, these signals can become unbalanced, results in increased blood vessel formation, which can lead to abnormal illness. Angiogenesis, e.g., is the cause of age-related wet macular-degeneration.[2]

The Chorioallantoic membrane (CAM) of chickens is a highly vascularized extraembryonic membrane that performs a variety of functions during embryonic development, including but not limited to gas exchange. The Chorioallantoic Membrane (CAM), which is visible, accessible, and rapidly growing, offers obvious benefits for researching and controlling vascular activities.[3]

Bellis perennis homoeopathy medicine acts upon the muscle fibres of the blood vasculature. First drug used in treatment injuries or wound to the deeper tissues, after major surgical work[4]. Deep trauma or septic wounds.[5]

When there is the trauma in the case, the tumour presumably had its origin from trauma (traumatism in the causation of tumour). In this type of case Dr. Burnett cured tumours with well-tried anti-traumatic Bellis perennis homoeopathic medicine. Potency used was mother tincture and 3X to treat tumours[6].

J. Compton Burnett has successfully treated with its help numerous cases of tumour's that started as blows.[7].

Bellis perennis having Antioxidant, Anticancer, Wound healing, Anti Haemorrhagic, Haemolytic, Neuroprotective, Anti ulcerogenic activity[8].

The goal of present research is to determine the angiogenesis effects of bellis perennis medications of various potencies on the Chorioallantoic membrane assay. In total, four groups of eggs were created (one control and three medicine treated groups). Each group done in duplicate. After 24, 48, & 72 h in various groups, images of the

CAM assay were taken. Under a microscope, the treated area was examined, and a histological analysis was done.

1.1 BACKGROUND AND JUSTIFICATION OF STUDY

- The formation of new blood vasculature is a factor in many immune, ischemic, inflammatory, infectious, and malignant diseases when angiogenesis is out of control.[9]
- Angiogenesis, in response to tissue injury, is a dynamic process that is highly regulated by signals from both serum and the surrounding extracellular matrix.[10]
- In this modern era, evidence base study holds the prime importance. Bellis perennis has strong action on blood vessels and tumours, So I like to prove Bellis perennis homoeopathic medicine have angiogenesis effect on Chorioallantoic membrane assay.
- J. Compton Burnett treated many tumour cases with Bellis perennis and clinically Bellis perennis have strong action on Psoriasis and Deep Traumatic Injuries. So, Bellis perennis has Anti-angiogenic effect on both Tumours & Psoriasis and same Bellis perennis has Angiogenic promoting effect on Deep Traumatic Injuries.
- Therefore, I would like to experimentally demonstrate the hormesis effect of Bellis perennis homoeopathic medicine. Higher potency has angiogenic effect on deep traumatic injury while lower potency has anti-angiogenic effect on tumours and psoriasis.

1.2 SCOPE OF THE STUDY

- Experimental evidence could be used to demonstrate the authenticity of homoeopathic Materia medica.
- Since its inception, every system of medicine has made an effort to prove that it is a scientific one. Dr. Samuel Hahnemann observed, felt, and embraced reality as he perceived it. Even in the 18th century, his analytical mind was a forerunner of modern evidence-based homoeopathy medicine.
- Bellis perennis effectiveness has been proven through experimentation using the Chorioallantoic membrane assay.
- This research will educate us how homoeopathic remedies work and explore Materia Medica using an experimental framework (IN OVO).

2. AIM AND OBJECTIVES

- To know the angiogenesis activity of the Bellis perennis 12X, 6CH, 30CH in Chorioallantoic membrane assay.
- In which potency Bellis perennis homoeopathic medicine producing Angiogenesis or Anti-angiogenesis effect on CAM assay.

3. REVIEW OF LITERATURE

3.1 ANGIOGENESIS

Angiogenesis refers to the growth of new blood vessels from existing one. It occurs in both healthy and diseased conditions throughout life, beginning in & lasting till death. No metabolically active tissues in the body are more than a few hundred micrometers away from a blood capillary created by the angiogenic process. Capillaries are required in all tissues for nutrition and metabolite diffusion exchange. Differences in metabolic activity results in corresponding changes in angiogenesis and as a result, in equal changes in capillarity. The involvement of O_2 in this control is vital. Hemodynamic factors are necessary for vascular network survival and vessel wall structural adaptability.[1]

Blood vessels emerge during embryonic development from mesoderm derived endothelial cell progenitors (Vasculogenesis). Endothelial sprouting and splitting help these vessels develop and remodel into the mature network (angiogenesis).[11]

Angiogenesis stimulation can be therapeutics in Peripheral arterial diseases, Ischemic cardiac diseases & wound-healing.

Depriving angiogenesis can be therapeutics in ophthalmic diseases, carcinoma, arthritis (rheumatoid), & some other conditions.

Blood vessels in healthy tissues develop and shrink in response to physiological needs. Exercises promotes angiogenesis in the skeletal muscles & the heart. Capillary regression is caused by a lack of activity. Capillaries in adipose tissue expand in obesity and contract during wt. decrease. Angiogenesis is evidently ongoing throughout life.

3.2 THE SOURCE OF BLOOD VESSELS

The first organ system to form in the embryo is the cardiovascular system. There is only one layer of endothelial cells on the luminal side of the circulatory system that comes into contact with blood, and these cells come from mesodermal. Angioblasts and hematopoietic stem cells are produced by mesodermal stem cells known as Hemangioblasts. Angioblasts can differentiate in to the endothelial cells, but they lack all of their distinguished patterns at this stage. Vasculogenesis is the development of blood vessels from angioblasts from beginning. It happens in the extra - embryonic and intraembryonic tissues of the embryo. Vasculogenesis is a vital process that involves cell-cell and cell-extracellular matrix interactions. Morphogens as well as development factors control the process' spatial and temporal direction. In this process, mesodermal stem cells differentiate into angioblasts and angioblasts migrate under the control of growth factors to form blood islands where endothelial cells are produced.

3.3 TYPES

- Sprouting angiogenesis
- Intussusceptive angiogenesis

SPROUTING ANGIOGENESIS

Enzymatic breakdown of the blood capillaries basement membrane, endothelial cell proliferation, directed EC migration, tubulogenesis (EC tube formation), vessel fusion, vessel pruning, and pericyte stabilisation are the fundamental steps in sprouting angiogenesis. When O2 sensing mechanisms identify a level of hypoxia that necessitates the growth of fresh blood vessels to meet the metabolic needs of parenchymal cells, sprouting angiogenesis is triggered in poorly perfused tissues. The majority of parenchymal cell types, including myocytes, hepatocytes, neurons, and

astrocytes, secrete VEGF in response to a hypoxic environment (VEGF-A). It doesn't seem like there are any redundant growth factor mechanisms that can take over VEGF-function A's in hypoxia induced angiogenesis.[1][12]

SPLITTING ANGIOGENESIS

Due to the vessel wall extending into the lumen, which causes a single vessel to divide in two, splitting angiogenesis is also known as intussusceptive angiogenesis.

In neonatal rats, intussusception was first observed.

Intussusceptive angiogenesis occurs in 4 stages.

1. The two opposing capillary walls form a zone of contact.
2. Endothelial cell junctions are reorganised, the bilayer of the vessel is punctured to allow growth factors and cells to enter the lumen.
3. A core of pericytes and myofibroblasts develops in between two new vessels at the point of contact. In order to create an ECM for the expansion of the vessel lumen, these cells start to lay collagen fibres into the core.
4. The core has been fleshed out with no changes to the basic structure. Splitting is important because it involves a reorganisation of existing cells. It allows for a significant increase in capillary density without a corresponding increase in the number of endothelial cells.

This is important during embryonic formation because there aren't enough resources to build a rich microvasculature with new cells every time a new vessel forms.[13]

Compared to sprouting angiogenesis, this type of angiogenesis is thought to be quicker and more effective Bec. it initially only needs to reorganise existing endothelial cells and is not dependent on endothelial migration or proliferation right away. While splitting angiogenesis occurs throughout life, it is particularly important for vascular

development in embryos because of their rapid growth and scarce resource requirements. However, intussusception primarily results in the development of new blood vessels where blood vessels already occur..[1][14]

3.4 PHYSIOLOGY OF ANGIOGENESIS

Mechanically induced stimulation

Mechanical stimulation of angiogenesis is not well understood. There is considerable debate about how shear stress acts on capillaries to cause angiogenesis, though current knowledge suggests that increased muscle contractions may increase angiogenesis.[15]

Nitric oxide production may boost during workout, exercise which could account for this. Vasodilation of blood vessels is induced by nitric oxide.

Chemically induced stimulation

Chemical stimuli for angiogenesis include growth factors like Vascular endothelial growth factor and fibroblast growth factor, as well as a number of angiogenic proteins like integrins and prostaglandins.

3.5 THERAPEUTICAL TARGET FOR ANGIOGENESIS

Angiogenesis might be used to combat diseases like cardiac diseases, which either have poor vascularization or have abnormal vasculature.[16]

The growth of fresh vessels in the human body can be inhibited or stimulated by particular substances, that might help treat such conditions. The mechanical characteristics of a tissue may be impacted by the existence of blood vessels where none should be, raising the risk of failure. A tissue that is attempting to repair or otherwise metabolic processes active may not be able to perform necessary functions such as

restoration (repair) if there are no blood vessels present. A local expansion of vasculature can be used to treat a variety of medical conditions, including ischemic infected wounds like diabetic ulcer, varicose ulcer, which are brought on by the inadequate development of blood vessels. This facilitates healing and the delivery of new nutrient to the area. Other conditions, due to ageing like macular degenerations, may be brought on by a localised increase in blood vessels that interferes with physiological function.

There are 2 main categories for contemporary clinical uses of angiogenesis: anti angiogenic treatment, which are where angiogenesis study began, and pro angiogenic treatment. Anti-angiogenic treatment are used in treatment of carcinoma and also in other malignant conditions. [17][18].

3.6 DISEASE CHARACTERISED BY EXCESSIVE-ANGIOGENESIS

- Numerous organs – carcinoma, infectious disease, auto-immune disease
- Blood vessels – venous malformations and arteriovenous malformations (AVMs), 22q11.2 deletion syndrome, cavernoma, transplantation arteriopathy (renal allograft rejection).
- Adiposity-based chronic disease – obesity
- Skin diseases – plaque psoriatic lesions, verruca (warts), dermatitis, keloidal scar, lobular capillary haemangioma, Kaposi's sarcoma-associated herpes virus in AIDS patients.
- Eyes – Vitreous wick syndrome, diabetic-retinopathy, retrolental fibroplasia, choroidal neovascularization (exudative age-related macular degeneration).
- Pulmonary – pulmonary arterial hypertension, bronchial asthma, ethmoidal polyps.

- Gastro intestinal tract – IBS, periodontal disease, ascites, peritoneal adhesions.
- Reproductive system - Dysfunctional uterine bleeding, PCOD, ovarian hyperstimulation syndrome.
- Bones and joint – osteoarthritis, synovial inflammation, osteomyelitis (bone infection).

3.7 DISEASE CHARACTERISED BY INHIBITION OF ANGIOGENESIS

- CNS – Alzheimer's disease (senile dementia), Lou Gehrig's disease, diabetic amyotrophy, cerebrovascular accident.
- Blood vessels – atherosclerotic cardiovascular disease, arterial hypertension, diabetes mellitus, coronary restenosis.
- Gastrointestinal tract– gastric and oral ulcer, regional enteritis.
- Skin – alopecia, Henoch-schonlein purpura, spider veins and phlebectasis
- Reproductive system – preeclampsia toxaemia (PET)
- Lung – respiratory distress syndrome, interstitial pulmonary fibrosis, emphysema (COPD).
- Kidney – Berger's disease.
- Bone – osteoporosis, impairment of fracture healing[9].

3.8 WOUND HEALING IN ANGIOGENESIS

During the healing process, angiogenesis capillaries sprouts infiltrate the fibrin wound clot and, within some few days, organise into a micro - vascular channel throughout the granulation tissue. Blood vessel density declines as collagen builds up in the granulation tissue to form scars. Endothelial cells, angiogenesis cytokines such as Fibroblast growth factor, vascular endothelial growth factor, transforming growth factor-β, angiopoietin, & mast cell tryptase, & ECM interact dynamically.[19]

3.9 TUMOR GROWTH AND ANGIOGENESIS

Because solid tumours require a blood supply to enlarge beyond a few mm in size, angiogenesis is crucial to the development of carcinoma. The formation of this blood supply may be induced by tumors through the release of chemical signals that promote angiogenesis. Additionally, tumours can stimulate the nearby normal cells to produce angiogenic signalling molecules.

Newly forming blood vessels that "feed" developing tumours with O2 & nutrient ions do this because it allows the tumour to grow or enlarge and allows carcinoma cells to enter nearby tissue, move throughout the body, and form new colonies of cancer cells; this is known as metastasis.

The tumors cannot develop and spread without a blood supply, so researchers have developed an angiogenesis-inhibiting agent or drug that prevents tumour angiogenesis. The aim of these drugs, also known as antiangiogenic agents, is to slow or reduce carcinoma growth by reducing its blood supply.[2].

Cancer cells have lost their ability to divide in a controlled manner. A malignant tumour is made up of a population of rapidly dividing and growing cancer cells that accumulate mutations over time.[20][21]

Tumours promote angiogenic activity by secreting growth factors & proteins such as vascular endothelial growth factors and basic fibroblast growth factors, which can cause capillaries growth within the tumor, which some researchers believe supplies needed nutrients, allowing the tumor to grow. Tumor blood vessels, in contrast to normal blood vessels, are enlarged, and their shape is irregular.[22][23]

3.10 PSORIASIS AND ANGIOGENESIS

In the onset of psoriasis, angiogenesis plays a significant role.

Early psoriasis causes the development of new blood vessels, which disappears once the condition is under control. When psoriatic lesions develop, several angiogenesis mediators, including VEGF, oxygen-depriving factors, angiopoietins, & pro-angiogenic cytokines like TNF, IL-8, & IL-17, are up-regulated. Strong blood vessel formation may be a result of contact and mediator-dependent factors produced by keratinocytes, mast cells, & immune cells in psoriatic lesions.[24]

3.11 CAM ASSAY

During avian development, the mesodermal layers of the chorion and allantois' combine to form the CAM. This structure quickly grows, creating a dense vascular network that acts as a conduit for the exchange of gases and waste products.

The CAM makes it possible to research tissue grafts, carcinoma development and metastasis, therapeutic agents and toxicologic evaluation, as well as angiogenesis and anti-angiogenesis molecules. The chorioallantoic membrane is a quicker, simple, and cost-effective model that enables the quick screening of many therapeutical experiments.

DEVELOPMENT OF EMBRYOS, MORPHOLOGICAL STRUCTURE, AND PHYSIOLOGICAL FUNCTIONS

The allantois of the chick embryo first appears as an evagination from the ventral wall of the endodermal hind gut at around three to four days of incubation. It push through the extra - embryonic body cavity and out of the embryo. From days 4 to 10, the allantoic vesicle expands rapidly, and the mesodermal layer of the allantois' fuses with the adjacent mesodermal layer of the chorion to form the chorioallantoic

membrane. Two allantois arteries and one allantoic vein connect this double layer's extremely rich vascular network to the embryos circulatory system.

The chorioallantoic membrane surface enlarge rapidly from 6 square centimetre on day six to 65 square centimetre on day fourteen, and feed vessels are becoming more numerous.

The development of CAM vascularization occurs in three stages.

- Several capillary sprouts enter the mesenchyme during the initial stages, fuse, & create the primary capillary plexus.
- In the stage 2, tissue pillars that express splitting microvascular development have taken the place of the Sprouts, which have disappeared.
- The growing pillars enlarge to form intercapillary meshes during final stage.

3.12 METHODS IN OVO AND EX OVO

IN OVO

As early as embryogenesis starts, fertilised domestic chicken eggs are set up in accordance with Hamburger and Hamilton and put into an incubator where they're managed to keep at 37 degrees Celsius in constant humidity.

After 1 to 3 millilitres of albumen are taken out on day3 to separate the Chorioallantoic membrane from the shell itself, a square window in the shell is created, exposing the underlying Chorioallantoic membrane vessels.

A sterile micropore tape is used to seal the window, and the incubation period lasts until the experimentation day. The area available for grafting, transplantation, & fine micro dissection is constrained, despite the fact that this method preserves a more physiological environment.

EX OVO

The embryo and its extraembryonic membranes could be transmitted to a Petri plate on day 3 of incubation, where Chorioallantoic membrane develops at the top as a flat membrane and extends to the dish's corner to form a two-dimensional monolayer that can accommodate multiple grafts.

Outside of shell, the embryos accessibility is greatly enhanced.

Long-term viability is often lower in shell-less cultures, and great care must be taken to keep the embryo from drying out.

3.13 ANGIOGENESIS AND ANTI-ANGIOGENESIS MOLECULE

Angiogenesis in the CAM have been reported to be enhanced and decreased by growth factor, hormonal changes, natural compounds, anti-carcinoma drugs, vapours, organic chemicals, pro angiogenic molecule, antibiotic, antibodies, & synthesised smaller molecule.

The test agent is usually inserted as small Whatman filter discs.

After 71–95 hours of incubation, an angiogenesis response manifests as increased vessel density around the implantation and radial vessel convergence—like spokes on a wheel—toward the centre.

The vessels surrounding the implantation become less dense after 71–95 hours and eventually vanish when such an angiostatic substance is evaluated.[25][26]

3.14 BELLIS PERENNIS (BRUISEWORT)

Common Names: English daisy, Bonewort, Bruisewort

DESCRIPTION

Bellis perennis is a herbaceous perennial with rhizomes that is grown as a tender biennial.

This plant prefers colder temperatures, cool, moist, rich soil, afternoon shade, and sun during the day. English daisies frequently wilt in lawns after escaping from flower gardens. This plant is not drought-resistant and is harmed by the hot weather. This plant blooms sporadically up until frost during the spring and early summer seasons. If grown in a suitable environment, it might be challenging to control this plant's growth. English Daisy enjoys a lot of fertility. This plant can reach maturity in as little as 2 years but can take up to 5 years. English Daisy grows slowly at first, then quickly and can become weedy.[27] its leaves range in length from 2 to 5 cm.

The most prevalent species of the daisy genus is bellis perennis, which belongs to the Asteraceae family. Despite the fact that many closely related plants go by the name "daisy," this particular variety is more popular, more widely grown, and has more medicinal benefits than the others.

Other names for this plant include woundwort and bruisewort because of its medicinal qualities. For the treatment of injuries and the bad effects of injuries, bruisewort is used in different medical systems. Although it is native to western, northern, and central Europe, this plant is also widely cultivated throughout the majority of temperate regions.[28]

MORPHOLOGY

A 10–25 cm tall, herbaceous perennial or annual plant with creeping rhizomes & a sparse strigose scape.

Basal, rosulate leaves with long, winged petioles.

Spathulate, 2 to 6 cm by 1 to 2.8 cm, with serrated to crenate margins; attenuate base

Capitula terminal, single, and 2 to 3 cm wide

A 5 to 6mm Involved with phyllaries that are 2-seriate, subequal, oblanceolate, leaf-like, surfaces pubescent, margins sparsely ciliate, midvein thin, translucent, and an obtuse, scarious, ciliate apex. 1 to 2 mm long strigillose cypselae. absence of Pappus[8].

HABITAT

Western Asia and Europe are where bellis perennis originally came from, but because of its rising notoriety and valuable properties, it has since naturalised throughout the world. In nourishing and wet soil, this plant thrives. In gardens, parks, along roadsides, in meadows, and even in cemeteries, common daisies can be seen growing in abundance. Due to its invasive nature, some individuals do, however, consider it to be a problematic plant.

PARTS COMMONLY USED

Newly dried flower heads are frequently used for therapeutic purposes. But occasionally may utilise its leaf also.

MEDICINAL PROPERTIES OF COMMON DAISY.

- Astringent
- Anti-inflammatory
- Antispasmodic
- Digestion
- Wound-healing
- purgatives
- moisturizer
- Expectorants[28]
- Vulnerary
- Ophthalmic and
- Homeostatic[8]

THERAPEUTIC ACTIVITY OF BELLIS PERENNIS

- Antioxidant activity
- Anxiolytic activity
- Antidepressant activity
- Anticancer activity
- Antimicrobial activity
- Neuroprotective activity
- Antihyperlipidemic activity
- Wound healing activity
- Gastric emptying inhibition activity
- Anti haemorrhagic activity
- Haemolytic activity
- Antiulcerogenic activity
- Skin-whitening activity

ANTI-CANCER ACTIVITY

Together with a well-known saponin, bellissaponin BS2, six acylated triterpenoid saponins known as bellisosides A to F that were isolated from the roots of bruisewort exhibited cytotoxic effects against by HL-60 human promyelocytic leukaemia cell.[8]

NEUROPROTECTIVE ACTIVITY

In models of seizures brought on by pilocarpine, an ethanol flower extract of Bruisewort may modify epileptogenic and promotes anti convulsion and neuro protective processes, according to the findings of animal experiments. Pilocarpine-treated adult Swiss mice experienced convulsions that progressed into convulsive

status-epilepticus, more than 87% of the animals experienced striatum and 75% of them experienced hippocampus damage. Following the ethanol flower extract pre-treatment, these indices were drastically decreased.

WOUND HEALING ACTIVITY

The conventional use of Bellis perennis for wound repair was supported by the discovery that a lotion made from of the n-butanol fraction of Bruisewort flower had the ability to heal wounds without scar formation in a spherical excision wound model in rats.[8]

ANTI HAEMORHAGIC ACTIVITY

Using homoeopathic medicines for treatment. In a double-blind, placebo controlled, randomised clinical trial involving 40 individual people, it was discovered that arnica and bellis perennis reduced postpartum blood loss in comparison to placebo.

OTHER USES

Bruisewort is a well-liked ornamental that is generally grown in gardens & parks. In childhood games, daisies are frequently used to create daisy chains. Ceratocystis ulmi, the causative agent of Dutch elm disease, was shown to be resistant to the plant's antifungal properties both in vitro and in vivo.[8]

3.15 BELLIS PERENNIS IN HOMOEOPATHY

ACTIONS OF MEDICINE

- BLOOD-VESSELS.
- **Nerves.**
- **Spleen.**
- Joints.

- Left side.[5]

PREPARATION

Tincture of whole plant

CLINICAL ACTIONS

- Boils
- Artery disease
- Acne
- The railway spine
- Psoriasis
- Gout and rheumatoid arthritis
- Spleen disorders
- Stasis
- Varicosis
- Tumours
- Traumatism.

CHARACTERISTIC SYMPTOMS

- Common name of "Wound-wort," & "Bruise-wort," shows one of the chief spheres of action of Bellis perennis, and combine it in action with its follow Compositae family, Arnica Montana and Calendula officinalis.
- J. Compton Burnett is the chief authority for its homoeopathic uses. "It acts very much like Arnica Montana, even to the production of erysipelas. "He has cured with its aid several cases of tumours originating in a blow". "Stasis" & "fag" are the key notes of its action. Exudations and swellings of many kinds. Fagged womb, Varicose veins.

- It has a noticeable effect on female sexual organs, particularly on a uterus and breasts that are engorged. It alleviates varicose veins, walking difficulties, and pregnancy-related problems.
- This may be taken as a keynote: "effects of cold or iced drinks when heated"; indigestion, amenorrhea, skin complaints (including psoriasis), rheumatism from this cause. Near and remote effects of blows, falls, accidents (trauma). It makes you feel tired out and want to lie down.
- C.J. Burnett gives it extremely highly as a treatment for debility following acute gout attacks and places it in the same category as vanadium, which it complements in degenerative diseases including plaque of the arteries & softening of brain.
- When used locally, it has been seen to cause pupil dilation. It is best to avoid giving it right before bed because it may result in insomnia and early morning awakenings 3am, or vice versa. “waking up too early in the morning and cannot get to sleep again" is a leading indication for its use.[7]

3.16 CURABILITY OF TUMOURS

Bellis Perennis and Ceanothus Americanus, two remedies, successfully treat an abdominal tumour.

The tumour probably developed as a result of a fall on the left side eight years prior that cracked the ribs; hence, the side had never been comfortable again, and for many months, this enormous mass had been developing & becoming more and more uncomfortable, eventually impeding locomotion. In addition to being immobile, the patient was also unable to turn over in bed due to weakness and the bulking mass. The

situation was not made any better by the fact that the patient was well past the age of sixty.

A slender-built man was crouching on his back and leaning slightly to the right when I finally went to examine the patient two days later. He was unable to turn over on his own, so my wife and I had to help him. This allowed us to perform a complete medical examination, which revealed a massive tumour in his left side that extended almost to the edge of his pelvis. The skin of the belly had dark spots, and inside the left Poupart's ligament were several lumps that felt like little potatoes and were probably glands. The picture of a hopeless malignant condition is completed by a fairly cachectic appearance and rather severe adynamia.

Inquiring carefully about the opinions of my six doctoral predecessors, I learned that their two-family advisors had always believed the tumour to be associated to the left kidney. And when that didn't work, they called in a reputable doctor who believed it could be cured. However, after his efforts had also failed, a good surgeon was called, and he believed it was completely incurable. Then a consultant doctor & consultant surgeon from Guy's arrived, & after much discussion, it was determined that the patient had cancer or, at the very least, a spleen-related tumour that was or had developed into a malignant tumour, and that the outcome would unavoidably be fatal—it was only a matter of time, in fact. Even after a thorough examination, I was unable to determine if the tumour was related to the spleen, kidney, or both. The tumour practically filled the left half of the abdomen and, without taking into account its history, appeared to be related to the spleen.

There was a prospect of recovery. From a purely diagnostic standpoint, there had been enough of diagnosis; however, I discovered that the medical care had been

limited to tonic and quasi-absorbent measures, perhaps quinine, iron, mercury, and iodine.

A more hopeless condition for medical treatment can scarcely be found, I believe, as any practical doctor or surgeon will admit.

First, how should we choose allopathic, homoeopathic, or other medications for such a case?

In cases that seem so hopeless and difficult, my personal strategy is to seize firmly onto a point that might be severe as a reasonable therapeutical starting point from which to execute a cure.

The case has a positive traumatic component, which is why my personal go-to and tried-and-true anti-traumatic is bellis-p; next, my own proving on this drug demonstrates that it has a clear affinity for the left hypochondrium; and finally, bellis perennis has already successfully treated a few tumours in my hands.

This strategy is at least a stop-gap until further thought is given to how to seize hold of any help-promising solution in the face of overwhelming odds. Bellis perennis as an anti-traumatic herb and ceanothus americanus as a splenic herb both came to mind, but which?: however *Bellis perennis Q* and *ceanothus 1X* were given in five drop doses every four hours in alternation; this was on Nov. After confessing that I believed the good man was doomed but was still resolved to attempt to rescue him, I alternately administered my two cures because I didn't know which was more likely to act rapidly. I soon came to regret this decision, as using two medications at once taught next to nothing.

After taking this drug for a while, the patient was able to turn over in bed and eventually climb out of bed by himself. In addition, 17 days after starting the

prescription, on November 29th, the patient and his wife arrived in a cab at my west-end offices.[6]

3.17 CASE OF OVARIAN TUMOUR

A hard, tender, encircled tumour the size of an orange is felt in the left iliac region while lying back and having muscles at ease. It gradually developed after a fall she had a year earlier. There are furfuraceaous patches on the pubes & skin of the neck. Dr. Burnett prescribed *Bellis perennis 3X.* in water 5gtt in thrice a day.[6]

3.18 TRAUMATIC SWELLING OF RIGHT BREAST CURED BY BELLIS ALONE

The instance of a young woman's breast swelling, specifically to neatly illustrate the curative spectrum of the DAISY in the treatment of tumours.

No skilled medical professional will dispute the crucial role that bruises, blows, and falls played in the development of tumours and cancer; as a result, our anti-traumatic care should account for a far larger portion of our treatment of growths from blows.[29].

3.19 CASE OF ANGINA PECTORIS

A northern gentleman in his mid-sixties came to see me with angina pectoris. Given that his left hepatic lobe was enlarged, I administered him Cardus Marianas Q for a month.

Pain is not as acute; it travels from the right shoulder over the chest and makes him puffy. Walking and chatting make the agony worse.

J. Compton Burnett prescribed *Bellis perennis Q* ten drops in water at bed time.

Very much better; all discomfort is gone. "I believe it (the bellis) has done my husband a great deal of good, as he has not complained of pain for some time now," his wife stated in a letter to the doctor. [30].

3.20 BRUISEWORT IN THE DISCOMFORT OF PREGNANCY

Some women find it very difficult to move around while they are pregnant since walking is so uncomfortable and nearly impossible. In these situations, the daisy quickly makes things right. Of course, I refer to situations in which mechanical issues are the root of the problem and may be fixed.

I sent a lady some bellis perennis 1, Bec. being far gone in the family way, she found locomotion so very tired; a very short walk quite overcame her, a fortnight or so thereafter I received the following report: - “the bellis did me so much good; I can walk quite well now, and do not get tired or stiff.”

In this case, its response was immediate, satisfactory, & truly specific, with no unfavourable effects. Why did I provide bellis in this circumstance? Simply because the mechanical pressure that caused the discomfort complained of caused the tissues to be compressed and in a condition that was very similar to a bruise. As a result, I recommended my old friend the bellis perennis, which acts on the tissues and the muscle fibres of the blood vessels to remove these mechanical obstructions.[31]

4. MATERIALS AND METHODS

4.1 STUDY SETTING:

The research was done at 2 different places

- Preparation of homoeopathic medicine bellis perennis made manually using scales of succussion (decimal and centesimal scale) was done in pharmacognosy lab, Sarada Krishna homoeopathic medical college and hospital, Kulasekharam.
- Evaluation of angiogenesis using chorioallantoic membrane assay is done at Trichy Research Institute of Biotechnology Pvt.Ltd, Tiruchirappalli – 620018.

4.2 STUDY DESIGN

- The experimental study done on evaluation of angiogenic effects of bellis perennis using the chorioallantoic membrane assay, under a light microscope, the treated area was examined, and a histological analysis was done on it.

4.3 INTERVENTION

- The remedy Bellis perennis 12X, 6CH, 30CH of 10µl using Whatman filter paper disc implant in the chorioallantoic membrane in the ratio of 1:11 (1µl of bellis perennis + 10µl of distilled water) diluted homoeopathic medicine in 3 different groups B, C, and D respectively. Each group done in duplicate.
- Control group (group A) treated with using Whatman filter paper disc implant in the chorioallantoic membrane in the ratio of 1:11 (1µl of dispensing alcohol + 10µl of distilled water).
- Eggs were split into 2 groups:
  - Control-group.

- Homoeopathy medicine treatment groups (Bellis perennis 12X, 6CH, 30CH).

4.4 METHOD OF PREPARATION OF HOMOEOPATHIC MEDICINE.

Homoeopathy medicine Bellis perennis 12X, 6CH, 30CH is prepared manually. Using bellis perennis mother tincture as a source. (Bellis perennis Q was obtained from GMP Certified Homoeopathic pharmacy Schwabe, India Pharmaceutical company batch no: 0151000)

DECIMAL SCALE PREPARATION

- 0.5ml of Bellis perennis homoeopathic medicine mother tincture is poured in the phial and then 4.5ml of rectified spirit is added to the phial. This 1/3rd of the phial remains empty for succussion. Then it is corked and ten downward strokes of equal strength are given. The Bellis perennis 1X is now ready.
- 0.5ml of Bellis perennis 1X is poured in the phial and then 4.5ml of rectified spirit is added to the phial. This 1/3rd of the phial remains empty for succession. Then it is corked and ten downward strokes of equal strength are given. The Bellis perennis 2X is now ready.
- The above process is to be continued up to Bellis perennis 12X homoeopathic medicine preparation.

CENTESIMAL SCALE PREPARATION

- 0.1ml of Bellis perennis homoeopathic medicine mother tincture is poured in the phial and then 9.9ml of rectified spirit is added to the phial. This 1/3rd of the phial remains empty for succussion. Then it is corked and ten downward strokes of equal strength are given. The Bellis perennis 1CH is now ready.
- 0.1ml of bellis perennis 1CH is poured in the phial and then 9.9ml of rectified spirit is added to the phial. This 1/3rd of the phial remains empty for succession.

Then it is corked and ten downward strokes of equal strength are given. The Bellis perennis 2CH is now ready.

- The above process is to be continued up to bellis perennis 30CH homoeopathic medicine preparation.[32]

4.5 CHOROALLONTOIC MEMBRANE ASSAY

MATERIALS AND METHOD

4.6 MATERIALS

- Fertilized domestic chicken eggs were purchased from poultry
- Whatman No.1 filter paper disc
- Micropore tape
- Haematoxylin and eosin stain
- Bouin's fixation solution
- Paraffin wax.

4.7 IN OVO CAM MODEL

PROCEDURE

- According to the protocol outlined in the "hen's egg test – chorioallantoic membrane (HET-CAM)[33]," 50 TO 55g fertilised domestic chicken eggs were incubated at 37 degrees Celsius in "humid atmosphere" (>60% humid) for 8days and were slowly moved at least three times a day).
- A 9^{th} day old eggs after the incubation period was complete, embryo is located by 'candling' and its position is marked on the shell with pencil. Wipe the shell with 70 percent alcohol.
- A hole has been made in the egg's small end to remove 0.5 to 1ml of albumin using needle to minimize adhesion of the shell membrane. Forceps were used

to separate the shell surrounding the embryonic air sac, and the shell membrane at the air sac's base was peeled away.

- On the 9th day, a Whatman No.1 filter paper disc loaded with 10µl volume of 12X, 6CH, and 30CH of Bellis Perennis medicine was carefully inoculated on the sterile surface of Chorioallantoic membrane. The window was sealed with a sterile micropore plaster & eggs were incubated for 24h, 48h, and 72h in various groups of eggs.
- CAM were exposed and photographed at 24h, 48h and 72h in various groups of eggs.
- Number of vessels was counted for three different time periods after the CAM was removed from eggs (24, 48 and 72 hour). Under a microscope, the number of vessels that were radially convergent towards the centre was counted and *Image J software* was used to analyse the photographs.

LIGHT MICROSCOPE ANALYSIS

- After 24, 48, and 72 hours of incubation, the carefully detached Bellis Perennis-treated area of the CAM was examined. The dissected membrane was placed on glass slides, and photograph of the capillary growth were captured using a light microscope at 4x and 10x magnifications.

HISTOLOGY

- After 72 hours of incubation, Bouin's fixative solution was flooded over the bellis perennis-treated area of the CAM.
- The membrane that surrounded the treated area was carefully removed, dried using a graduated sequence of alcohol from (50%, 70%, 90%, and 100%), and then imbedded in paraffin wax. This procedure required the use of forceps and surgical scissors.

- Rotating microtomes were used to slice vertical cross-sections of tissue that were 7 micrometres thick. Before to staining with haematoxylin and eosin, sections were cleaned with xylene solution and treated with alcohol in descending order (100%, 90%, 70%, & 50%).
- The histological slides were mounted with DPX, evaluated qualitatively using light-microscope at a 40x magnification, & photographs were taken using a camcorder connected to light-microscope at a 10x magnification.[34]

PREPARATION OF BELLIS PERENNIS MEDICINE

Figure 1 - Bellis perennis mother tincture as a source of medicine preparation

Figure 2 – Micropipette 100µl, 200µl, 500µl, 1000µl

Figure 3- Preparation of Bellis perennis medicine in the process of succussion done in the pharmacognosy lab.

Figure 4 - Bellis perennis is prepared from 1X to 12X.

Figure 5 - Bellis perennis is prepared from 1C to 30C.

Figure 6 - Bellis perennis is prepared from both the decimal (1X to 12X) and centesimal scales (1C to 30C).

Figure 7 - Different scales of potencies of Bellis perennis used for the chorioallontoic membrane assay.

Figure 8 - Fertilized domestic chicken eggs are kept for incubation in an egg incubator.

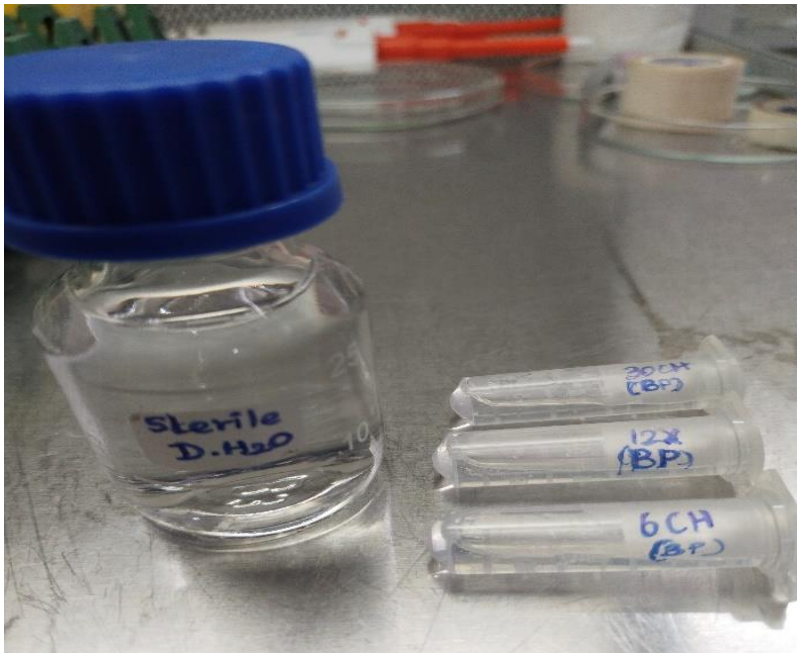


Figure 9 - Sterile distilled water and Bellis perennis samples

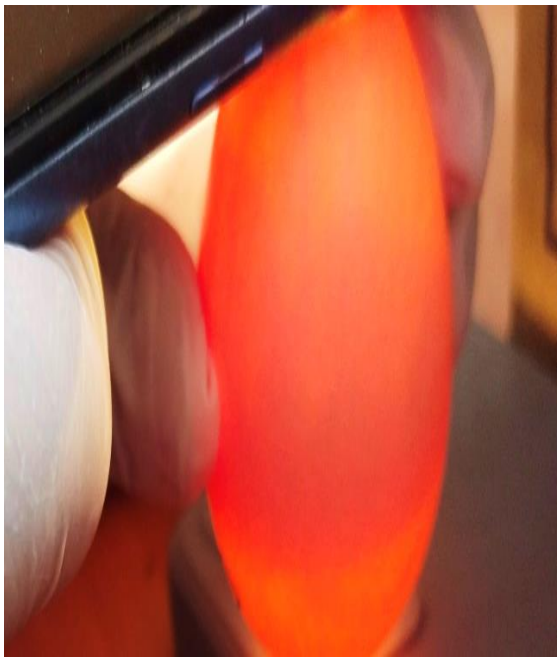


Figure 10 - Embryo is located by the candling method.

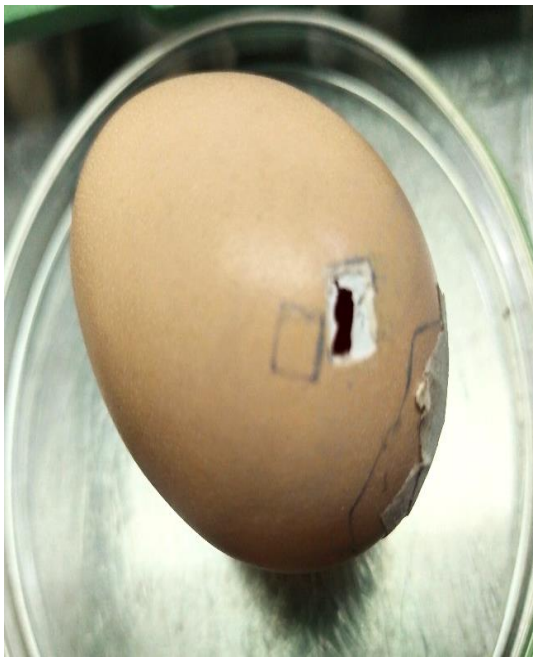


Figure 11 - Opening a window in an egg shell to innoculate medicine.

Figure 12 - CAM is inoculated with a sample of the Bellis Perennis drug using a micropipette.

Figure 13 - The CAM's detached Bellis Perennis-treated region was looked at. Images of the capillary growth were taken under a light microscope at 4 and 10x magnification using a light microscope while the excised membrane was kept on glass slides.

5. OBSERVATION AND RESULTS

5.1 CHORIOALLANTOIC MEMBRANE (CAM) ASSAY – ANGIOGENIC ACTIVITY

Angiogenesis is an essential process both in healthy and diseased states. Normally, vasodilatation and an increase in vascular permeability accompany this process.

Using a chorioallantoic membrane assay, bellis perennis 6CH, 30CH, and 12X were assessed for their capacity to promote and inhibit angiogenesis.

The highly vascularized extra embryonic membrane known as the chorioallantoic membrane of the chick embryo has several functions.

5.2.1 CONTROL GROUP

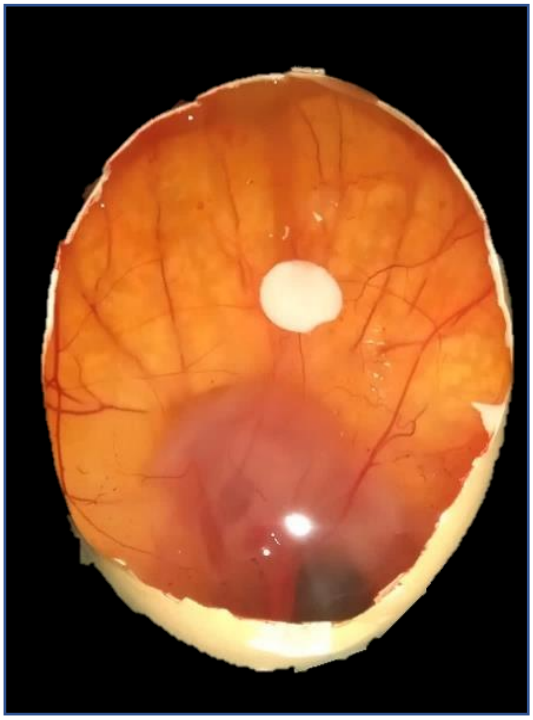


Figure 14 – CONTROL **24 HOURS**

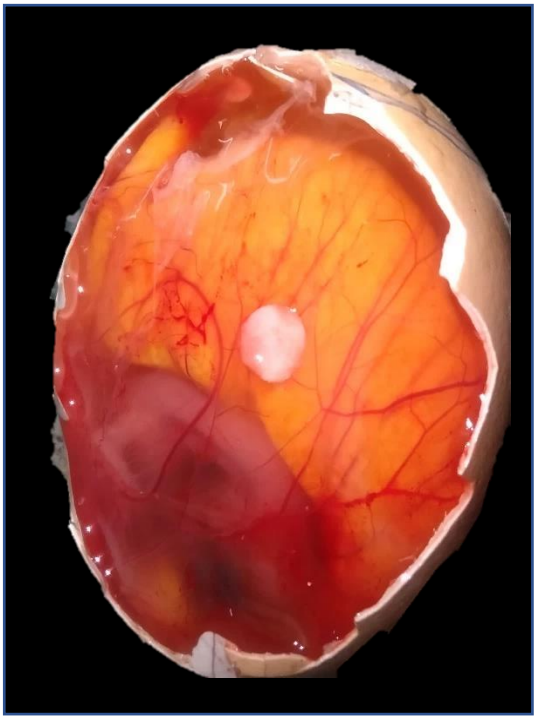


Figure 15 – CONTROL **48 HOURS**

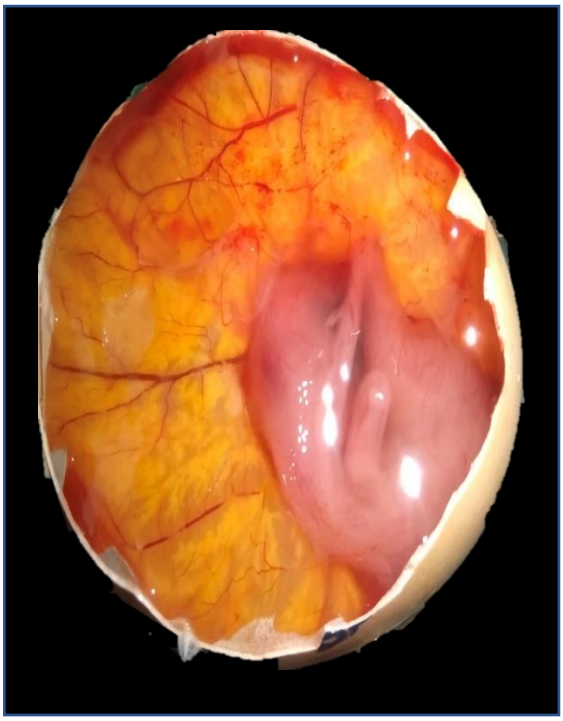


Figure 16 – CONTROL **72 HOURS**

5.2.2 CONTROL GROUP – LIGHT MICROSCOPIC VIEW

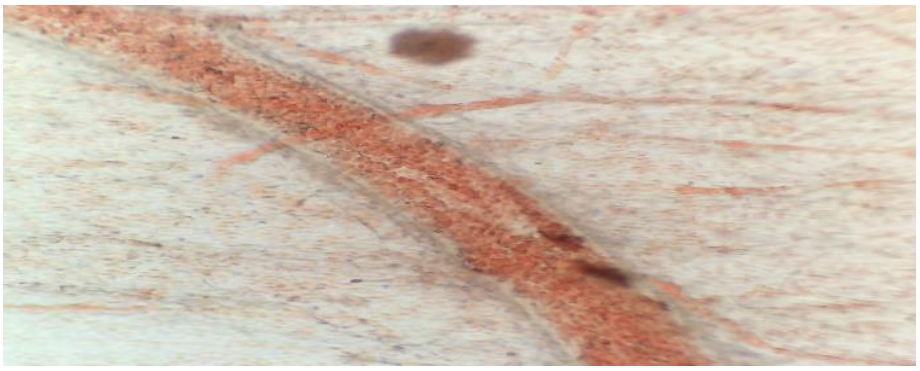


Figure 17 – CONTROL **24 HOURS**

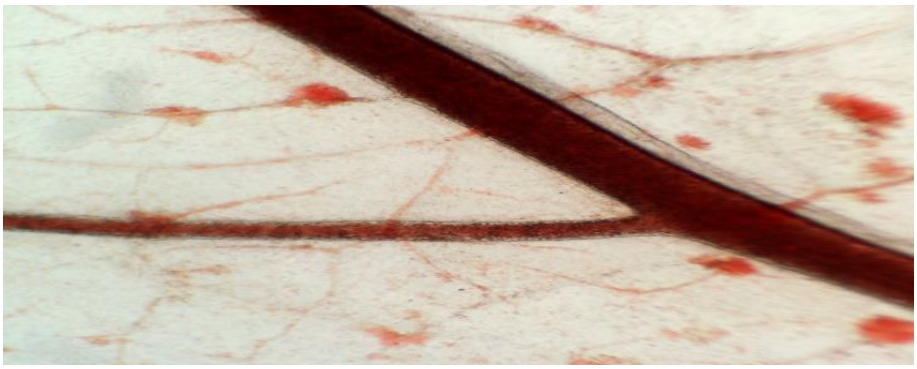


Figure 18 – CONTROL **48 HOURS**

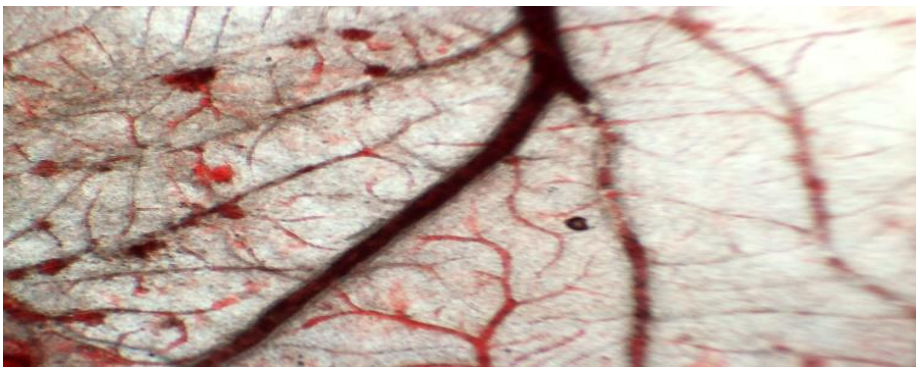
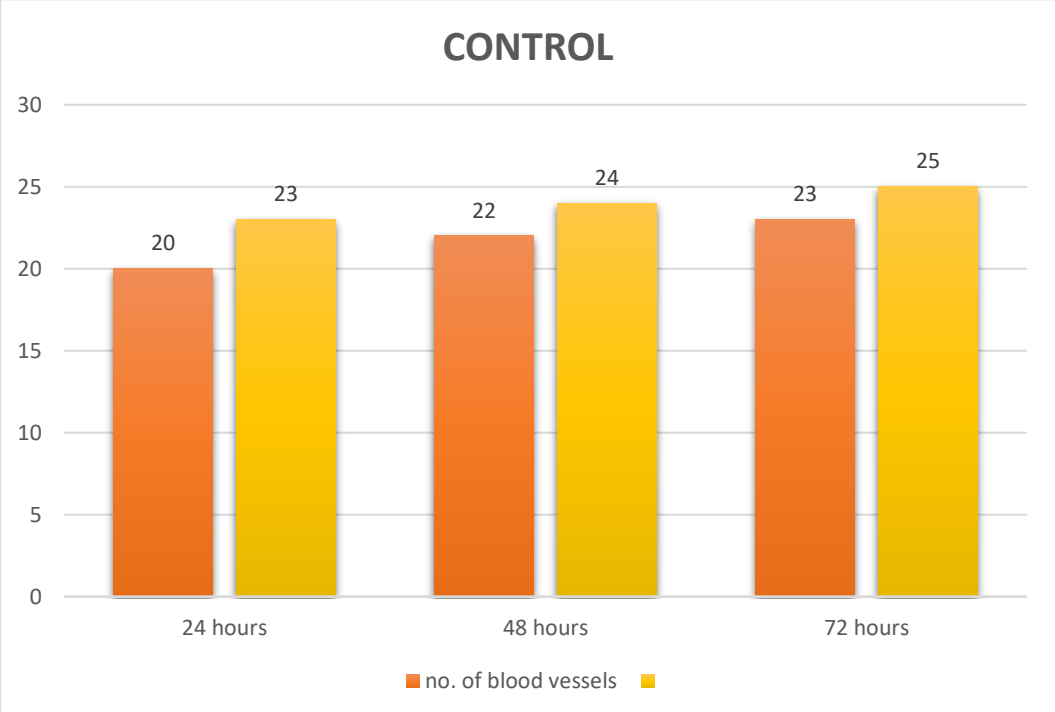


Figure 19 – CONTROL **72 HOURS**

TABLE 1 – NUMBER OF BLOOD VESSELS IN CONTROL

INCUBATION PERIOD	NO OF BLOOD VESSELS	
24 HOURS	20	23
48 HOURS	22	24
72 HOURS	23	25



GRAPH 1 – NUMBER OF BLOOD VESSELS IN CONTROL

5.3.1 CHICK EMBRYO TREATED WITH BELLIS PERENNIS 12X

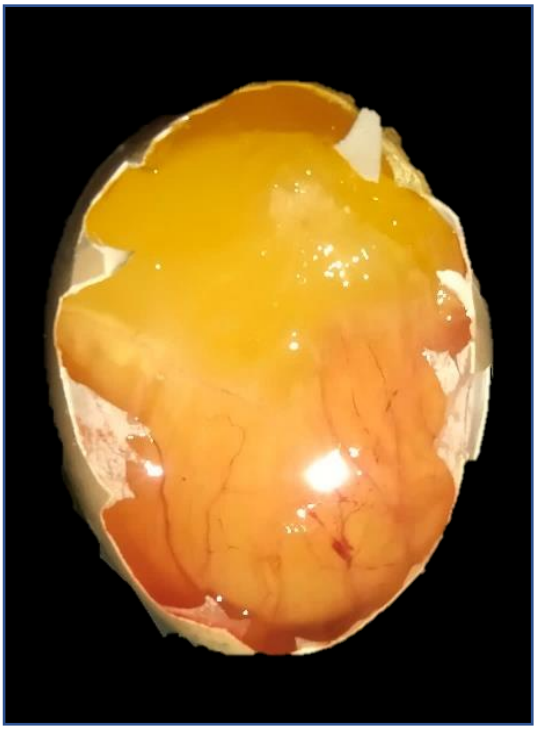


Figure 20 – 12X **24HOURS**

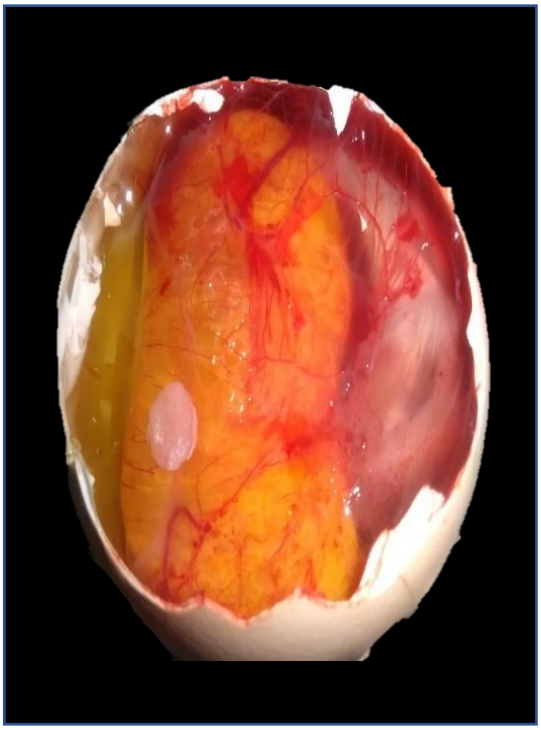


Figure 21 – 12X **48HOURS**

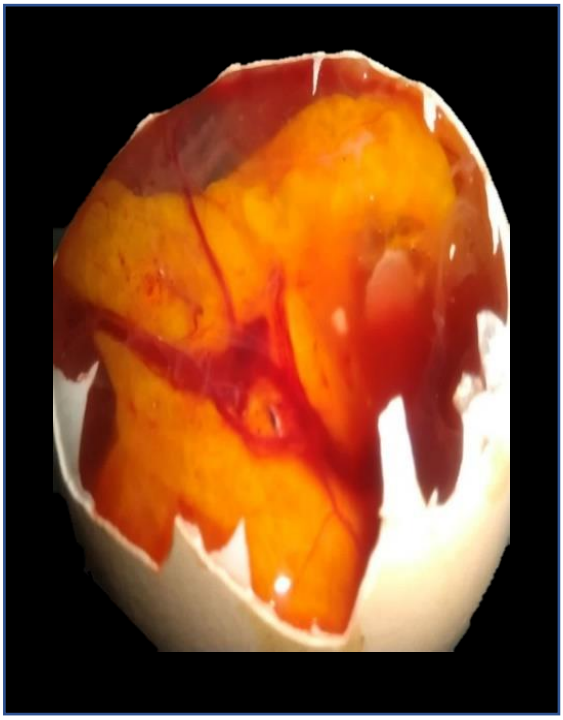


Figure 22 – 12X **72HOURS**

5.3.2 LIGHT MICROSCOPIC VIEW – Bellis Perennis 12X

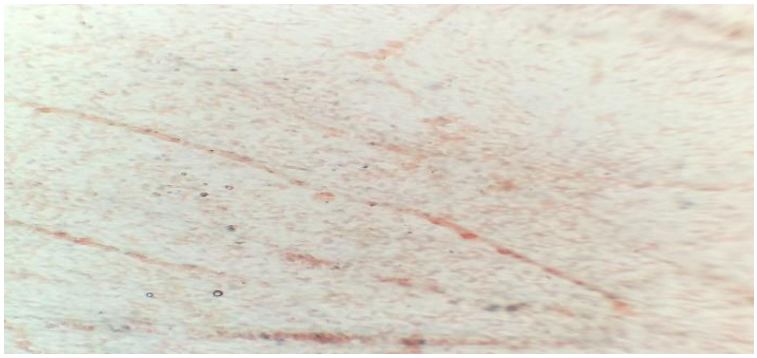


Figure 23 – 12X **24HOURS**

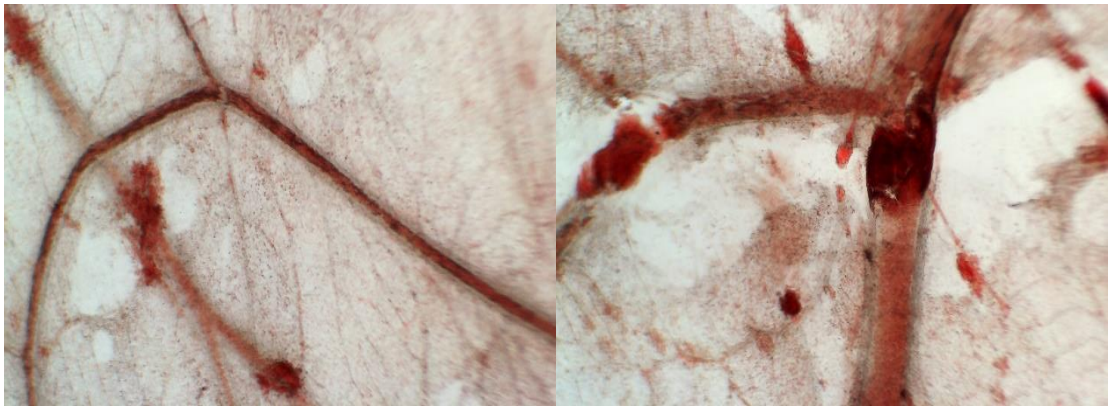


Figure 24– 12X **48 HOURS** LIGHT MICROSCOPE 4 and 10x magnification

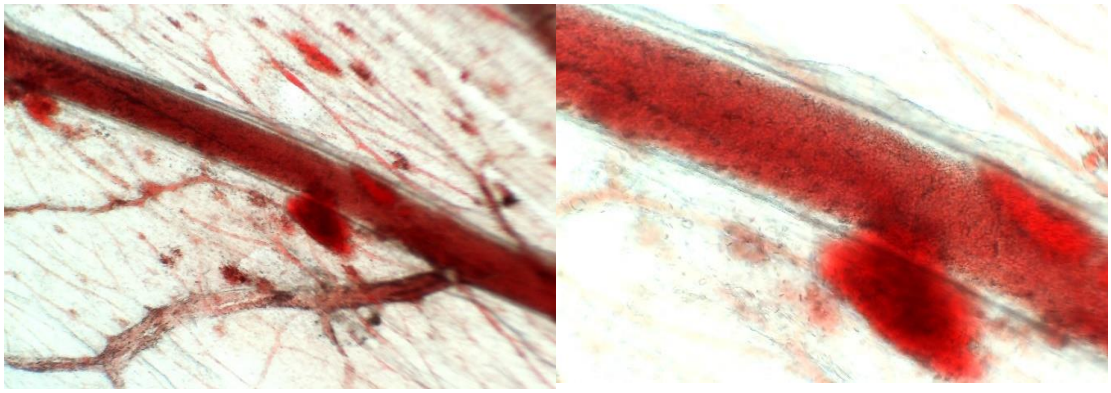
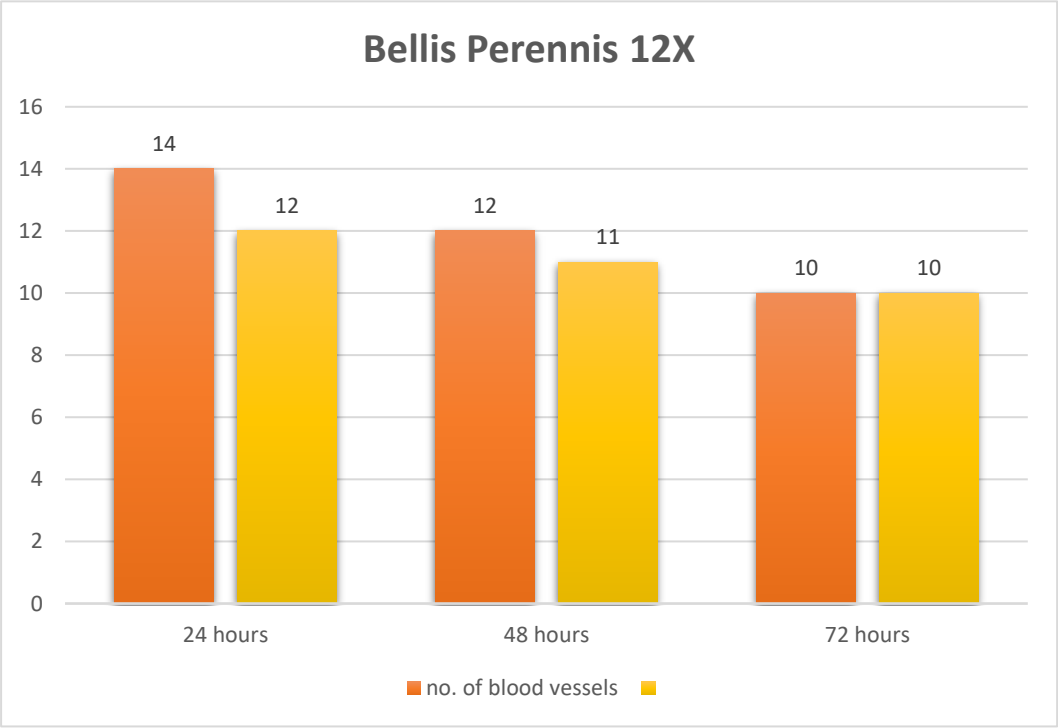


Figure 25– 12X **72 HOURS** LIGHT MICROSCOPE 4 and 10x magnification

TABLE 2 – NUMBER OF BLOOD VESSELS IN BELLIS PERENNIS 12X

INCUBATION PERIOD	NO OF BLOOD VESSELS	
24 HOURS	14	12
48 HOURS	12	11
72 HOURS	10	10



GRAPH 2 – NUMBER OF BLOOD VESSELS IN 12X

5.4.1 CHICK EMBRYO TREATED WITH BELLIS PERENNIS 6CH

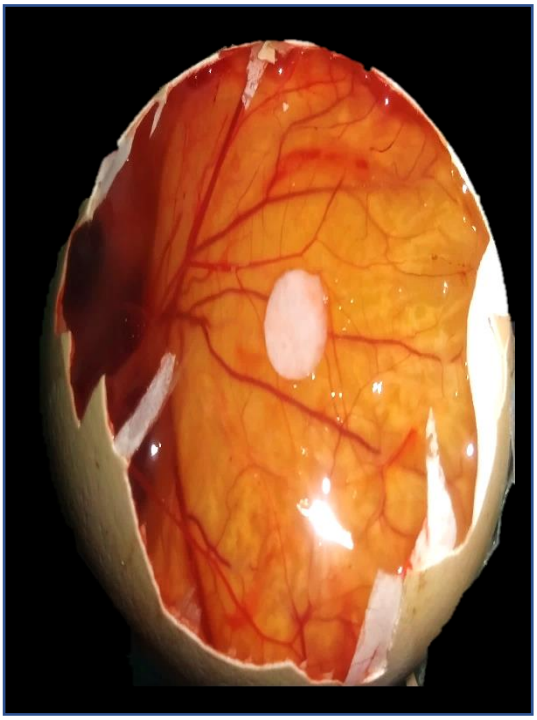


Figure 26 - 6CH **24HOURS**

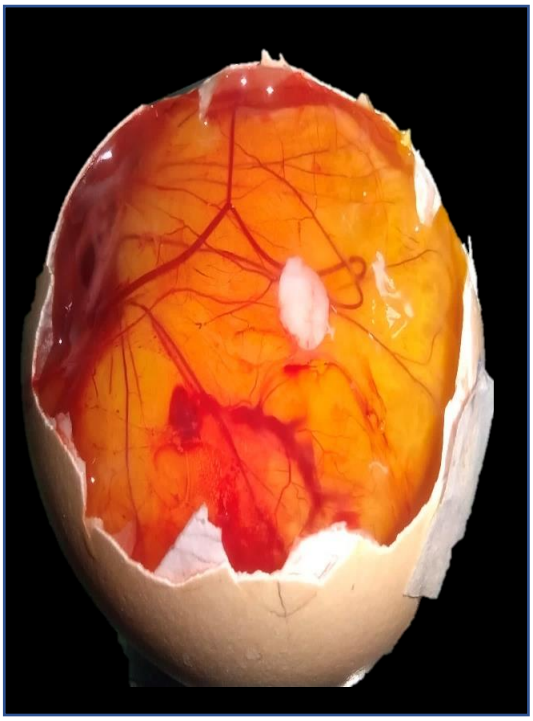


Figure 27– 6CH **48HOURS**

Figure 28– 6CH **72HOURS**

5.4.2 LIGHT MICROSCOPIC VIEW – Bellis Perennis 6CH

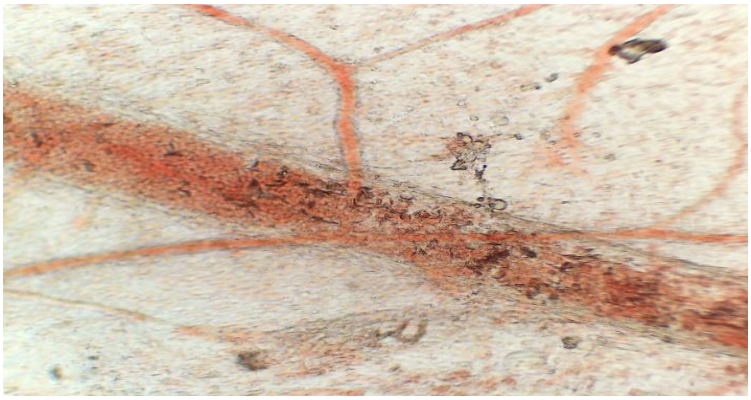


Figure 29– 6CH **24HOURS**

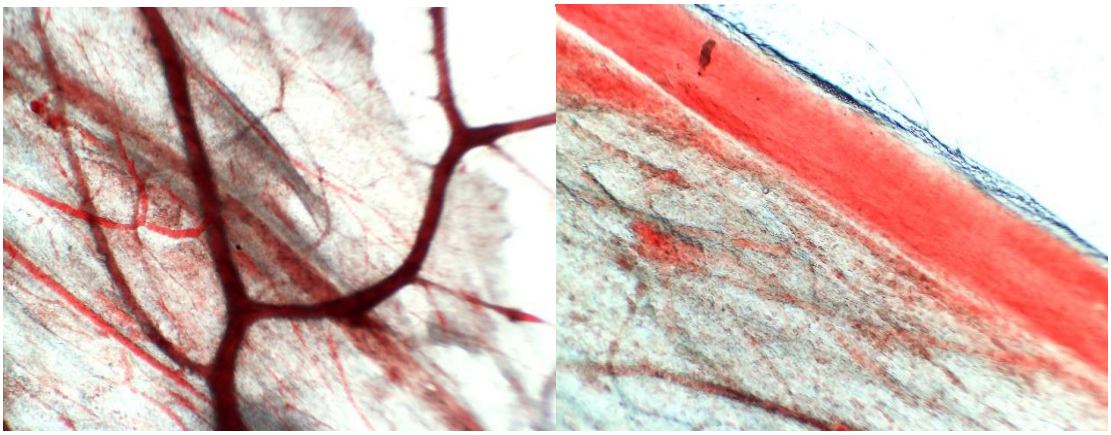


Figure 30– 6CH **48HOURS** LIGHT MICROSCOPE 4 and 10x magnification

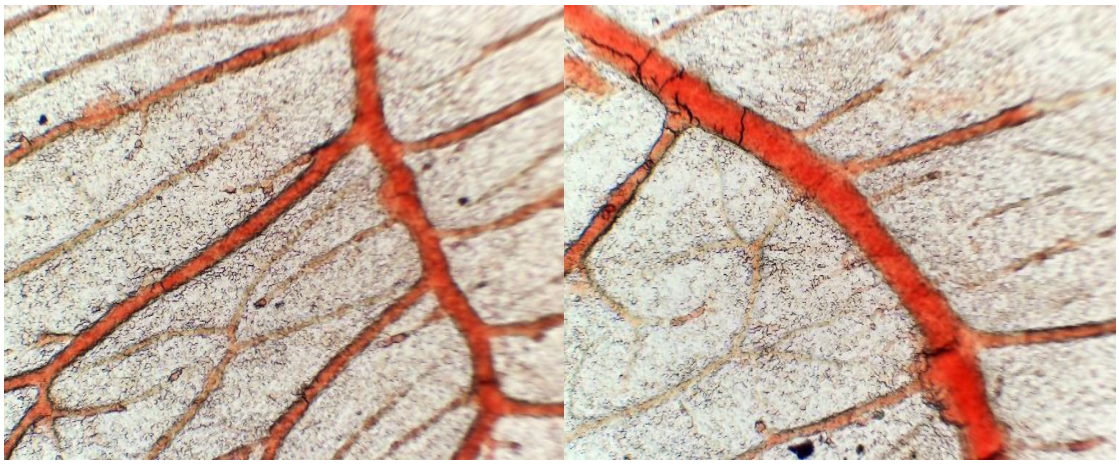
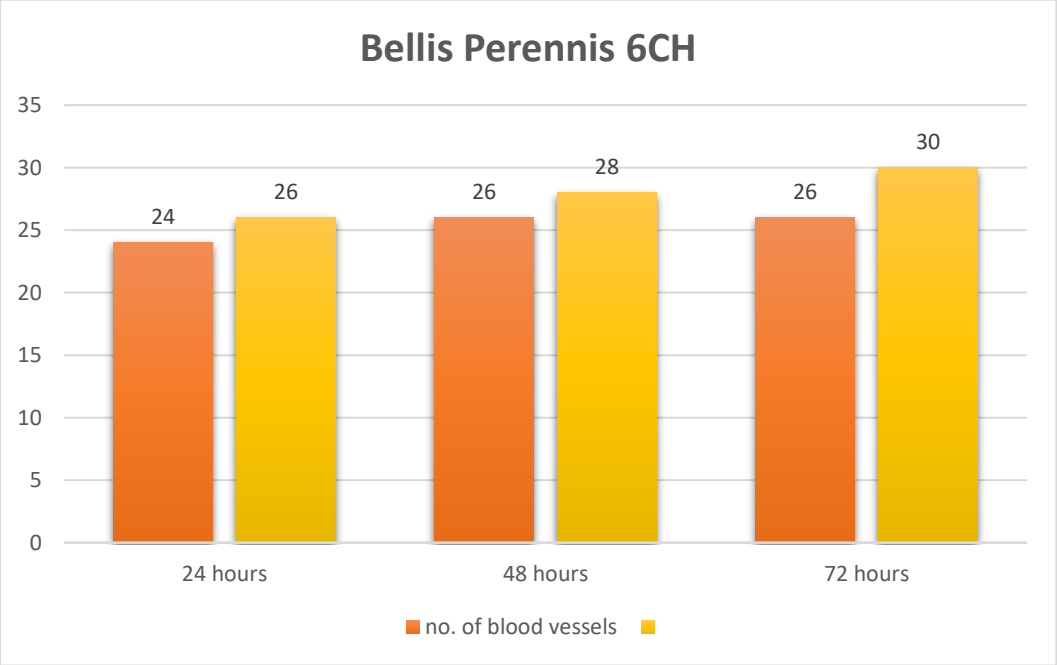


Figure 31 – 6CH **72HOURS** LIGHT MICROSCOPE 4 and 10x magnification

TABLE 3 – NUMBER OF BLOOD VESSELS IN BELLIS PERENNIS 6CH

INCUBATION PERIOD	NO OF BLOOD VESSELS	
24 HOURS	24	26
48 HOURS	26	28
72 HOURS	26	30



GRAPH 3 – NUMBER OF BLOOD VESSELS IN BELLIS PERENNIS 6CH

5.5.1 CHICK EMBRYO TREATED WITH 30CH

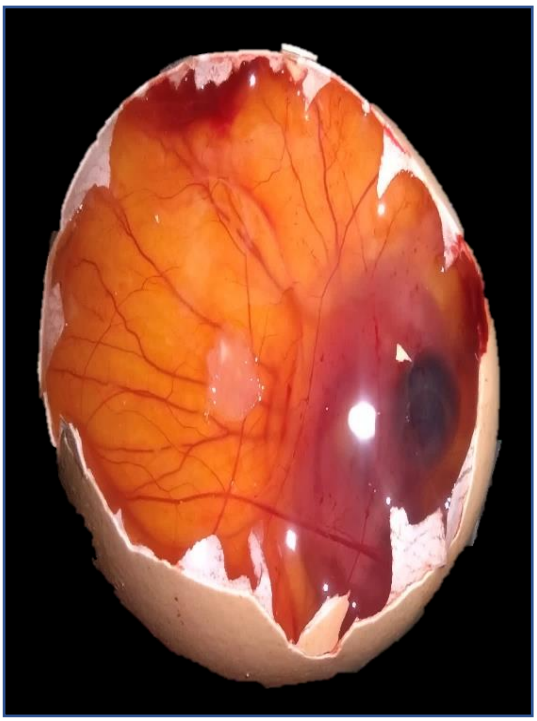


Figure 32 – 30CH **24HOURS**

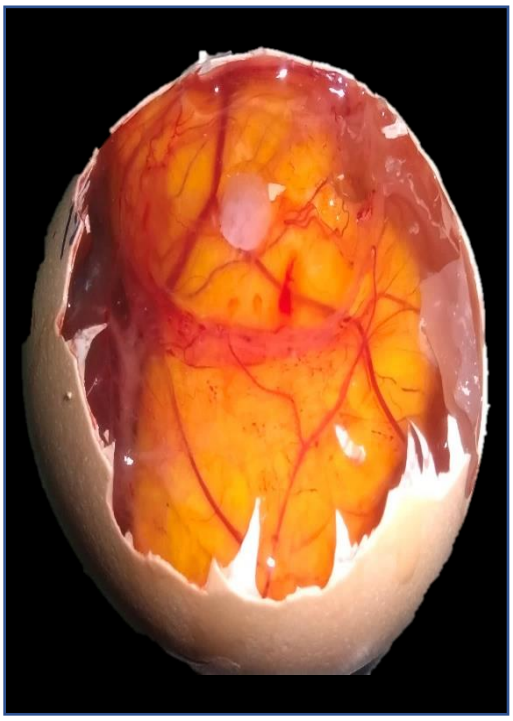


Figure 33– 30CH **48HOURS**

Figure 34– 30CH **72HOURS**

5.5.2 LIGHT MICROSCOPE VIEW – Bellis Perennis 30CH

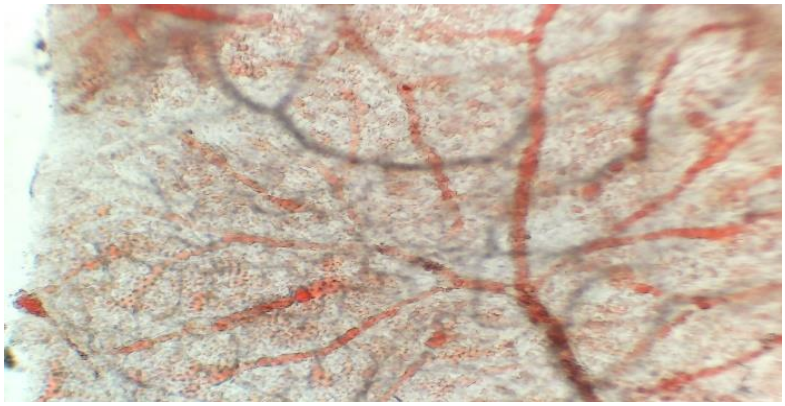


Figure 35– 30CH **24HOURS**

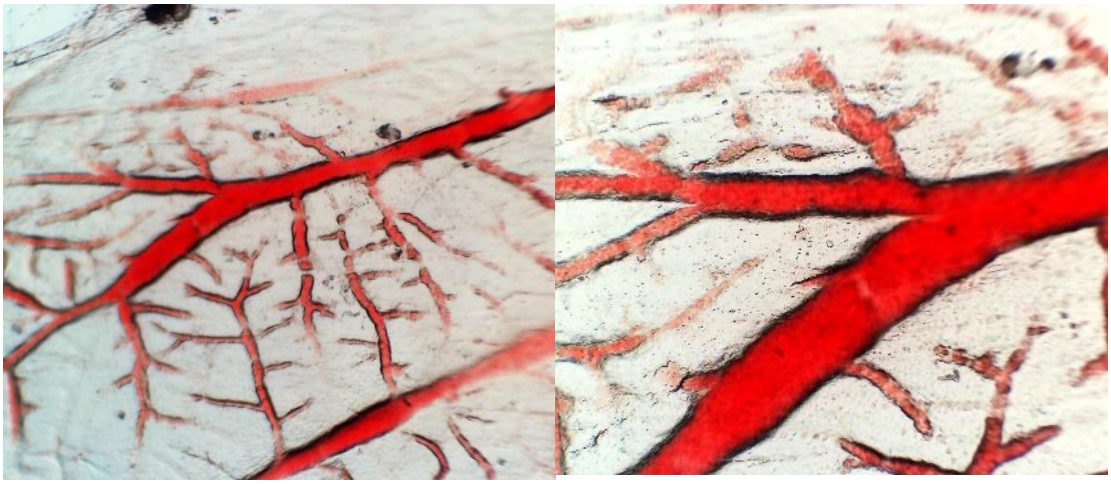


Figure 36– 30CH **48HOURS** LIGHT MICROSCOPE

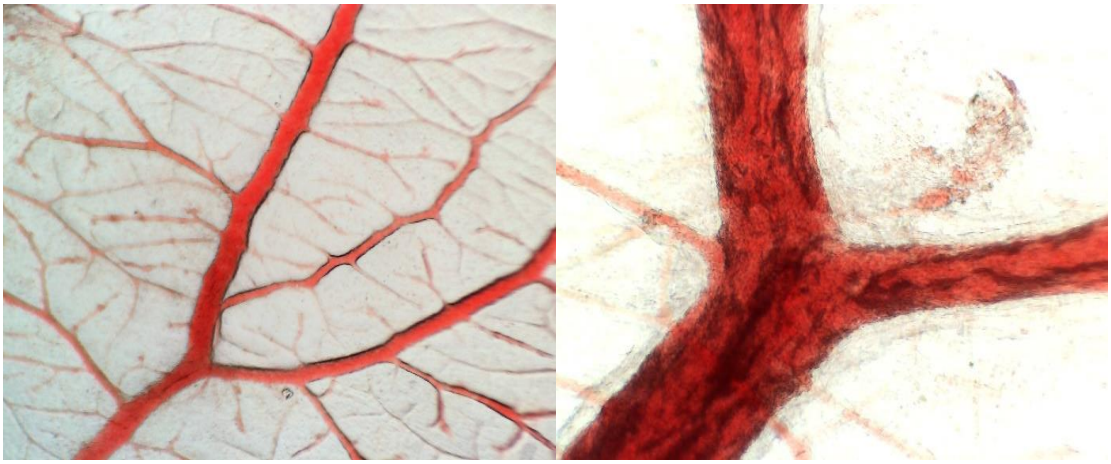
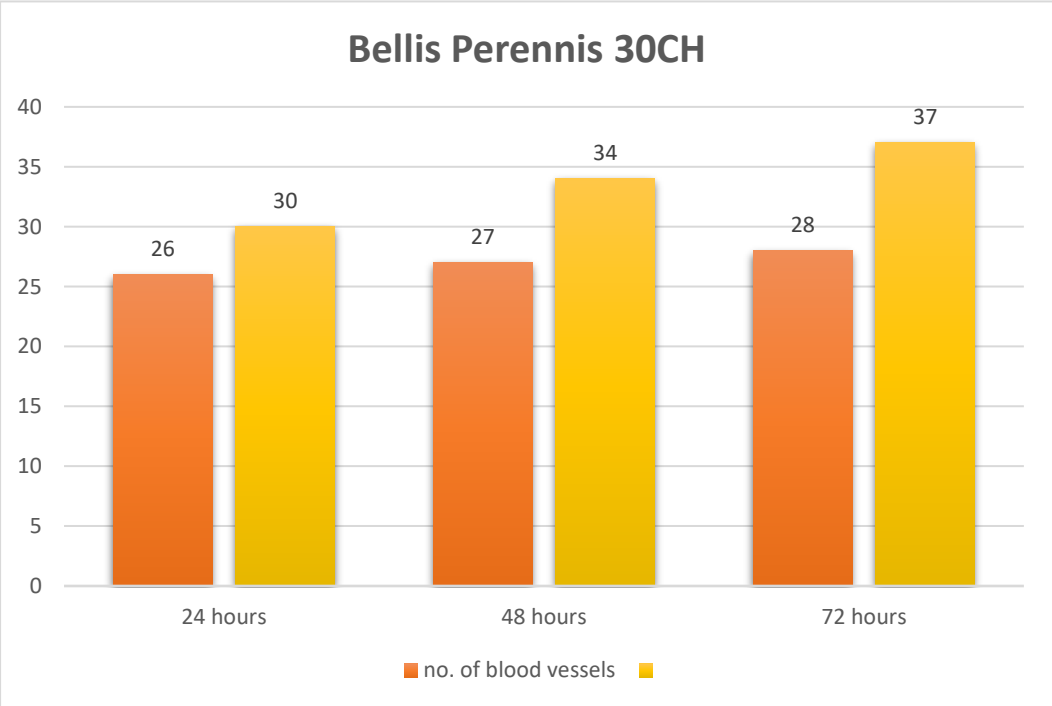


Figure 37– 30CH **72HOURS** LIGHT MICROSCOPE 4 and 10X magnification

TABLE 4 – NUMBER OF BLOOD VESSELS IN BELLIS PERENNIS 30CH

INCUBATION PERIOD	NO OF BLOOD VESSELS	
24 HOURS	26	30
48 HOURS	27	34
72 HOURS	28	37



GRAPH 4 – NUMBER OF BLOOD VESSELS IN BELLIS PERENNIS 30CH

Figure 38 - The Bellis Perennis-treated area of the CAM dissected in a petri dish. Images of the capillary growth were taken with a light microscope while the dissected membrane was kept on glass slides.

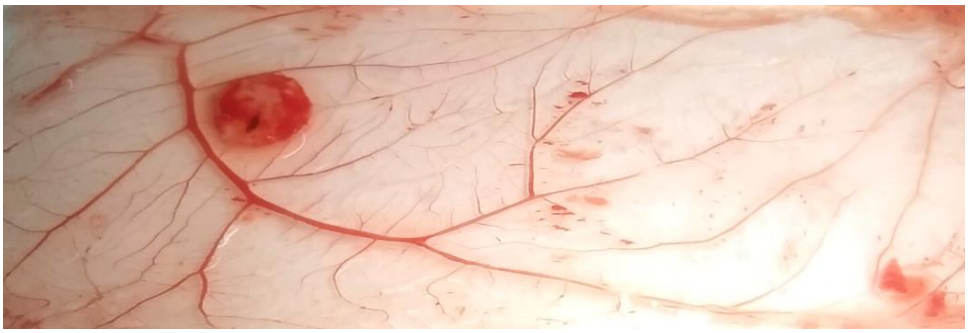


Figure 39 - Glass slides used for light microscopy and blood vessel counting

HISTOLOGICAL OBSERVATIONS OF CAM

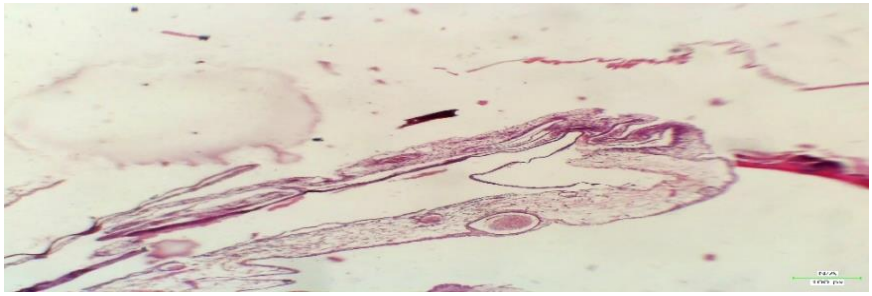


Figure 40 – ***Control 72hours Shows Thin Chorionic and Allantoic Epithelial Layers with Sub Epithelial Capillary Network***

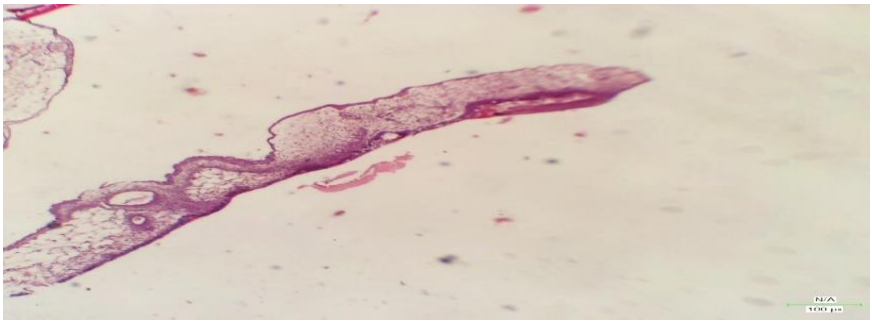


Figure 41 – ***12X 72hours Shows Thin Chorionic and Allantoic Epithelial Layers with lesser number of sub epithelial capillary network***

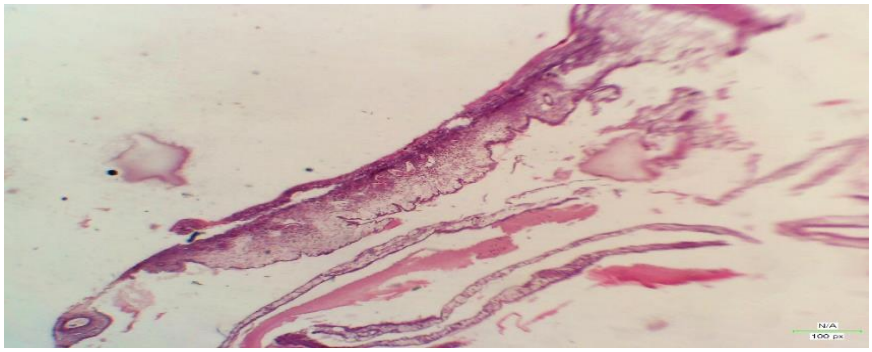


Figure 42 – ***6CH 72hours Shows Presence of Lesser Number of Small Vessels and Slight Thickness at the Primary Stratum***

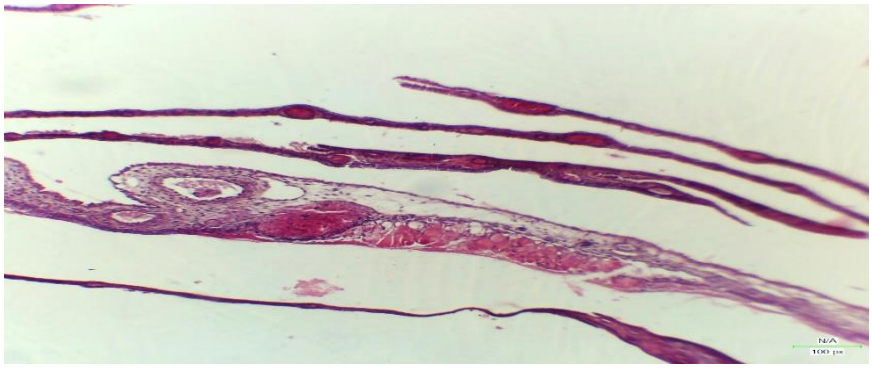
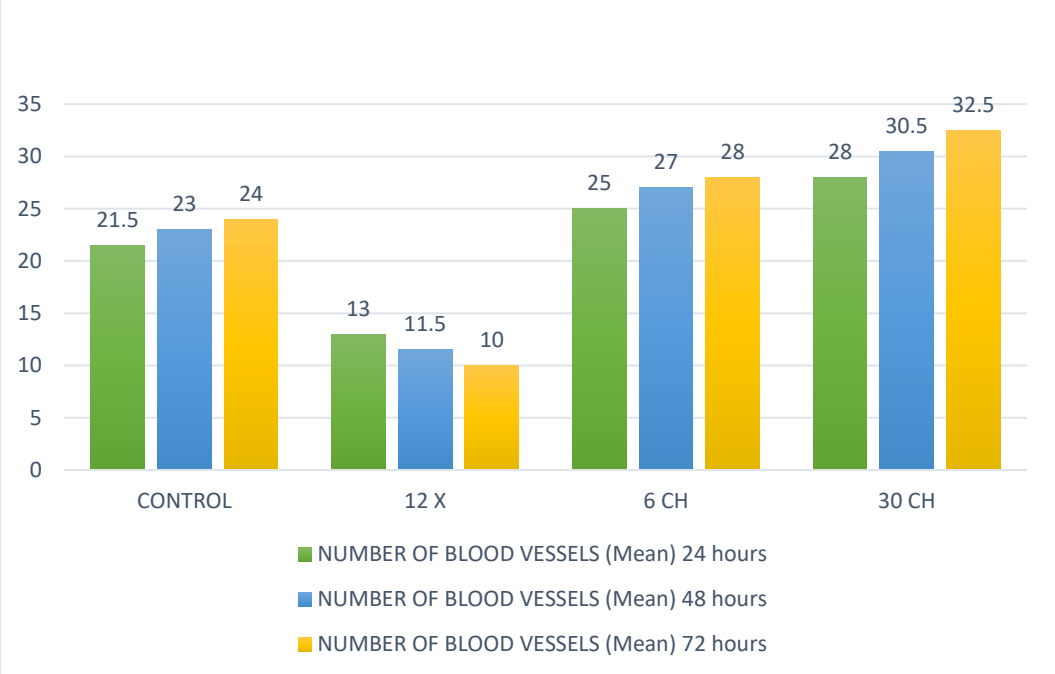


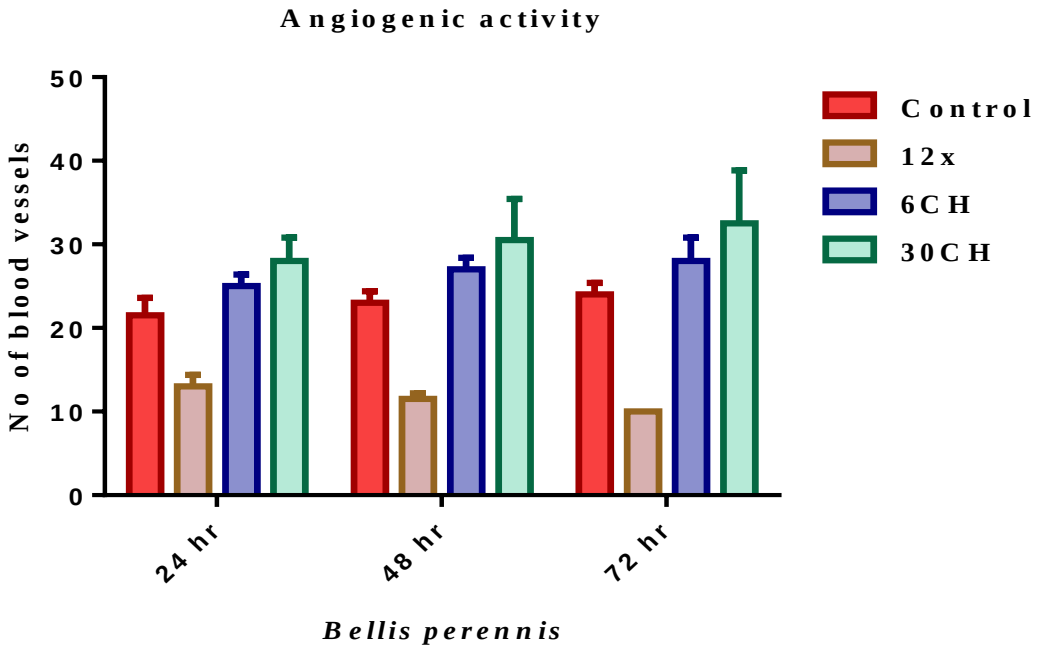
Figure 43 – ***30CH 72hours Shows the Presence of Numerous Large Vessels at the Surrounding of Large One with Increased Thickness at the Primary Stratum***

TABLE 5 – MEAN NUMBER OF BLOOD VESSELS IN CAM TREATED WITH BELLIS PERENNIS 12X, 6CH AND 30CH (24, 48, AND 72 H)

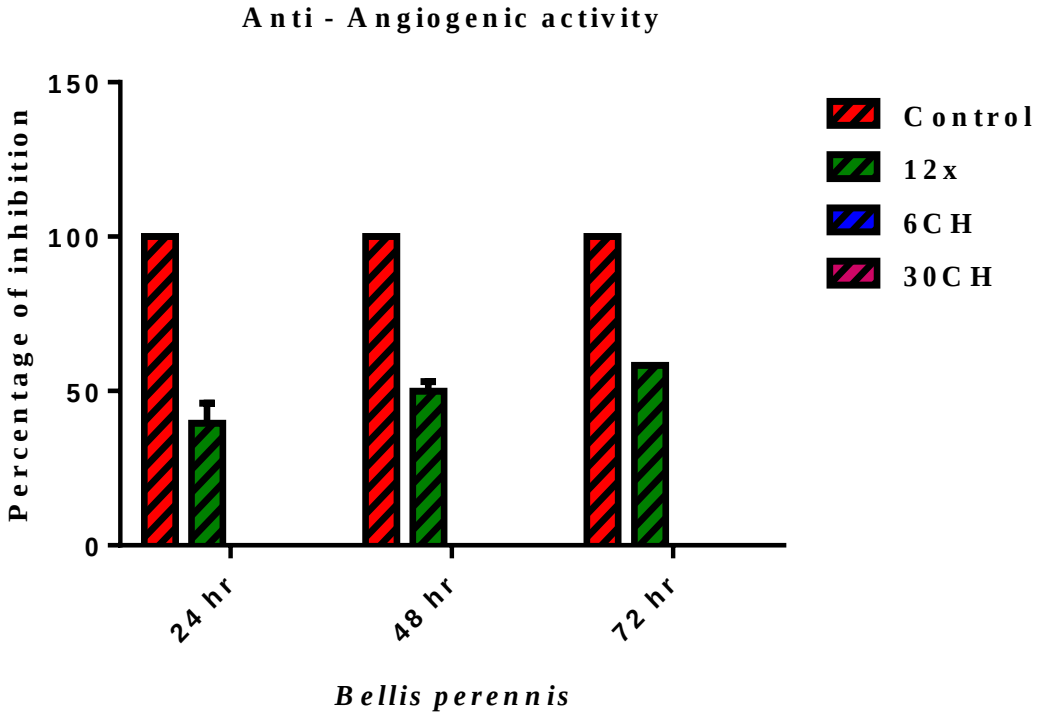
	NUMBER OF BLOOD VESSELS (Mean)		
	24 hours	**48 hours**	**72 hours**
CONTROL	21.5	23	24
12 X	13	11.5	10
6 CH	25	27	28
30 CH	28	30.5	32.5



GRAPH 5 – MEAN VALUE OF NUMBER OF BLOOD VESSELS IN 24, 48, 72 H



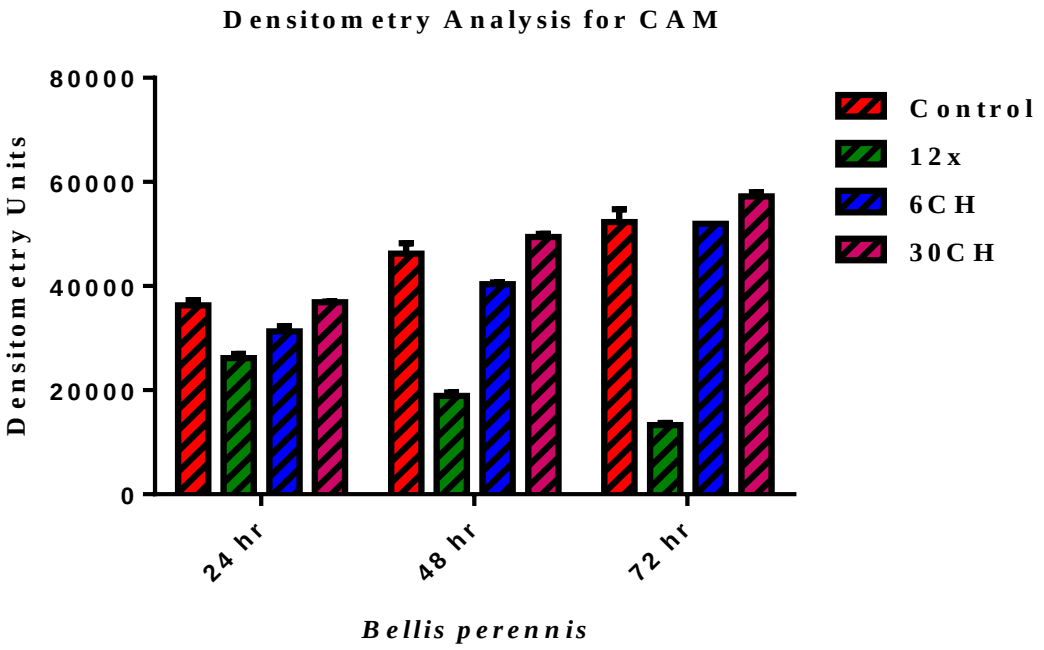
GRAPH 6 – ANGIOGENIC ACTIVITY OF BELLIS PERENNIS 12X, 6CH, 30CH



GRAPH 7 – ANTI – ANGIOGENIC ACTIVITY OF BELLIS PERENNIS 12X COMPARED WITH CONTROL

TABLE 6 – DENSITOMETRY ANALYSIS OF CAM USING A LIGHT MICROSCOPE

S. No	Sample details		Densitometry values in duplicates		Mean Value (%)
1.	Control	24 hours	36992.73	35543.13	36267.9
2.	*Bellis perennis* treated 12X		25592.31	26743.34	26167.8
3.	*Bellis perennis* treated 6CH		31992.66	30584.72	31288.7
4.	*Bellis perennis* treated 30CH		36992.731	36833.15	36912.9
5.	Control	48 hours	47644.99	44777.82	46211.4
6.	*Bellis perennis* treated 12X		18452.66	19365.24	18909
7.	*Bellis perennis* treated 6CH		40092.32	40584.81	40338.6
8.	*Bellis perennis* treated 30CH		48992.11	49833.59	49412.9
9.	Control	72 hours	54031.25	50624.931	52328.1
10.	*Bellis perennis* treated 12X		12992.97	13584.26	13288.6
11.	*Bellis perennis* treated 6CH		51992.731	51992.731	51992.7
12.	*Bellis perennis* treated 30CH		56644.99	57777.2	57211.1



GRAPH 8 – DENSITOMETRY ANALYSIS OF CAM

TABLE 7 – MEAN OF DENSITOMETRY ANALYSIS OF CAM

	Densitometry Mean		
	24 hours	**48 hours**	**72 hours**
CONTROL	36267.9	46211.4	52328.1
12 X	26167.8	18909	13288.6
6 CH	31288.7	40338.6	51992.7
30 CH	36912.9	49412.9	57211.1

GRAPH 9 – MEAN OF DENSITOMETRY ANALYSIS OF CAM

6. STATISTICAL ANALYSIS

TABLE 8 – NO OF BLOOD VESSELS AND PERCENTAGE OF INHIBITION

S. No	Sample details		No. of blood vessels		Percentage of inhibition		Mean Value (%)
1.	Control	24 hours	20	23	100	100	100
2.	*Bellis perennis* treated 12X		14	12	34.88	44.18	39.53
3.	*Bellis perennis* treated 6CH		24	26	0	0	0
4.	*Bellis perennis* treated 30CH		26	30	0	0	0
5.	Control	48 hours	22	24	100	100	100
6.	*Bellis perennis* treated 12X		12	11	47.82	52.17	50
7.	*Bellis perennis* treated 6CH		26	28	0	0	0
8.	*Bellis perennis* treated 30CH		27	34	0	0	0
9.	Control	72 hours	23	25	100	100	100
10.	*Bellis perennis* treated 12X		10	10	58.33	58.33	58.33
11.	*Bellis perennis* treated 6CH		26	30	0	0	0
12.	*Bellis perennis* treated 30CH		28	37	0	0	0

PERCENTAGE OF INHIBITION is calculated by the formula

$$\frac{\text{No of the vessel in CAM treated with normal saline} - \text{No of the vessel in CAM treated with drug sample}}{\text{No of vessel in CAM treated with normal saline}} \times 100$$

Table 9 – STANDARD DEVIATION TABLE

STANDARD DEVIATION TABLE							
S. No	**Sample details**		**No. of blood vessels**		**Mean Value (%)**	**STDEV**	**Mean ± STDEV**
1.	Control	24 hours	20	23	21.5	2.12132	21±2.12
2.	*Bellis perennis* treated 12X		14	12	13	1.414214	13±1.41
3.	*Bellis perennis* treated 6CH		24	26	25	1.414214	25±1.41
4.	*Bellis perennis* treated 30CH		26	30	28	2.828427	28±2.82
5.	Control	48 hours	22	24	23	1.414214	23±1.41
6.	*Bellis perennis* treated 12X		12	11	11.5	0.707107	11.5±0.7
7.	*Bellis perennis* treated 6CH		26	28	27	1.414214	27±1.41
8.	*Bellis perennis* treated 30CH		27	34	30.5	4.949747	30.5±4.94
9.	Control	72 hours	23	25	24	1.414214	24±1.41
10.	*Bellis perennis* treated 12X		10	10	10	0	10±0
11.	*Bellis perennis* treated 6CH		26	30	28	2.828427	28±2.82
12.	*Bellis perennis* treated 30CH		28	37	32.5	6.363961	32.5±6.36

TABLE 10 – TWO-WAY ANOVA TABLE

Table Analyzed	**Data 1**				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant ?	
Interaction	18.44	< 0.0001	****	Yes	
Row Factor	11.42	< 0.0001	****	Yes	
Column Factor	69.81	< 0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	7.903e+008	6	1.317e+008	F (6, 12) = 110.3	P < 0.0001
Row Factor	4.896e+008	2	2.448e+008	F (2, 12) = 204.9	P < 0.0001
Column Factor	2.992e+009	3	9.974e+008	F (3, 12) = 834.9	P < 0.0001
Residual	1.434e+007	12	1.195e+006		

RESULT

- Percentage of inhibition is more on Bellis Perennis 12X compared to control and Bellis perennis 6CH and 30CH. There is no percentage of inhibition on Bellis perennis 6CH and 30CH.
- In two-way ANOVA table P value <0.0001 the result is statistically significant, and thus rejects the null hypothesis in favour of the alternative hypothesis.
- Bellis perennis 6CH and 30CH has angiogenic activity on chorioallantoic membrane and Bellis perennis 12X has anti-angiogenic activity on chorioallantoic membrane.
- Hence alternative hypothesis is proved.

7. DISCUSSION

Homoeopathy is a branch of medicine that has been successfully utilized by individuals. However, there is a lack of evidence-based research. The effect of homoeopathic medicine is still unclear. More literature is available, but it is less evident. Having this in mind, this study was initiated.

Many publications say *Bellis Perennis* homoeopathic medicine has strong action on clinical conditions like malignant tumours, psoriasis, and wound healing. But I have doubts about how one medicine could have both angiogenesis-promoting and inhibiting factors and there is no scientific evidence for that.

Only by researching *Bellis Perennis'* angiogenetic effects will one be able to determine the drug's scientific validity.

When there is the trauma in the case, the tumour presumably had its origin from trauma (traumatism in the causation of tumour). In this type of case Dr. Burnett cured tumours with well-tried anti-traumatic Bellis perennis homoeopathic medicine. Potency used was mother tincture and 3X to treat tumours and also Bellis perennis has a strong action on blood vessels, making it the most commonly used remedy in day-to-day life for injury or wound healing.

Bellis perennis in a large dose (lower potency) has the properties to inhibit angiogenesis due to the particle presence in the medicine, and the same medicine in a small dose (higher potency) has action on wound healing (higher potency).

A dosage-response phenomena called hormesis is characterized by small dose stimulating and large dose inhibiting. The phenomenon is applied in bellis perennis large dose inhibiting the angiogenesis and small dose promoting the angiogenesis.

This opposite effect is happening due to the dynamization of medicine. The large dose has the higher materialistic particles, and the small dose has the dynamization, which has no materialistic particles. while reducing the material particles in the large dose by the process of potentization. While reaching higher and higher potencies, the material constituents will reduce, and the action of the remedy will promote angiogenesis.

According to Arndt-Schulz law, for every substance, small doses stimulate, moderated doses inhibit, large doses kill. Large dose inhibiting and small dose promoting the angiogenesis. The smaller the dose of the medicine, the greater its effectiveness.

While the mathematical calculations for 12X and 6CH are identical, the methods used to prepare the decimal and centesimal scales vary. Both the medicine 12X and the medicine 6CH have different angiogenesis activities. This variation can be due to preparation of medicine on different scales.

Angiogenesis is the process of which new blood vessels are developed from existing ones, it is vital to many physiological and pathological events.

In this research, we examined the angiogenic capacity of the Bellis perennis using an In Ovo CAM test. Because it is seen as a transitional stage between a single cell culture & a complex mammalian model, the assay's key advantage is that it may be used as a rapid way to measure angiogenesis response. The use of chorioallantoic membrane for scoring tissue responses to angiogenesis activity of biological materials precisely and in a manner similar to responses seen in mammalian model responses, as

well as for assisting in the maintenance of test materials at the administration place, is another significant advantage.

According to the findings of this study on Chorioallantoic membrane assay, *Bellis perennis* has both angiogenesis-promoting and inhibiting properties. *Bellis perennis* 12X has angiogenesis-inhibiting properties, and Bellis perennis 6CH and 30CH can induce neovascularization.

Therefore, in the current study, we conducted experiments on various groups of eggs for 24, 48, and 72 hours to examine the angiogenic activity of *Bellis perennis* Bec. at this period, newly developed vessels will stabilize. Interestingly we discovered, both *Bellis perennis* 6CH and 30CH showed significant angiogenic-effects at 72hours of incubation, while *Bellis perennis* 12X showed significant anti-angiogenic effects, indicating that the angiogenic activity of *Bellis perennis* depends on length of incubation period.

Bellis perennis 30CH appears to have a greater ability than 6CH concentration to induce the sprouting of new blood vessels from the existing ones, according to an examination of the vessel growth pattern under a microscope. Bellis perennis can thereby induce neo-vascularization in a potency-dependent way; the 30CH potency may therefore be more suited, resulting in an earlier and more reliable angiogenesis.

Some foreign substance placed on the CAM can cause an inflammatory reaction, which can then lead to a secondary Vaso proliferative reaction and a false positive result. To solve this problem, we thoroughly examined the histology after adding *Bellis perennis.* The changes in histology and cellular morphological changes in treated area showed that *Bellis perennis* can start the growth of new blood vessels without causing any localised inflammation. A *Bellis perennis* of 30CH exhibits

significantly high angiogenesis responses by hastening the development of new blood vessels by sprouts from the main one. Additionally, the thickness of the Chorioallantoic membrane at the treated area can be used to analyse the angiogenesis or anti-angiogenic effects of a compound since the development and formation of new blood vasculature will increase tissue thickness at the stroma. As a result of the formation of capillary like tubes and other structure, our study revealed that 30CH of *Bellis perennis* is able to utilize maximum angiogenesis response at the stroma region very close to chorionic epithelium rather than mesoderm, resulting in increased tissue thickness[34].

8. FUTURE PROSPECTS

- This research will inspire the researcher to answer various questions and gain understanding of this topic.
- Further studies can be carried out:
    1. Higher potencies of Bellis Perennis.
    2. Treatment with other homoeopathic remedies.
    3. Analyse deeper mechanism of action like RT-PCR, Gelatin zymography, Immunohistochemistry.
    4. Reproducibility of the study can be done.

9. CONCLUSION

- The current research shows that Bellis perennis has the ability to both induce vascularization through sprouting and to inhibit angiogenesis in the treated area. This is because of the homoeopathic medicine Bellies Perennis' hormesis effect and its work on Arndt-Schulz law.
- A factor that inhibits angiogenesis is present in the bellies perennis 12X, while a factor that promotes it is present in the bellies perennis 6CH and 30CH.
- Increased tissue thickening and cellular morphological changes of the chorioallantoic membrane with Bellis perennis the treatment promotes Bellis perennis potency-dependent angiogenic ability.
- Direct observation of blood vessel formation and their growth on the treated area shows that the concentration and incubation period of bellis perennis impact its ability to induce angiogenesis, particularly its sprouting effect.
- Bellis perennis 30CH is discovered to exhibit a notable angiogenic response after 72 hours of incubation.
- At 72 hours after incubation, bellis perennis 12X is discovered to have the ability to inhibit the angiogenic response.
- Overall, our findings suggest that Bellis perennis may be useful in angiogenic therapeutics and may be more effectively used in the treatment of wound healing, which requires the formation of new vessels, as well as tumours, which require anti-angiogenic activity.

10. SUMMARY

- This study clearly demonstrates the efficacy of the homoeopathic medicine *Bellis perennis.*
- *Bellis perennis* 30CH and 6CH clearly have angiogenesis-promoting factors, whereas 12x clearly has angiogenesis-inhibiting factors.
- This study illustrates how homoeopathic medicine has a hormesis effect and the Arndt-Schulz law.
- The vessel growth pattern was examined using a microscope clearly shows that *Bellis perennis* 30CH has a greater potential than Bellis perennis 6CH concentration to stimulate the sprouting of new vessels from the existing ones.
- *Bellis perennis* has the ability to initiate the neovascularization without causing localised inflammation, as evidenced by the *Histological sections* and in the treated area, there was also altered cellular morphology.
- It is obvious that analysing the thickness of the Chorioallantoic membrane in treated area can be used to determine whether a substance has promoting or anti-angiogenesis effect because the growth and expansion of new blood vessels will thicken the tissue in stroma region.
- It is one of the experimental studies to demonstrate the validity of the *Homoeopathic medical system.*

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