MATERIAL AND METHODS

SECTION I

(i) Source of animals and Maintenance:

Adult wall lizards, *Hemidactylus flaviviridis*, of both the sexes with intact tails weighing 10±2 g were procured from local animal dealer. They were maintained in wooden cages with glass slider on one side, in the animal house with 12 12 light-dark cycles. Water was supplied to the animals *ad libitum* all the time and cockroaches were fed 2-3 times in a week In all the experiments, the animals were acclimatized for a period of seven days prior to the experimentation The experiments were conducted in the months of March-July and Sept-Nov Animals were maintained in a temperature controlled room at 30 ± 2^0 C as this temperature is necessary to produce optimum tail growth in lizards The tails of the lizards were autotomized by pinching off the tail by exerting mild thumb pressure leaving three segments intact from the vent

(ii) Experimental protocol:

During the period of study, the experiments were conducted in two series In the first series, the animals were treated with the drug four days prior to autotomy and the treatment was continued after autotomy for six days. In the second series of experiment, the drugs were administered at different stages of tail regeneration *viz* prior to amputation, at wound epithelial stage, early blastema and differentiation stage, to study the stage specific effects. Immediately after amputation, the process of wound healing initiates, followed by the formation of wound epithelium The wound epithelium appears as a smooth shining surface and is accompanied by the process of dedifferentiation Blastemic stage is characterized by conical aggregation of cells, called blastemal cells (approx 1-2 mm in length from the stump) which have been formed as a result of dedifferentiation process The blastema further grows in size and later on differentiates to replace the missing structures

SECTION II

Drugs and Dosage

(i) Fibroblast Growth Factor-2 (FGF-2 or bFGF) [High Dose]:
Intraperitoneal (i p) administration 50 µg/kg body weight prepared in 0.6% saline, immediately before use

(ii) Fibroblast Growth Factor-2 [Low Dose]:
Intraperitoneal (i.p) administration. 25 µg/kg body weight prepared in 0 6% saline, fresh before use.
In loco administration 10 µg/kg body weight prepared in 0.6% saline, fresh before use

(iii) AntiFibroblast Growth Factor-2
Intraperitoneal (i.p) administration 25 mg/kg body weight prepared in 0.6% saline, freshly before use
In loco administration 10 mg/kg body weight prepared in 0 6% physiological saline, fresh before use

(iv) Epidermal Growth Factor:
Intraperitoneal (i p.) administration: 25 µg/kg body weight prepared in 0.6% physiological saline, fresh before use
In loco administration 10 µg/kg body weight prepared in 0.6% saline, fresh before use

All the growth factors *viz* FGF-2, AntiFGF-2 and EGF were purchased from Sigma Chemicals Co., St Louis, MO, U.S A All other chemicals were of AR grade and procured from Sisco research laboratories Pvt Ltd., Mumbai, INDIA, Qualigens fine chemicals, Mumbai, INDIA

SECTION III

(i) Morphometric measurements of tail growth

The growth in the length of the tail regenerate was measured using millimeter scale at fixed intervals. The time taken to reach the various stages of tail regeneration, such as wound epithelium (WE), early blastema and differentiation was recorded (Figure i).

(ii) Histoflouroscence studies;

For the nucleic acids (DNA and RNA) localization, metachromatic flourochrome Acridine Orange was used. The tail regenerate was removed immediately after sacrificing the animals, blotted dry, and transferred to a cryostat microtome mainatined at -20^0 C. The tissues were embedded in OCT compound (Tissue Tek-II) and sectioned at 10µm in longitudinal plane The sections were exposed to 0 1% Acridine Orange in 0 1M phosphate buffer (pH-6 0) and observed under fluorescence microscope (Leica, DMRB) with 410 nm excitation filter and 510 nm emission cut off filter. The Acridine Orange induces specific yellow emission for DNA and flame red emission for RNA at the ultraviolet range (Culling, 1974).

(iii) Estimation of Antioxidants.

a) Estimation of superoxide dismutase:

Blood: Blood was collected from different experimental and control groups of lizards by cardiac puncture under hypothermic anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes and serum separated out Erythrocyte sediment was washed thrice with phosphate buffered saline (PBS) and hemolysate was prepared with 1g/dl Hemoglobin concentration The superoxide dismutase (SOD) activity was estimated according to the method of Kakkar *et al.,* (1984).

Tissue: Tissues were collected after sacrificing the animals and were blotted. 4% tissue homogenate was prepared in 0.89% KCl in chilled mortar and pestle and then centrifuged at 3000 rpm for 15 min. The enzyme activity was measured in the tissues by the method by Kakkar *et al.,* (1984)

(b) Estimation of catalase activity

Blood: Blood was collected and hemolysate prepared as described earlier, but with Hb concentration 5g/dl This was further used to estimate catalase activity as described by Hugo (1987)

Tissue: 10% tissue homogenate was prepared in chilled mortar and pestle with PBS and centrifuged at 3000 rpm for 15 min The enzyme activity was measured by the method described by **Gerald Cohen *et al.*, (1970).**

(c) Estimation of reduced Glutathione·

Blood: Blood was collected from the experimental and control animals as described earlier The reduced glutathione level was estimated in whole blood according to the method of Bentler and Gelbart (1985)

Tissue: 10% tissue homogenate was prepared in PBS in chilled mortar and pestle and centrifuged at 3000 rpm for 15 min. The supernatant was used to estimate reduced glutathione by the method of Bentler and Gelbart (1985).

(d) Estimation of Malondialdehyde·

Blood: Blood was collected from respective animals and control animals as described. Blood was centrifuged at 3000 rpm for 10 min. and erythrocyte sediment was washed thrice using PBS The malonedialdehyde level was estimated according to the method by Stocks and Dormandy (1971).

Tissue: 10% tissue homogenate was prepared using PBS in chilled mortar and pestle and then centrifuged. The MDA level was estimated using the methodology described by Stocks and Dormandy (1971)

(iv) Estimation of Nucleic Acids

Tail regenerate was obtained after sacrificing the experimental and control lizards 20% homogenate was prepared using PBS in chilled mortar and pestle. The nucleic acids were estimated according to the method described by Schneider (1957).

(v) Estimation of proteins.

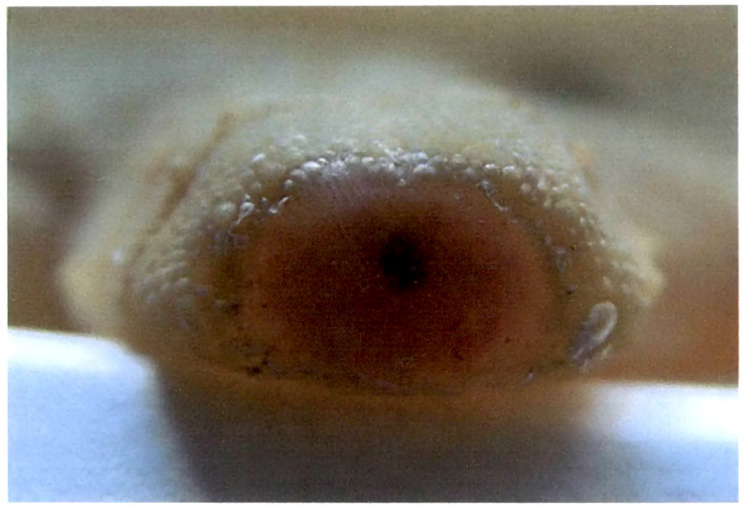
Tail regenerate was harvested from the experimental and control lizards. 10% tissue homogenate was prepared using PBS and protein content was estimated as per the method of Lowry *et al.*, (1951)

SECTION IV

Statistical Analysis

The significance level of the experimental and control group was evaluated by Student's't' test, with a 95% confidence limit. For multiple group comparison and difference between the groups the data were subjected to One Way Analysis (ANOVA) followed by Duncan's Multiple Range Test **(Duncan, 1955)** using SPSS-PC Statistical Analysis Package A 'p' value of 0.05 or less was accepted as being statistically significant

Figure i. Stages of tail regeneration in *Hemidactylus flaviviridis*



Wound Epithelial stage

Early Blastema stage

Differentiation stage