Angeles University Foundation
School of Medicine

DETERMINATION OF THE ANTI-ANGIOGENIC ACTIVITY OF WHITE ANGEL (*Holarrhena antidysenterica*) LEAF EXTRACT USING THE CHORIOALLANTOIC MEMBRANE ASSAY

CANCER

- The leading cause of death worldwide according to the World Health Organization.

- A major concern of not only to the public but also the medical field and other international health organizations.

- Involves the process of ANGIOGENESIS.

ANGIOGENESIS

It is a process of creating new capillary blood vessels through sprouting from already existing blood vessels in a process involving the migration and proliferation of endothelial cells from preexisting vessels, from which tumor angiogenesis is the angiogenic process that is observed in cancer.

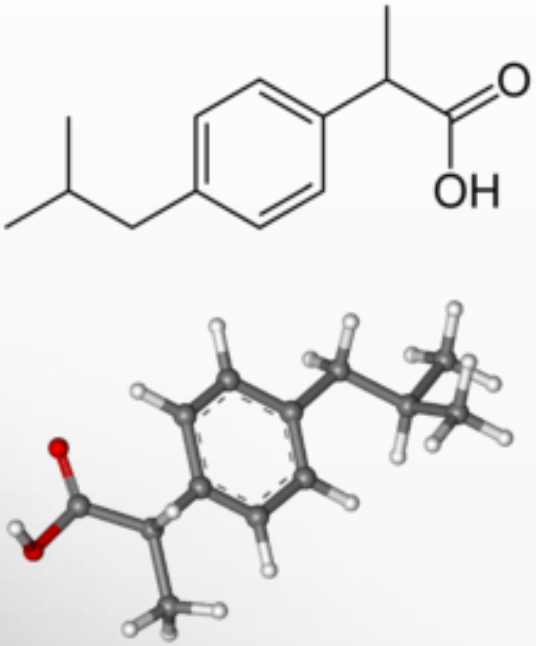
*Carmeliet, P. and R. K. Jain (2000). Angiogenesis in cancer and other diseases. Nature 407(6801): 249-257. Retrieved last December 19, 2013 from http:// ncbi.nlm.nih.gov/pubmed/11001068

White Angel
(Holarrhena antidysenterica)

- family Apocynaceae
  - *Catharantus roseus*
  - *Calotropis procera*

- it is an important plant used in indigenous systems of medicine remedy for various diseases

IBUPROFEN

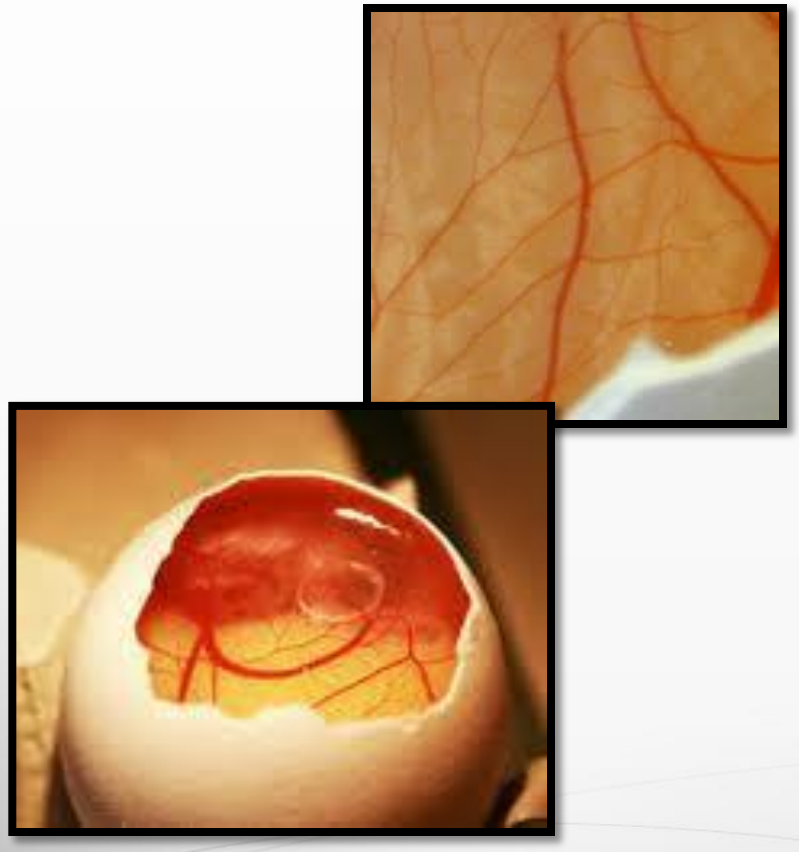


- Ibuprofen inhibits angiogenesis through direct effects on endothelial cells.

- Ibuprofen inhibits VEGF by reducing HIFs under hypoxic conditions.

*Jones, M., Wang H., Peskar, B., Levin E., Itani R., Sarfeh U., Tamawski A. (1999). Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight to mechanisms and implications for cancer growth and ulcer healing. Nat Med. 1999 Dec;5(12):1418-23.

CAM Assay



The researchers have chosen this assay because of its high reproducibility, simplicity and cost-effectiveness.

Ribatti, D. (2010). The chick embryo chorioallantoic membrane as an in vivo assay to study antiangiogenesis. *Pharmaceuticals*, 3, 482-513. doi: 10.3390/ph3030482

OBJECTIVE OF THE STUDY

- To determine if the leaf extracts of *Holarrhena antidysenterica* can inhibit the formation of new blood vessels using duck chorioallantoic membrane assay.

METHODOLOGY

- White Angel Leaves
- Fertilized mallard duck eggs

Input

Process

- Extraction
- Chorioallantoic Membrane Assay
- Visual Assessment and Photography
- Statistical Analysis

- Significant differences between the branch point densities of the four (4) different groups

Output

METHODOLOGY

Four (4) groups:
Environmental control group
Positive control group (Ibuprofen + NSS)
Negative control (ethanol + NSS)
Test group (19.28 µg/mL HA leaf extract + NSS)

Branch point density = $\frac{\text{number of branch point in a blood vessel segment}}{\text{length of blood vessel segment}}$

Statistical comparison using **One –way ANOVA** and **Scheffe test**

RESULTS

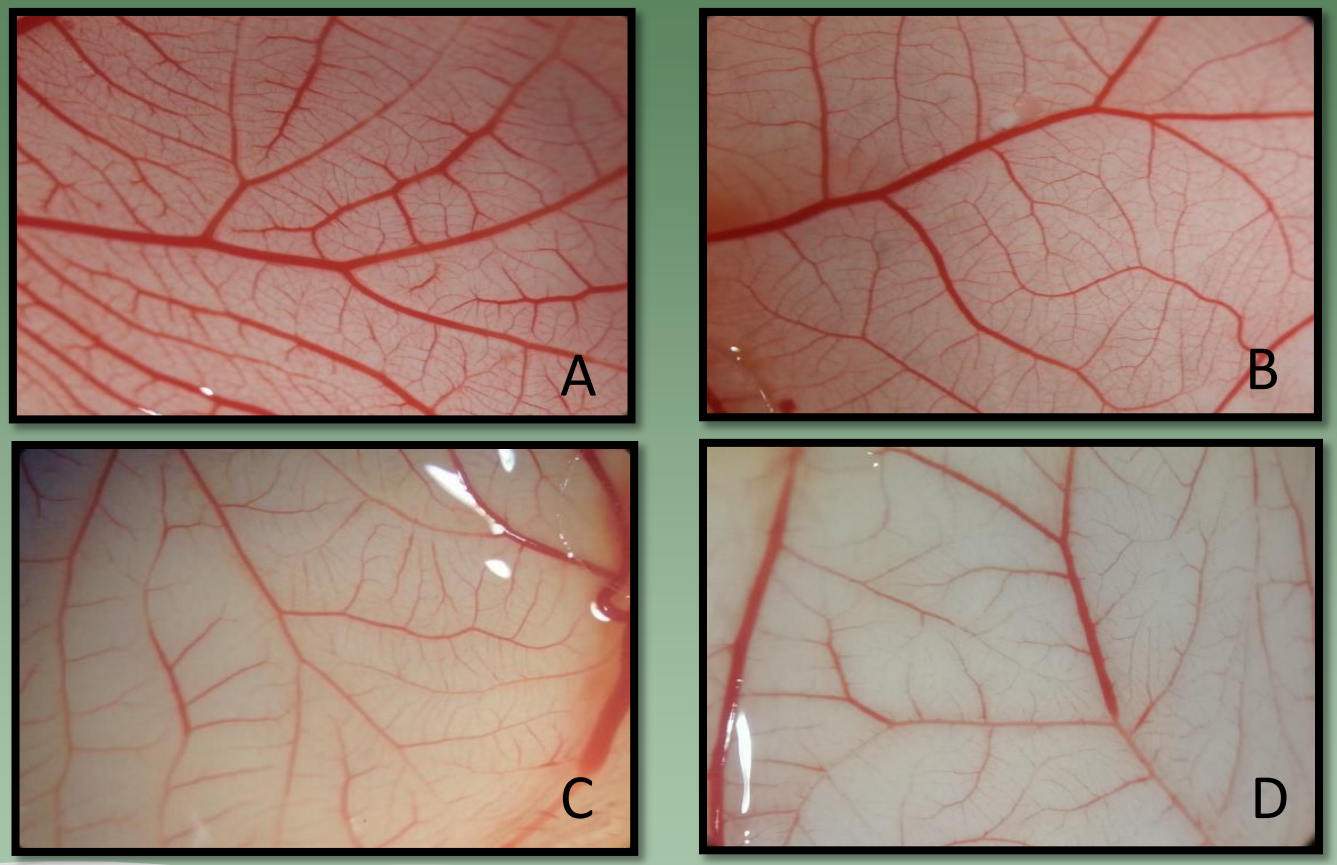
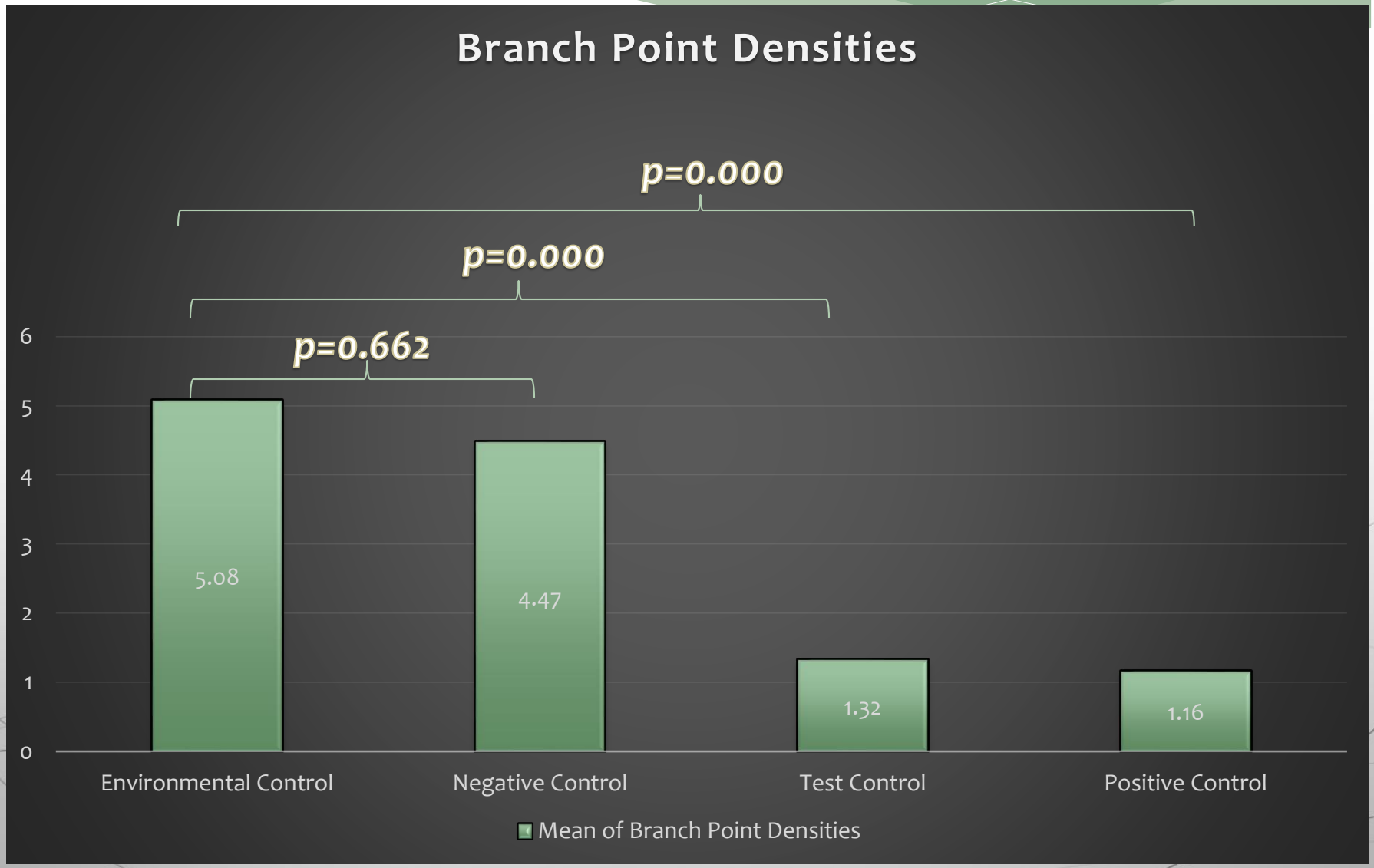


Figure 1. Vascular areas of CAM exposed to (A) environmental control (untouched eggs), (B) negative control administered with ethanol + NSS, (C) test control administered with 19.28 µg/mL plant extract + NSS, and (D) positive control administered with Ibuprofen + NSS

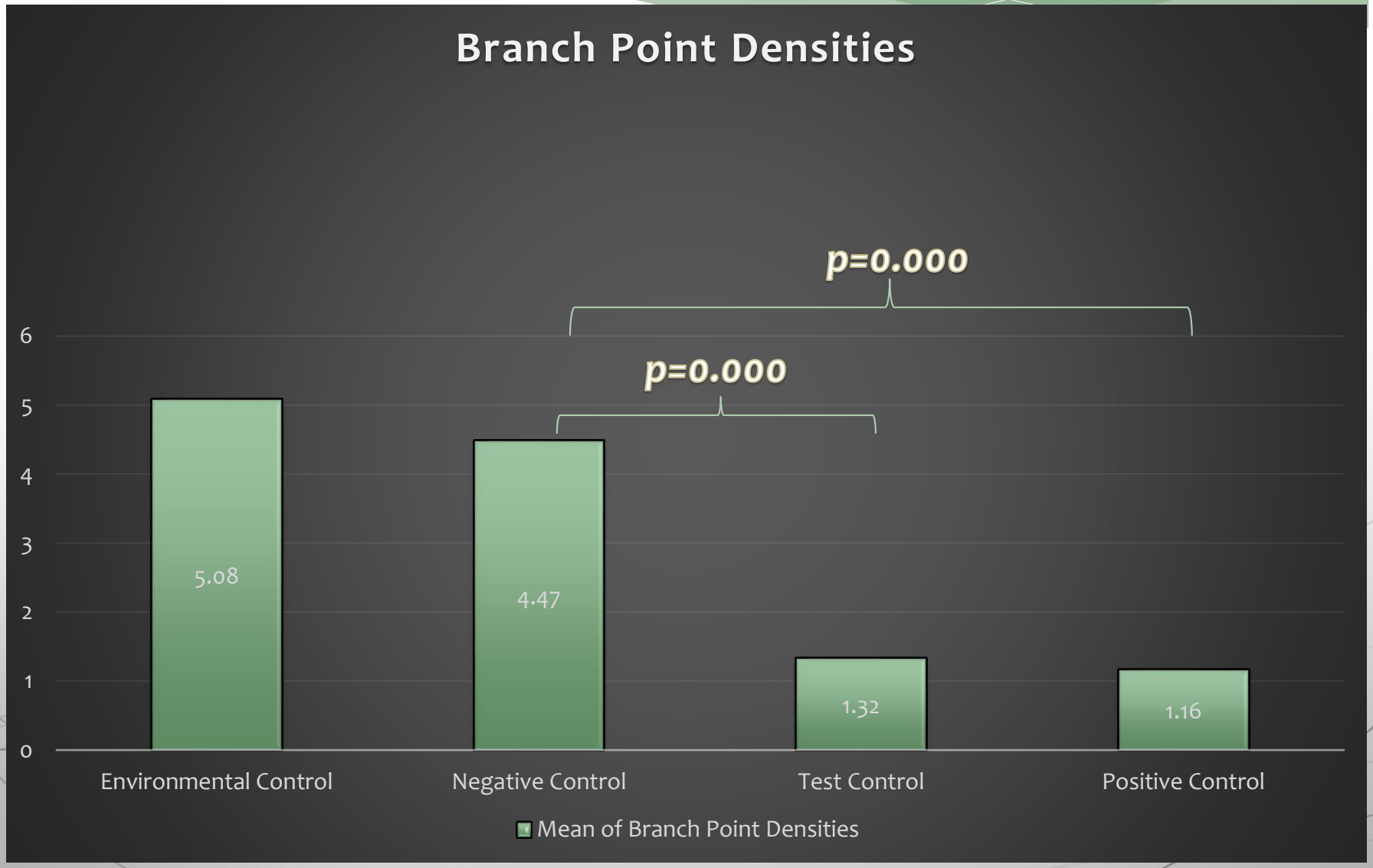
RESULTS

GROUP	Branch Point Densities
Environmental (A)	5.08 ± 1.010
Ethanol + NSS (B)	4.47 ± .847
19.28 µg/mL HALE + NSS (C)	1.321 ± .332
Ibuprofen + NSS (D)	1.16 ± .650

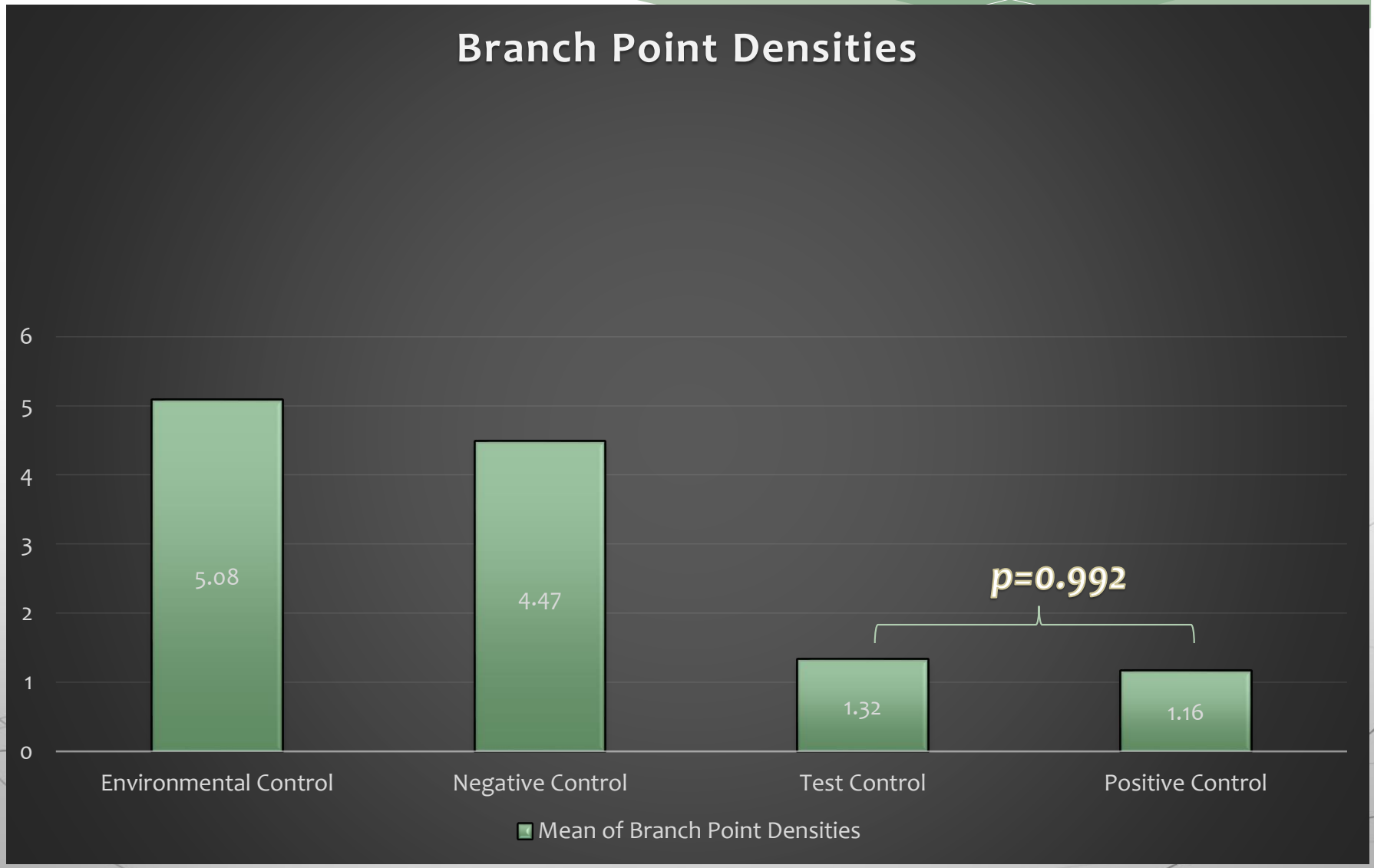
RESULTS


Branch Point Densities
p=0.000
p=0.000
p=0.662
6
5
4
3
2
1
0
5.08
4.47
1.32
1.16
Environmental Control
Negative Control
Test Control
Positive Control
Mean of Branch Point Densities

RESULTS


Branch Point Densities
p=0.000
p=0.000
6
5
4
3
2
1
0
5.08
4.47
1.32
1.16
Environmental Control
Negative Control
Test Control
Positive Control
Mean of Branch Point Densities

RESULTS


Branch Point Densities
6
5
4
3
2
1
0
5.08
4.47
1.32
1.16
p=0.992
Environmental Control
Negative Control
Test Control
Positive Control
Mean of Branch Point Densities

CONCLUSION AND RECOMMENDATIONS

Conclusion

- It has been observed that the *H. antidysenterica* leaf extract *significantly inhibits* the development of capillary networks in CAM.

Recommendation

- Identify exact phytochemical component/s of the *H. antidysenterica* leaf extracts that are responsible for its angiosuppressive property and determine their possible mechanisms of actions.
- Determine the extent of effect of the *H. antidysenterica* leaf extracts by establishing concentration gradients.

THANK YOU