

Effect of Aceclofenac Sodium on Angiogenesis by Using Chorioallantoic Membrane (CAM) Assay

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DESCRIPTION

The chick chorioallantoic membrane (CAM) is a possibly three-dimensional representation that can be used for in vivo as well as in situ studies. It's relatively easily available and consistencies in quality render it an appropriate biological model for use in experiments requiring live tissues. The aim of current research was to determine the angiogenic/antiangiogenic effect of aceclofenac sodium and to dictate the effectual dose of aceclofenac sodium for angiogenesis.

Fertilized eggs (5days old) of chicken were obtained from a local hatchery. They were incubating at 37°C with humidness of 55-60%. Then, on fifth day of incubation, a opening about 2cm of diameter was generate by removing the shell and then inner shell membrane. It was done under aseptic conditions 5 groups will be formed. Group A was kept as control and was given 0.1ml PBS, 0.64mg/0.1ml in group B, 0.32mg/0.1ml, 0.16mg/0.1ml, and 0.08mg/0.1ml in group C, D and E respectively. Then pH of all solutions were checked with pH meter and were adjusted to 6-7.4. In order to reduce chance of contamination all the prepare silutions were filter by 0.2 μ syringe filters.

On 6th day, the prepare dilutions of aceclofenac sodium were inject and eggs were again fasten with paraffin film under seterile environment.. These were put back in incubator for following 24 hours. After twenty four hours, eggs were removed out from incubator and picture of all groups i.e. control as well as those deal with all concentrations of aceclofenec were produce using a DSL camera. The growth of blood vessels and other chacteristics were perceived by used Adobe Photoshop version 7.0, then these picture were shift to scan probing image processing (SPIP) software 6.6.2. The diameter of primary, secondary and tertirary blood vessels were measured. The framework described the extant of inhibition of naturally developing CAM include, the diameter and branching system of blood vessels measured as per mm, and categories of blood vessels, i.e. primary, secondary and tertiary blood vessels.

Data were examined on SPSS statistical software version 22.0 using One Way ANOVA. Application of aceclofenec on chorioallantoic membrane at 6th of incubation showed angiogenic effects in high concentration and anti-angiogenic effect in low concentrations. The results shows noticeable changes in building of CAMs, thining

of primary, secondary and tertiary blood vessels, and decline in abbot curve. The significant amount of aceclofenec used may show anti-angiogenic activity in the identical fashion those notice in in vitro and explain its clinical effectiveness.

The development of the CAM is similar to that of the allantois in mammals. Its growth starts from day 3 of embryonic development. Development of the allantois occurs extra embryonically from the ventral wall of the endodermal hindgut. Partial fusion of the chorion and allantois occurs between days 5 and 6. By day 10, there is an extensive formation of capillary network. The complete differentiation of the CAM is complete by day 13. Here, the embryo is grown outside of the shell. In this method, the eggs are first kept in inside a humidified incubator for up to a period of 3 days, to ensure that the position of the embryo is opposite to the position where the egg will be subsequently cracked. A small hole is made on the side of the air chamber to equilibrate the pressure, followed by the cracking of the egg on a petri-dish. This method is ideal for visualizing the growing embryo and their manipulation without limitations in accessing the embryo during the different stages of development. However the process requires aseptic conditions. There are also problems associated with the handling of the embryo, as the yolk membrane is prone to rupture both during and after the culture.

Here, the embryo is grown within the confines of the egg shell. In this method, fertilized eggs are rotated inside an incubator for three days in order to prevent the embryo from sticking to the membranes of the shell. A hole is then created on the eggshell and wrapped with a film to prevent dehydration and infections. The egg is then maintained in a static position until further use. This step prevents the CAM from sticking to the shell membrane. At day 7 post-fertilisation, the hole is extended in order to access the CAM. This method offers several advantages over the ex-vivo method as the physiological environment for the developing embryo remains virtually unchanged. It is easier to maintain sterility as well the integrity of the CAM and the embryo when they are present inside the shell. However good technical skills are required for this method. The presence of the shell around the developing embryo makes access to the embryo difficult. There are also limitations in the observing and imaging of the developing embryo.

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Received: February 02, 2021, **Accepted:** February 16, 2021, **Published:** February 23, 2021

Citation: Nazeer A (2021) Effect of Aceclofenac Sodium on Angiogenesis by Using Chorioallantoic Membrane (CAM) Assay. J Appl Pharm. 13:e001.

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