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Research Article

ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF THE REGENERATING TISSUES OF AN EARTHWORM, EUDRILUS EUGENIAE

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ABSTRACT

The earthworm *Eudrilus eugeniae* is segmented worm which have the remarkable ability of regeneration. Organogenesis and animal adaptation upon loss of body parts are crucial events in regeneration of all traits and both are uncovered myths in biology. Earthworms so called ecological engineers are the sources of food, medicine and so on. In the present study the earthworm regenerated from the posterior end within a week. The regenerating tissue when tested for total antioxidant capacity and free radical scavenging activity, showed a remarkable result with high antioxidant and free radical scavenging activity. Free radical scavenging activity of *Eudrilus eugeniae* tissue using DPPH method was found to increase with increase in concentration of the sample. Thus from the current study it can be concluded that *Eudrilus eugeniae* can be utilized as good source of antioxidant potential and free radical scavenging activity.

INTRODUCTION

The phenomenon of regeneration is a fascinating field in novel biology. The field may promise to implement the progress of the success in near future up to the clinical set up. Regeneration is a biological phenomenon in which the lost organ or tissue are restored to its nearly identical structure with the help of associated cell tissues and organs, .the remarkable reparative process includes the recognition and recapitulation of missing structure with the functional integration between the newly formed and pre-existing tissues in order to direct physiological and structural alterations .furthermore the process of regeneration involving would healing as an initiative process in which is accomplished by cellular proliferation at the site of amputation.

The regeneration ability to replace the lost body parts in animal varies greatly (Vorontsova *et al*., 1960; Goss, 1969; Brusca and Brusca, 2003) and understanding the differences in regeneration ability is a key question in biology (Goss, 1969; Morgan, 1901; Elder, 1979; Reichmann, 1984). In 1901, T.H. Morgan, documented two modes of animal regeneration: morphallaxis and epimorphosis. In the case of epimorphosis, regeneration occurs by the proliferation of cells at the amputation site. Epimorphosis occurs mainly via de-differentiation and subsequent re-differentiation of cells, or by the proliferation of reserve population of stem cells. Examples include limb regeneration in amphibians (Brockes *et al*., 2001) and compensatory regeneration of liver in mammals (Stocum, 2004; Taub, 2004).

Eisenia fetida(Moment, 1949) and *P excavatus* have the capacity to regenerate both anterior and posterior segment; Capitella sp. II and Mediomastus sp., regenerate only the posterior segment, and they fail to regenerate anterior parts (Bely, 2006).

The mechanism determining the differences in the ability of earthworm segments regeneration is still obscure. Amputation at the anterior segments completely removes mouth, brain, and other germs cell associated organs and it is remarkable to note that ingestion of nutrition is not possible upon anterior segments amputation till the time of mouth parts is regenerated completely. The interested model animals for the present study are the earthworms, *E eugeniae.* The regeneration studies were reported in the following earthworms such as *Eisenia andrei, Eisenia fetida, Lumbricus rubellus* (Blakemore, 1999; Gates, 1972; Blakemore, 1998) and *Ptychodera flava* (Rychel and Swalla, 2008). But there was no report on regeneration studies in *E eugeniae*.Antioxidants are involved in the defence mechanism of the organism against the pathologies associated to the attack of free radicals. Endogenous antioxidants include

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both enzymatic and non enzymatic compounds. When endogenous factors cannot ensure a rigorous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical compounds. Exogenous antioxidants can either be derived from natural sources or be synthetic compounds, like butyl hydroxyl anisole, butyl hydroxyl toluene, gallates, etc. (Litescu t al, 2011).

In the present study the regenerated tissue was assayed for antioxidant activities.

MATERIALS AND METHODS

Culture and Maintenance of Earthworms

The earthworms, *E. eugeniae*, were maintained in a tub containing soil, cow dung, and leaf litter at an ambient temperature. *Eudrilus eugeniae* was collected from Karnataka Compost Development Corporation. They were maintained in separate plastic tubs containing soil, cow dung and decayed leaf litters in the ratio of 1:4:2. The earthworms were kept in the Indian Academy Biochemistry laboratory and allowed to multiply minimum of ten days in order to allow them to adapt to experimental conditions (28 ± 2°C) and to get acclimatization of the laboratory condition.

Dissection of earthworm, *E eugeniae*

The earthworm, *E eugeniae* was fixed with 10% formaldehyde solution for 15 minutes on a Petri plate. After fixation, the worms were taken out from the Petri plate and kept on a dissection board with dorsal up position. Using the sterile surgical blade the earthworm was symmetrically dissected out. The earthworm was amputated in post clitellar portion in different ways –anterior ½, anterior ¾ anterior ¼ anterior, posterior ½, posterior ¼, and posterior ¾ respectively. After making cuts in the earthworm segments, they were allowed to regenerate. In all the groups, the worms were initially amputated at the tail segments and were kept for regeneration until for one week. The adult worm without any cuts was acting as control. The earthworms which were amputated at different sites were carefully monitored on day 5th, 9th and 13th day for the size of the anterior and posterior bud (Fig 1, 2).

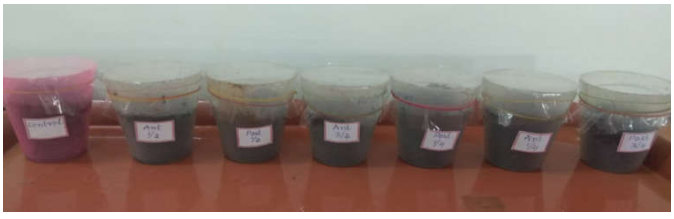

Fig 1 Earthworm samples after amputation in different containers.

Fig 2 The regeneration process overview

Determination of Total Antioxidant Capacity

The anti-oxidant activity of the portion was evaluated by the phospho molybdenum method according to the procedure of Prieto *et al*, 1999. The assay was based on the reduction of Mo (V) by the extract and the subsequent formation of a green phosphate / Mo (V) complex at acidic pH. The regenerated earthworm sample 0.3 ml was mixed with 2.5 ml of phospho molybdenum reagent. The absorbance of the reaction mixture was measured after 15 minutes at 695 nm on UV double beam spectrometer. The L-Ascorbic acid was used as reference standard. The antioxidant activity of sample was expressed as percentage.

Total anti-oxidant assay (%) = [(A Control –A Sample)/ A control] x 100

Determination of DPPH radicals scavenging activity

The free radical scavenging activity of the earthworm regenerated sample was measured in terms of hydrogen donating or free radical scavenging ability using the stable free radical DPPH .The free radical scavenging activity was estimated by DPPH method.1Mm solution of DPPH in ethanol and also 1mg /1ml extract solution in ethanol was prepared and 1.5 ml of this solution was added to 1.5 ml of DPPH. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared by taking 3ml ethanol and control was prepared by taking 3ml DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation:

% DPPH radical scavenging= [(Control OD- sample OD)/ Control OD] X 100

RESULTS AND DISCUSSION

Regeneration of Earthworm Tissues

The earthworms which were cut at anterior were not regenerated when cultured and maintained for two weeks duration. But *Eudrilus eugeniae* started regenerating after 7 to 9 days from the posterior region. The regeneration was observed in all the earthworm samples which were cut at the posterior regions (Fig 3.1, 3.2).

Fig 3.1 The container containing posterior 3/4th cut earthworm.

Fig 3.2 Regenerated tissues at posterior end

Anti Oxidant Actvity

Total antioxidant activity of *Eudrilus eugeniae* tissue sample at different concentration is given in table-1. Total anti-oxidant activity of *Eudrilus eugeniae* regenerated tissue extract was expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentrations of Eudrilus *eugeniae* (Fig 4).The observed Scavenging effect of earthworm extract, and standard on the total antioxidant activity decreases in the following order: *Eudrilus eugeniae* regenerated tissue extract >L ascorbic acid >control earthworm extract. Among this, *Eudrilus eugeniae* possess high antioxidant potential as compared with the ascorbic acid.

Table 1 Total antioxidant activity of *Eudrilus eugeniae* extract at different concentrations.

Concentration(µg/ml)	% of Inhibition	
	Ascorbic acid	earthworm tissue
40	0.167	0.189
80	0.29	0.407
120	0.386	0.49
160	0.586	0.6
200	0.732	0.9

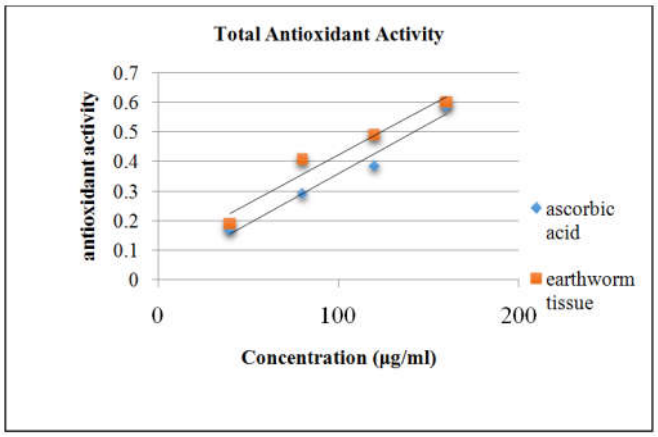

Figure 4 Total anti oxidant assay activity of *Eudrilus eugeniae* extract at different concentrations.

DPPH radicals scavenging activity

Free radical scavenging activity of *Eudrilus eugeniae* regenerated tissue at different concentrations is given in Table 2 .The antioxidant activity was found to be maximum for the 100µg/ml of earthworm extract that was used in the DPPH assay. Free radical scavenging activity of the *Eudrilus eugeniae* on DPPH radicals was found to increase with increase in the concentration (Fig 5). *Eudrilus eugeniae* possesses potential scavenging activity as compared with ascorbic acid.

Table 2 DPPH scavenging activity of *Eudrilus eugeniae* extract at different concentrations.

Concentration(µg/ml)	% of Inhibition	
	Ascorbic acid	earthworm tissue
20	0.666	0.821
40	0.758	0.849
60	0.807	0.905
80	0.935	1.025
100	1.025	1.314

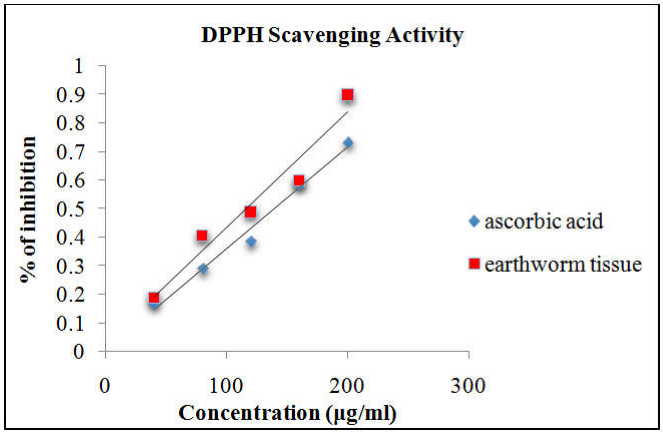

Figure 5 Free radical scavenging activity of *Eudrilus eugeniae* extract at different concentrations.

CONCLUSION

On the basis of the results of this study, it clearly indicates that both the samples - ascorbic acid and *Eudrilus eugeniae* regenerated tissue possessed antioxidant activities. The present investigation confirmed that the free radical scavenging activity of *Eudrilus eugeniae* regenerated tissue was found to be higher than that of the standard. Thus the regenerating tissue of *Eudrilus eugeniae* was found to have high antioxidant potential and free radical scavenging activities. The genetic and molecular tools are addressing this regeneration event in a rapid fashion nowadays. The knowledge obtained in the field of regeneration helps in better understanding the process and has a practical impact in the field of medicine in a near future.

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