

TOXICITY EVALUATION OF AQUEOUS EXTRACT OF BARK AND LEAF OF *FICUS RACEMOSA* IN EARLY DEVELOPMENT OF CHICK EMBRYO

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ABSTRACT

Single dose treatment of various concentrations of aqueous extracts of leaves and stem bark of *Ficus racemosa* were administered at various hours of chick embryo development to determine the toxic effects leading to mortality and morphological abnormalities. The extracts were administered at 48, 72 and 96 hrs of development since toxicity studies were directed towards early development of circulatory system, especially chorioallantoic membrane vasculature. The final effect of the extracts was observed at 144 hrs of development. Window method was used for the administration of the extracts. The results indicate that increase in the concentration was more lethal or induced abnormalities. High mortality was observed at high doses (1.0, 1.5 and 3.0 mg/egg) of

the leaf extracts. Initiation of doses at early hrs of development also influenced percent mortality. Bark extracts (all the doses 0.5 mg, 1.5 mg and 3.0 mg per egg) showed 100% mortality irrespective of initiation of doses. The dead embryos showed significant inhibition in growth and differentiation.

KEYWORDS: *Ficus racemosa*, chick embryo, toxic effects, mortality, window method.

INTRODUCTION

The toxic effects of *Boerhavia diffusa* (leaves) and *Pterocarpus santalins* (bark) on chick circulatory system of chorioallantoic membrane have already been tested in our

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laboratory.^[1,2] In the present work we have tested toxicological effects of a plant which is used by Ayurvedic, Unani, Siddha and Homeopathy systems of traditional medicines.^[3] It is used as astringent, carminative, stomachic, vermifugal and is believed to be a good remedy for visceral obstructions. Fruit extract of this plant is used in leprosy, diarrhoea, circulatory and respiratory disorders and menorrhagia.^[4, 5, 6]

Bark of *F. racemosa* is acrid, cooling, galactagogue and good for gynaecological disorders. The stem bark is used to treat menorrhagia, leucorrhoea, gonorrhoea, urinary diseases, hemorrhage and skin diseases, dysentery, diarrhea, urological disorders, diabetes, hiccough, leprosy, asthma and piles.^[3, 7, 8, 9, 10, 11, 12] According to Unani system of medicine *F. racemosa* leaves are astringent to bowels and good in case of bronchitis, used to treat dysentery, bilious infection and as a mouthwash in spongy gums.^[13] The tender leaf buds are applied on the skin, in the form of paste, to improve the complexion. A decoction of the leaves is a good wash for wounds and ulcers. The infusion of bark and leaves is also employed as mouth wash to spongy gums and internally in dysentery, menorrhagia, effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis and haemoptysis.^[3]

The leaf of *F. racemosa* contains sterols, triterpenoids (Lanosterol) and alkaloids, tannins and flavonoids. Stem-bark gives gluconol acetate, β -sitosterol, leucocyanidin-3-O- β -D-glucopyranoside, leucopelargonidin-3-O- β -D-glucopyranoside, leucopelargonidin-3-O- α -L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate and α -amyrin acetate. From trunk bark, lupenol, β -sitosterol and stigmasterol were isolated.^[14]

For this reason we have tested aqueous extracts of both, bark and leaf, of *Ficus racemosa* in the present study so that if it has any teratogenic or toxic effects that need to be known by the users.

MATERIALS AND METHODS

1. Plant Material and extract preparation

Leaves and stem bark of properly identified *Ficus racemosa* were obtained from campus of Shivaji University, Kolhapur, MS, India. The leaves and barks were cleaned, shade dried, powdered mechanically and then strained through muslin cloth. The powder was extracted using routine methods to get aqueous extract. The yield of aqueous extract of leaves was 8.1 % and bark was 11.3%. The dried samples of extracts were dissolved in Hanks Balanced Salt

Solution (HBSS-HIMEDIA, India) to prepare the stock solutions so that suitable concentrations can be used for the applications.

2. Incubation of eggs

In the present work fertilized eggs of *Gallus gallus murghi* were obtained from Quality poultry products, Malgaon, Tal. Miraj, Dist. Sangli, MS, India. Eggs of similar size and weight were selected and checked for damage. The shells were disinfected with 70% alcohol. Interests being in the Chorio-allantoic membrane (CAM) vasculogenesis and angiogenesis development, treatment hours were selected at 48, 72 and 96 hrs according to the development of CAM and vitelline veins. The eggs were incubated in aseptic conditions in vertical positions such that blunt end of the egg was always upwards. The eggs were incubated at 37.5°C temperature and relative humidity was maintained at 70-75%. The treatment doses were administered at above stated hours and development was continued up to 144 hrs i.e. on completion of CAM development and blood vessels with capillary networking.

3. Dose administration by Window Method^[15]

After scheduled period of incubations (table1) the windows were prepared in eggs under aseptic conditions and extracts of *F. racemosa* were spread on the embryonic plates in the final volume of 0.5 ml HBSS. Different concentrations, adjusted in the final volume of 0.5 ml, were spread on the embryonic plate uniformly in different embryos in segregates. All the treatments were given in final volume of 0.5ml of HBSS with scheduled concentrations of extracts. Normal (untreated) embryos were maintained as normal group. Embryos of operative control were sham operated for window preparation and embryos of HBSS control received 0.5ml of HBSS free of extract. The HBSS and all the doses were brought to 37°C before administration. The window made for administration was sealed with sterilized adhesive tapes and the embryos were immediately transferred to the incubator to continue further incubation hrs adjusting the experimental time slot as described in table 1 until completion of 144hrs. The whole process of administration of treatment doses did not take more than 15 min.

Table 1: Exposure schedule of extracts of *F. racemosa* at different developmental stages of chick embryos

Group	Developmental stage in hrs	Corresponding HH stage	Time of exposure to treatment of doses in hrs			Final observation at hrs
			48	72	96	
I	48	12	√	---	---	144
II	72	20	---	√	--	144
III	96	24	---	---	√	144

On 144 hrs of incubation, the shells were removed and the embryos were observed for mortality and associated abnormalities.

RESULT AND DISCUSSIONS

Mortality is the ultimate biomarker of toxic effects, in that it indicates the lethal action of compound. Abnormalities among embryos can be used to indicate the teratogenic potency of compounds,^[16, 17, 18, 19] and measure general status of embryo. Yolk sac or organ weights reflect if compounds interfere with growth.^[20]

In the present investigation, single dose treatment of various concentrations of aqueous extracts of leaf and bark (0.3 mg, 0.5 mg, 1.0 mg, 1.5 mg and 3.0 mg for leaf extract and 0.1 mg 0.5 mg, 1.0 mg and 1.5 mg for bark extract) of *F. racemosa* were initiated at different hrs of development (as stated in the materials and methods) to determine the toxicity leading to mortality and morphological abnormalities with hemorrhage, vascular damage. The embryos were observed for mortality in the following interval of development as shown in Table 2. Total weights and abnormalities were noted if any.

Table 2: Average mortality for the aqueous extracts of leaves of *Ficus glomerata* Roxb

Sr. No	Extract treatment (mg/egg)	Experimental condition of treatment and percent mortality at 144 hrs of development						Abnormalities at 144 hrs
		Normal	Sham control	HBSS	48 hrs	72 hrs	96 hrs	
1	0.3	10% at all hours of treatment (normal embryo)	8-10% at all hours of treatment (normal embryos)	5-6% at all hours of treatment (normal embryos)	0	55	70	Normal embryos
2	0.5				0	25	40	Normal embryos
3	1.0				0	100	100	Abnormal torsion and twisting of body of embryo, extended and thin neck, internal hemorrhage in brain and body region, hemorrhage on CAM, less developed CAM
4	1.5				80	100	100	
5	3.0				100	100	100	

Normal embryos showed 5 % mortality i.e. 95 % survival and no abnormalities at 144 hrs of development and at the time of hatching. While Sham operated control group showed 9.3 to 9.7% mortality with 90.7 to 90.3% survivals. All the embryos of operative control groups were normal. HBSS control embryos showed 4.5-5% mortality at all developmental hours studied. It was observed that due to HBSS mortality was decreased by 5.5-5% as compared to the mortality observed in normal and 4.8- 4.7 % as compared with Sham/operative control at 144hrs of development. The aqueous extracts of the bark of *F. racemosa* were lethal at all studied concentration doses and no embryos survived even at the lowest concentrations of 0.1mg/ 0.5ml of HBSS. The dead embryos when looked at 144hrs after opening the eggs, were of earlier stages (HH stage 24-26, HH stage at 144 hrs should be 28-29) ^[21] and were abnormal in overall development viz., abnormal torsion, internal hemorrhage in brain and other regions of the body, extended neck region, hemorrhage on CAM at periphery, less developed and structured vasculature on CAM etc.

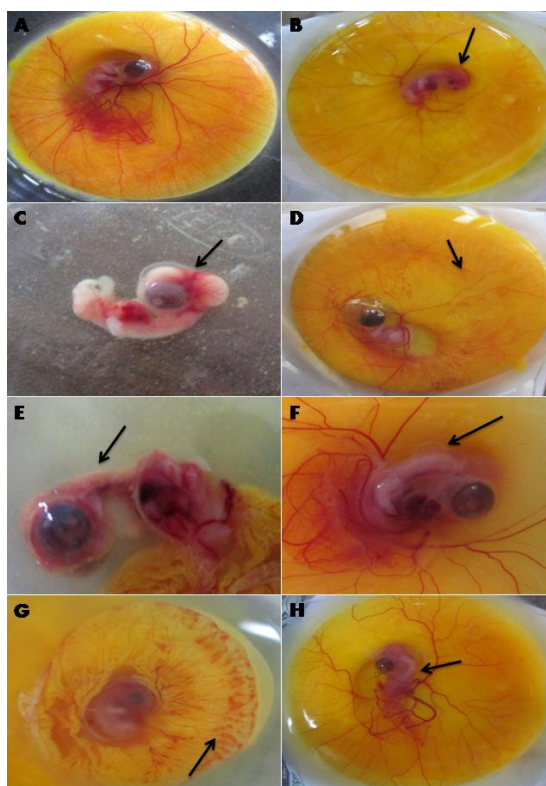


Figure 1: Morphological abnormalities induced by aqueous extract of *Ficus racemosa* leaves during early development of chick embryo. A: Normal embryo, B: Brain and eye development altered by 1.0mg/egg at 72 hrs treatment. C: Internal hemorrhage in brain by 1.5 mg/egg dose treatment at 96 hrs (dead embryo). D: Poorly developed CAM vasculature by 3.0mg/egg at 48 hrs treatment (dead embryo). E, F: Extended and thin neck region by 1.0mg/egg at 72 hrs and 1.5mg/egg at 48 hrs respectively G: Hemorrhage on CAM with

deformed vasculature by 3.0mg at 72 hrs treatment (dead embryo). H: Abnormal torsion by 3.0mg/egg at 48 hrs of treatment.

For the aqueous extract of leaves of *F. racemosa* initiation of 0.3 mg dose although at 48 hrs resulted in 0% mortality, at higher hours i.e. at 72 hrs was more toxic resulting in 55 % mortality and dose initiation at 96 hrs showed higher mortality i.e. 70%. At the dose initiation of 0.5mg at 48 hrs, no mortality was observed, all the embryos were alive and also no abnormality of any kind is reported (0% mortality). Thus the mortality was improved over the normal and HBSS treated embryos. At 72 hrs, the dose initiation of 0.5mg of aqueous extract showed 25% mortality. At 96 hrs of dose initiation, mortality increased to 40%. For the aqueous extract of 1.0 mg, dose initiation at 48 hrs resulted in no mortality (0% mortality), all embryos alive. However at 72 hrs of dose initiation and at 96 hrs of dose initiation, all the embryos were dead (100% mortality) and the dose lethal. The higher doses of 1.5mg and 3.0 mg at all hours of dose initiation i.e. 48 hrs, 72 hrs and 96 hrs of development proved to be lethal to all the embryos, showing 100% mortality (Table 2).

When the embryos were analyzed at 144 hrs for mortality and deformities, it was observed that wherever the embryos were dead, they were of lesser HH stage (HH stage 24-26, normal HH stage at 144 hrs of incubation should be 28-29). The embryos were visually abnormal in many ways as described earlier (Fig 1). Thus all the doses of aqueous extract of bark of *F. racemosa* showed 100% lethal effect in all stages of chick embryo development and thus bark is not advisable in the developmental status. Such a high toxicity could be possible due to extremely high concentration of potassium ions in it and rich contents of phenolic compounds^[22]

While aqueous extracts of leaves (1.0 mg, 1.5 mg and 3.0 mg/egg) showed 100% mortality with 48, 72 and 96 hrs of treatment, the lower dose (0.5mg/egg) was free of mortality at 48 hrs of treatment. But at 72 hrs, 25% mortality was observed with no abnormalities and at 96 hrs of treatment mortality was higher by 15% with no abnormalities. These results indicate that the late stages of development are more susceptible to the extract than early stages of development. Those embryos that tolerated the treatment and survived were normal in all respects of development.

The results show that the toxins in the barks may be reaching in some concentrations in leaves leading to mortality. Since all the doses of bark are lethal and abnormalities in

embryos observed with bark extracts and leaf extracts are similar or same. Hemorrhages in the body organs indicate the toxins probably interfere with the vasculature development which is also true in case of CAM vasculature.

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