

The present study hypothesized that the amputated parts of earthworm treated with valproic acid (VPA) would have slower rate of regeneration as compared to the amputated parts not treated with valproic acid. The insight of this study would encourage the use of valproic acid in regenerative medicine based approaches and in treating damaged tissues or even cancer.

Materials and Methods:

Sixty adults and healthy earthworms (*Eisenia fetida*) were taken and cultured in cup and pot.

Fifteen earthworms were taken and categorized into three groups. Earthworms from each group were amputated at 40th segment from the head region. Group A constituted untreated worms (Control). Group B and Group C constituted the earthworms which were with treated 1mM and 2mM concentrations of valproic acid respectively. For amputation, the worms were placed on ice cool pack and the chosen segment was amputated using a surgical blade. After amputating valproic acid was applied by drop method at amputation site every alternate day. Each part of amputated worm was kept in separate cups and the cups were accordingly labeled.

Results and Discussion:

It is well known that parts of earthworms can survive if they are cut off. The regeneration process in *Eisenia fetida* followed four different distinct stages (Fig. 1-4) and accordingly a time schedule. It began with a small rounded bud formation which lacked pigmentation. The bud appeared after an average of 4-5 days in the worms of Group A (Tables 1 and 2), after an average of 6-7 days in Group B (Tables 3 and 4) and after an average of 8-9 days in

Group C (Tables 5 and 6). The bud elongated gradually to form a blastema after 6 to 7 days in Group A, 8 to 9 days in Group B and 10 to 11 days in Group C. Meanwhile the colour of the outgrowth became reddish and segmentation appeared. The regeneration got completed in 30 to 35 days in Group A, 32 to 37 days in Group B and 33 to 40 days in Group C (Tables 1-6).

When the 40th segment of earthworm was amputated, both anterior portion having mouth and clitellar part and posterior portion having anus and rest of the body parts survived and regenerated successfully. It was observed that posterior part regenerated faster than anterior part. Rashmi et al (2015) also reported similar findings.

It was observed that clitellum was not restored after regeneration in posterior part which was similar to the observation made by Rashmi et al (2015). Pigmentation was only seen in posterior parts and was not visible in anterior parts.

Untreated earthworms regenerated faster than the VPA treated earthworms. There was no increase in the number of segments in both anterior and posterior parts i.e., no overshooting of segments took place. Anterior parts showed around 37 regenerated segments (hypomeric regeneration) which were less than the lost segments in all groups, Group A, Group B and Group C. In Group A, posterior parts regenerated equal number of segments i.e., 38 to 40 segments. Rashmi et al., (2015) also reported similar results. In Group B and Group C, the posterior parts showed less number of regenerated segments i.e., nearly 24 to 27 segments which were less than the lost segments. Savigny (1826) also observed similar condition.



Fig. 1. Bud Formation



Fig. 2. Blastema Formation



Fig. 3. Segmentation



Fig. 4. Complete Regeneration

Table 1. Time taken (in days) for regeneration of anterior part in Group A worms (control). Values are Mean \pm S.E.

	N = 5
Bud	5.4 \pm 0.48
Blastema	7.4 \pm 0.48
Pigmentation & segmentation	13.2 \pm 0.74
Complete regeneration	35.2 \pm 0.74

Table 2. Time taken (in days) for regeneration of posterior part in Group A worms (control). Values are Mean \pm S.E.

	N = 5
Bud	4.4 \pm 0.48
Blastema	6 \pm 0.63
Pigmentation & segmentation	11.8 \pm 0.74
Complete regeneration	30 \pm 0.63

Table 3. Time taken (in days) for regeneration of anterior part treated with 1mM VPA (Group B). Values are Mean \pm S.E.

	N = 5
Bud	7.2 \pm 0.74
Blastema	9.2 \pm 0.74
Pigmentation & segmentation	16 \pm 0.63
Complete regeneration	37.4 \pm 0.8

Table 4. Time taken (in days) for regeneration of posterior part treated with 1mM VPA (Group B). Values are Mean \pm S.E.

	N = 5
Bud	6.2 \pm 0.4
Blastema	8 \pm 0.63
Pigmentation & segmentation	14.6 \pm 0.8
Complete regeneration	32 \pm 0.63

Table 5. Time taken (in days) for regeneration of anterior part treated with 2mM VPA (Group C). Values are Mean \pm S.E.

	N = 5
Bud	9.6 \pm 0.48
Blastema	11.4 0.48
Pigmentation and segmentation	17.2 \pm 0.74
Complete regeneration	40 \pm 0.63

Table 6. Time taken (in days) for regeneration of posterior part treated with 2mM VPA (Group C). Values are Mean \pm S.E.

	N = 5
Bud	8.2 \pm 0.4
Blastema	10 \pm 0.63
Pigmentation & segmentation	16 \pm 0.63
Complete regeneration	32.8 \pm 0.74

Conclusion:

In the present study, it was found that Valproic acid slowed the rate of regeneration of lost segments in *Eisenia fetida*. Posterior part of the amputated worms regenerated faster than the anterior part of the amputated worms. No overshooting in the number of segments were found in all groups. Posterior parts of control earthworms showed equimeric results but treated earthworms showed hypomeric results.

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