EVALUATION OF THE HERB-DRUG INTERACTION IN CHICK EMBRYO FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

The present study investigates the interaction between Boerhavia diffusa (Punarnava capsules) and Diclofenac sodium powder using chick embryo model for anti-inflammatory activity. Boerhavia diffusa (Nyctaginaceae) is one of the significant indigenous medicinal plant with a broad spectrum of therapeutic activity. The medicinal value of this herb has been reported for its anti-diabetic and diuretic properties. In different areas of the world, it has also been used for analgesic, anti-inflammatory, and carminative activities. The present study was aimed at evaluation of herb-drug interaction between Boerhavia diffusa and Diclofenac sodium for the anti-inflammatory activity using chick embryo model. The inflammation was induced by placing whatman filter paper disc on the chorio-allantoic membrane of an 8-day chick embryo. This model is comparatively cheap, requires little space to perform and only small quantities of the drugs are required. Concurrently no interaction study has been performed on this selected herb and drug.

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INTRODUCTION

Inflammation is a body defense reaction in order to eliminate or limit the spread of injurious agents which is a local response to injury of living mammalian tissues. Various components to an inflammatory reaction represent edema formation, leukocyte infiltration and granuloma formation that can contribute to the associated symptoms and tissue injury. [1]

*Boerhavia diffusa* is the plant used in present study (Family; Nictaginaceae) is commonly known as punarnava mainly an herbaceous creeping weed and is widely distributed in the tropical and temperate region of the world. [2] As per Ayurvedic claims it exhibits a wide range of medicinal properties. The whole plant of *B. diffusa* has been employed for the treatment of various disorders like gastrointestinal disorders, liver disorders and heart diseases. In earlier studies of some groups it has shown to have anti-inflammatory, antimicrobial, immunosuppressive, hepatoprotective, antitumorogenic, antiasthmatic activities and laxative, diuretic, antiurethritis, anticonvulsant, antifibrinolytic, antimetodal, antibacterial properties. [3-6]

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) of the phenyl acetic acid class with analgesic, anti-inflammatory, and antipyretic properties. [7]

Experimental studies on the incubated egg, for example the embryo and the chorio-allantoic membrane (CAM), are carried out frequently. The first system supplying the embryo is the yolk-sac blood vessel system. [8]

The avian chorio-allantoic membrane (CAM) is the outermost extra embryonic membrane lining the non-cellular eggshell membrane. It is formed by fusion of the splanchnic and the somatic mesoderm of the allantois and the chorion. By day 12th of incubation the fused CAM develops and covers the entire surface of the inner shell membrane of the chicken. At day 21st the chick will normally hatch. The CAM serves as a support for the extra embryonic respiratory capillaries it actively transports sodium and chloride from the allantoic sac and calcium from the eggshell into the embryonic vasculature and forms part of the wall of the allantoic sac that collects excretory products.

For studying biological processes such as tumor transplant experiments, toxicity, more recently angiogenesis, acute and chronic inflammatory responses the CAM is a common method because of low cost, the simplicity of the surgical procedure, and the possibility to continuously observe the test site without disrupting it, [9] The chick embryo chorio-allantoic membrane (CAM) was also used as an in vivo wound healing model. [10]

**Figure 1: Candling of egg.**

With the help of candling living normal embryos show (figure 1): [11]

- Clearly defined blood vessels with no hemorrhagic areas evident
- Some body movement when stimulated by the candling light
- A generally healthy appearance
- Dead embryo show a ring of blood outlined on the inner surface of the shell.

Fertile eggs of a cross strain were used (Sudanese bantam-English White Leghorn). [12] These were incubated at 36-37°C, the eggs were turned twice daily. In preliminary tests, the initial incubation period varied between 8 and 12 days. The rationale behind selection of chick embryo CAM model was the advantages such as easy availability, less expensive and no protocol approval with IAEC over methods involving the use of laboratory animals. One disadvantage is that the slope of the dose-response curve appears to be inherently shallow and therefore the test may not be suitable for precise biological assay.

MATERIALS AND METHODS COLLECTION OF DRUG AND HERB

The drug Diclofenac sodium was collected from Nutraplus India limited, and herbal formulation of Punarnava (*Boerhavia diffusa*) capsules was collected from Himalaya herbal health care

EXPERIMENTAL ANIMALS

Fertilized Chick Embryo of 2 days old were procured from Central Poultry Development Organization for chick embryo model. Fertile eggs of a cross strain were used (Sudanese bantam-English White Leghorn). These were incubated at 36-37°C; a moist atmosphere was maintained by placing dishes of water on alternate shelves in the incubator; the eggs were turned twice daily.

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CHEMICALS AND BIOMATERIALS
DMSO, Diclofenac sodium, Boerhavia diffusa, Sterile filter paper disk.

EXPERIMENTAL DESIGN
Chick embryo chorio-allantoic membrane model

The inflammatory reaction was induced by placing whatman filter paper disc on the chorio-allantoic membrane of an 8-day chick embryo followed by re-incubation in situ for 4 days. Drug and herb are dissolved in DMSO.

Treatment groups are as followed:
Group I: induced saline (0.1 ml)
Group II: disease control induced paper disc with saline (0.1 ml).
Group III: induced paper disc with drug sodium diclofenac (1mg).
Group IV: paper disc with herb Boerhavia-diffusa (1mg). [13]
Group V: interaction group paper disc with drug Diclofenac (1mg) and Boerhavia-diffusa (1mg).

DISC

Whatman No. 1 filter paper was used as Filter paper discs. Any disc with rough or uneven edges was discarded; the discs were washed in distilled water to remove fragments and then sterilized in Universal screw-topped bottles, approximately 50 discs/bottle, by autoclaving (15 lb/sq. in./20 min). Four smaller discs, each approximately 5 mm diameter, were implanted in each egg.

TECHNIQUE

1. The technique used for dropping the chorio-allantoic membrane was a modification of that described by Beveridge & Burnet (1946). [14]
2. The egg was mounted on a stand, with the drilled area of shell uppermost; a straight Hagedorn's needle was gently inserted under one corner of the smaller triangle of shell and this triangle was raised and removed, care being taken to avoid puncturing the shell membrane during this procedure.
3. A drop of sterile saline was placed on the exposed shell membrane and a small slit made in this membrane (and not through the closely adjacent chorio-allantoic membrane) by gentle downward pressure with the Hagedorn's needle.
4. By means of a rubber teat, suction was applied to the hole drilled over the air sac and the chorio-allantoic membrane fell away from the shell membrane, drawing in the drop of saline (Figs. 2 b). The shell and shell membrane circumscribed by the larger triangle were then removed, and the sterile filter paper disc inserted and carefully lowered on to the exposed membrane (Figs. 2 c).
5. The opening in the shell was sealed with Sellotape and the hole over the air sac region sealed with molten paraffin wax (Fig. 2 d). The eggs were then reincubated at 370 for 4-6 days, without turning.

6. At the end of this period, the chorio-allantoic membrane was exposed by cutting around the long circumference of the egg with a pair of curved scissors; the chorio-allantoic membrane remained in the top half of the shell, together with the filter paper disc. The membrane was gently eased out of the shell using forceps.
7. The filter paper disc and the underlying portion of thickened membrane were dissected out and the disc, together with the underlying granulation tissue, was placed on a plastic spotting tile and dried overnight at. The dry disc plus granulation tissue was subsequently weighed.
8. Specific effects of the drug and herb on the growth of embryos were also observed.

Figure 2: (a) Chick embryo showing sacs and membrane; (b) Air sac evacuated and chorio-allantoic membrane dropped; (c) Filter paper disc placed on the chorio-allantoic membrane; (d) Tri-angular opening through shell and shell membrane sealed with sellotape.
RESULT AND DISCUSSION

The result obtained from present study indicate that all the treatment groups showed a dose related reduction in the amount of granulation tissue growing on the undersurface of the implanted disc. Diclofenac sodium was the most potent of the drugs studied; it caused significant inhibition of granulation tissue as compared to combination treatment group. Growths of chick embryo were also affected. It has also been observed that growth of diclofenac treated embryos was normal (fig.5). The disintegrated embryos were observed in the group treated with Punarnava extract (fig.6). This may be due to the effect of herb on the embryonic development. The treatment group, with both Punarnava extract and Diclofenac sodium showed slow development of embryos (fig.7). In order to assess the actual amount of granulation tissue present, the weight of the dried filter paper disc was subtracted from the total weight of the dried disc plus granulation tissue.

Figure 3: Vehicle Control.

Figure 4: Disease Control.

Figure 5: Diclofenac sodium.
CONCLUSION

The presence of a filter paper disc on the chorio-allantoic membrane of an 8-day chick-embryo, when incubated at 37° for 4 days, produced an inflammatory reaction on the membrane. This inflammatory response was significantly reduced when diclofenac sodium 1mg, *Boerhavia diffusa* 1mg and combination group was impregnated into the disc prior to implantation. Specific effects of the drug and herb on the embryos were observed. The test, being economical of materials and laboratory space, is suitable for the routine screening of potential anti-inflammatory compounds at fixed dose levels.

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