

UGC- Minor Research Project

Title: “Study of co-administration of Pentoxifylline with the plant extract *Tridax procumbens* on the early development of blood vessels in chick embryo”

Principal Investigator: Dr.K.D.Pendharkar

Funding agency: University Grants Commission.

SUMMARY OF THE PROJECT:

Minor Research Project entitled, “Study of co-administration of Pentoxifylline with the plant extract *Tridax procumbens* on the early development of blood vessels in chick embryo” was undertaken with the objectives as follows.

1. To observe the early development of chick embryo under normal conditions by making whole mount slides.

To study the pentoxifylline treated embryos to see the development at vascular level by making whole mount slides.

3. To study the *Tridax* extract treated embryos to see the overall development at vascular level by making whole mount slides.

4. Study of protein and cholesterol content of the tissue by quantitative estimation.

5. To study the histology of development to find out effect of *Tridax* and pentoxifylline.

The eggs were kept for incubation, after 24 hrs the rotations were given to them manually time to time .After 36 hrs incubation a fine hole was made at the narrow base of the egg and some albumen removed and the hole was sealed with adhesive transparent tape. The broad end was opened by tapping it with blunt end of forceps then after removal of the pieces of shell. Then the embryo was positioned by need base removal of some albumen. The embryo was treated with of Pentoxifylline and Plant extracts.

The treated eggs were covered with the transparent cello tape carefully (maximum efforts were taken to avoid infections) and with control egg kept for incubation the chemical treatment procedure done very fast to avoid any break in incubation.

The eggs were taken out after 24 hrs and then the cello tape removed and photographs were taken to see the effect. Sometimes the eggs were taken in the bowl containing 0.9% saline the embryo with vascular network cut circularly and taken on watch glass for some observations.

The chick eggs were incubated at 37°C in the laboratory up to 36 hrs. The doses of pentoxifylline & *Tridax* extract were given to developing embryo; blood vessels containing tissues were separated from embryo after treatment and fixed in Cornoy’s fixative.

After fixation the tissue were transferred in absolute alcohol for dehydration, then cleaned in xylene & embedded in usual manner; the blocks were cut at 7 microns. Then de-prefixed slides were transferred into the absolute alcohol.

Then stained in mercury Bromo-Phenol Blue for 30min to 2 hrs. Then transferred in 1% aqueous acetic acid for 5 min. After washing in 1% aqueous acetic acid were cleaned in xylene and mounted in DPX.

The dried slides containing sections were observed under microscope and photographs were taken to maintain record.

The embryonic tissues (1.5mg) were blotted so as to remove excess water content and weighed on sensitive balance.

The sample was dried in oven at 80°C and weighed. The dried samples were crushed and with Na₂SO₄ (anhydrous) separately in mortar and pestle. The crushed samples were mixed separately with mixture of chloroform and methanol (2:1v/v) and filtered.

Equal volume of KCL was added to filtrate so as to remove non-lipid contaminants and release the bound acidic lipids. The KCL washed supernatant was separated and residue weighed. Weight of lipid is calculated by the formula.