EVALUATION OF EFFECTS OF DIFFERENT EXPERIMENTAL COMPOUNDS ON MIDGUT LIPASE ACTIVITY IN *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE)

Eslam Alipour*, Reza Farshbaf Pour Abad*, Motafa Valizadeh and Davoud Mohammadi*****

* PhD Student and Professor, Department of plant protection Faculty of Agriculture, University of Tabriz, IRAN.
** Professor, Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Tabriz, IRAN.
*** Asistant Professor, Department of Plant Protection, Faculty of Science, University of Tarbiat Moallem-e-Azarbaijan, IRAN.

[Alipour, E., Farsbaf Pour Abad, R., Valizadeh, M. & Mohammadi, D. 2013. Evaluation of effects of different experimental compounds on midgut lipase activity in *Galleria mellonella* L. (Lepidoptera: Pyralidae). Munis Entomology & Zoology, 8 (1): 167-174**]**

ABSTRACT: In insects, lipase enzymes have key roles in utilizing, storing and transmitting lipids. Experimental compounds such as metal salts like potassium phosphate, magnesium nitrate and NaCl, buffers like EDTA and Tris, SDS and ethanol are commonly used in laboratory studies, and lack of knowledge to their possible effects on enzyme activity can lead to inaccurate conclusions in studies involve these enzymes. In this study, effects of these compounds on midgut lipase activity of *Galleria mellonella* that is an ideal insect for physiologic researchs for It's simple rearing and large larvae, are evaluated. In order to this, The last instar larvae were dissected, samples were obtained and the effects of these compounds on enzyme activity were measured spectrophotometrically in 5 different concentrations. As obtained results, enzyme activity was reduced by 75% ethanol, 5 mMol magnesium nitrate and various concentrations of SDS, and was increased by NaCl and potassium phosphate in 5 mMol concentration. Concentrations up to 4 mMol of NaCl, potassium phosphate and magnesium nitrate, and also concentrations of 25, 50, 85 and 96 % of ethanol had no effect on enzyme activity.

KEY WORDS: Lipase, Enzyme activity, Metal ions, Ethanol, *Galleria mellonella*.

Lipase enzymes generally are defined as triacylglycerol hydrolases (EC 3.1.1.3) that break carboxylester bonds in diacylglycerols, galactolipids and phospholipids and more specially in triacylglycerols, So, they play a key role in controlling the absorption, transport and utilization of lipids (Horn et al., 2009). In fact, lipases are a group of enzymes that hydrolyse triacylglycerols to di and monoacylglycerols with free fatty acids as coproducts (Mrdakovic et al., 2008; Sharma et al., 2001).

In addition, lipases also catalize esterification and degradation of acids, bases and amins (Hasan et al., 2009).

In insects, these enzymes have key roles in utilizing, storing and transmitting lipids and also they are important in basic physiological prosseses of reproduction, development, defending against pathogens and oxidative stress, and pheromone signalling (Horn et al., 2009). Although many researchs have been carried out on animal, microorganism and plant lipases there are little information about insect lipases and most of researchs performed on insect lipase focused on fat bodies (Orscelik et al., 2008). There has been considerable interest in the investigation on midgut enzymes since larval midgut is a possible target for insect control, as a primary interface between insect and environment (Mrdakovic et al., 2008).

Greater wax moth, *Galleria mellonella*, is present in everywhere bees are kept. Their spread in areas with low tempretures mainly is limited (Vosughi & Nabian, 1374). Most damages occur in tropical areas, because they can be active longer in this areas (Esmaeeli, 1372). This insect is used commonly in physiologic, toxicologic and pathologic investigations and also as a artificial host in mass rearing of diptera and hymenoptera, and at the same time, is almost the most important pest of bee products and makes serious losses to commercially beekeeping (Vosughi & Nabian, 1374). It's simple rearing and large larvae made it ideal insect for physiologic researchs.

Experimental compounds such as metal salts like potassium phosphate, magnesium nitrate and NaCl, buffers like EDTA and Tris, SDS and ethanol are commonly used in laboratory studies, and lack of knowledge to their possible effects on enzyme activity can lead to inaccurate conclusions in studies involve these enzymes. In this experiment, effects of these compounds on midgut lipase activity of *G. mellonella* are evaluated.

MATERIAL AND METHODS

Insect rearing

Egg masses of *G. mellonella* were prepared from a colony in department of plant protection of Tabriz university in the amount needed to form a new colony and were transferred to the rearing unit and rearing of insects was performed feeding on artificial diet prepared according to the method presented by Poinar (1975). insects purificated for 3 generations and then used in the experiments. labratory conditions in the rearing prosses were temperature of 26±2 °c, relative humidity of 50±5% and photoperiod of 16:8 (L:D).

Enzyme specimen preparation

Last instar larvae were dissected according to the method presented by Cohen (1993) in order to separate midguts for use in the experiments. removed midguts were transferred to 1.5 ml microtubes containing 1 ml cold phosphate buffer after being washed in the same buffer. microtubes were transferred to the refrigerator (-20 °c) after homogenization and kept there to be used in the next steps.

In order to preparation of enzyme specimens, the mentioned microtubes were centrifugated at 4 °c and 10^4 round/min for 5 min, then their supernatants were transferred to other microtubes and were employed in the enzyme activity assays.

Enzyme activity assays

Lipase enzyme activity was measured by related detection kit (lipase kit, pars azmun iran co.) spectrophotometrically according to the method presented by manufacturer of kit.

Evaluating effects of various compounds on enzyme activity

In order to this evaluation, various concentrations of these compounds (1-5 mMol) were prepared with distilled water and pH set at 6.5 using HCl and NaOH. Then 300 µl of each above solutions mixed with 30 µl of enzyme solution in the 0.5 ml tubes and enzyme activity was measured 5 min after mixing of solutions. 300 µl of distilled water mixed with 30 µl of enzyme solution was considered as control. Possible effects of ethanol were evaluated in 5 various concentrations 96, 85, 75, 50 and 25 % as well using distilled water as control. 50 µl of enzyme solution added to each microtubes containing 0.5 ml of ethanol and to the control and enzyme activity was measured after 10 min incubation at the room

temperature (23-25 °c). All experiments were conducted in a completely randomized design. four replications was used for each treatment and each replication included 2 midguts.

Statistical analisis

Analisis of variance was conducted using software MStat-C. mean comparisions were performed with duncan's multiple range test method using this software at levels 1 and 5 %. Excel software was used for drawing diagrams. Data normality test was also performed and appropriate data convertion was performed in cases was required.

RESULTS AND DISCUSSION

Effect of various concentrations of ethanol on enzyme activity

The effects of different concentrations of ethanol on enzyme activity showed significant differences at 5 %. Enzyme activity was reduced with decreasing concentrations of ethanol down to 75 % as ethanol 75 % made the most reduction in the enzyme activity. The enzyme activity showed an increasing trend with next reductions in ethanol concentration as ethanol 25 % had minimum effect on enzyme activity (Fig. 1).

Given that ethanol 75 % among different concentrations of ethanol releases the most oH into environment and released oH reduces enzyme activity, results of this study that show the most reduction in the enzyme activity for ethanol 75 % can be justified.

There are little investigations in this field. In the study of Castro-ochoa et al. (2005) on properties of lipase enzyme produced by bactria, *Bacillus thermoleovorans* CCR11 the enzyme activity was consistent in the presence of organic solvents except butanol.

Effect of various concentrations of NaCl on enzyme activity

Significant differences was obtained between the effects of different concentrations of NaCl on enzyme activity. Concentrations of 1, 2, 3 and 4 mMol NaCl did not show any significant effect on enzyme activity but 5 mMol NaCl increased enzyme activity significantly (Fig. 2).

Effect of metal ions on various enzymes have been studied by many researchers. Metal ions can enhance or reduce enzyme activity depending on its type (Hasan et al., 2009). Metal ions generally form complexes with ionized fatty acids, changing their solubility and behavior at interfaces. Release of fatty acids to the medium is rate determining and could be affected by metal ions. However, the effects of metal ions depend on particular lipase (Hasan et al., 2009).

In the study of Zibaee et al. (2008) on lipase enzyme activity of midgut and salivary glands of *Chilo suppressalis*, adding NaCl reduced activity of this enzyme. In the study performed by Grillo et al. (2007) on lipid metabolism and midgut triacylglycerol lipase role in *Rodnius prolixus*, activity of this enzyme was affected by NaCl concentration as it was more activated by increasing its concentration. Studies of Hiol et al. (1999) on properties of extracellular lipase of mold *Hiemalis f. hiemalis* showed that Na^+ ion enhances activity of this enzyme. In the investigations of Kumar et al. (2005) on properties of lipase of bacteria *Bacillus coagulans* BTS-3 was shown that Na^+ ion has not any effect on its activity.

Effect of various concentrations of Tris on enzyme activity

Figure 3 shows comparision of means and change trend of effects of this treatment. Difference between effects of various concentrations of Tris on enzyme activity was insignificant.

Effect of various concentrations of potassium phosphate on enzyme activity

Effects of different concentrations of potassium phosphate on enzyme activity had significant differences at 5 %. Concentrations up to 4 mMol had not any significant effect on enzyme activity, although they had an increasing trend, while concentration of 5 mMol significantly increased enzyme activity (Fig. 4).

Results of this study showed some differences and similarities compared to previous researches in this field. In the work of Zibaee et al. (2008) on midgut and salivary gland lipase activity in *C. suppressalis* it was showed that its activity was decreased in presence of KCl which released K^+ ion after being ionized. Researches of Kumar et al. (2005) on lipase properties of bactria *Bacillus coagulans* BTS -3 showed that K^+ ion increases its activity.

Effect of various concentrations of magnesium nitrate on enzyme activity

Effects of different concentrations of magnesium nitrate on enzyme activity showed significant differences at 1%. Concentrations up to 4 mMol had not any significant effect on enzyme activity while it's 5 mMol concentration decreased enzyme activity significantly (Fig. 5).

There are considerable differences between the studies of other researchers. In the work of Zibaee et al. (2008) on midgut and salivary gland lipase of *C. suppressalis* Mg^{2+} ion released from magnesium nitrate decreased this enzyme's activity. In the studies of Hiol et al. (1999) on properties of extracellular lipase of *Hiemalis f. hiemalis* was shown that Mg^{2+} ion increases enzyme activity. Investigations of Kumar et al. (2005) on properties of lipase of bactria *B. coagulans* BTS-3 showed that Mg^{2+} ion increases it's activity. In the researches performed by Cote & Shareck (2008) on two lipase enzyme produced by bactria *Streptomyces coelicolor* was not shown any significant reduction by Mg^{2+} ion on their activity. In the work of Karadzic et al. (2006) on properties of alkalin lipase produced by *Pseudomonas aeruginosa* was shown that this enzyme is inhibited slightly by Mg^{2+}.

Effect of varius concentrations of SDS on enzyme activity

Effects of different concentrations of SDS on enzyme activity showed significant differences at 1%. Enzyme activity has a decreasing trend with increase in SDS concentration. There was not any significant difference between SDS concentration of 1 mMol and control and also between its concentrations 4 and 5 mMol (Fig. 6).

SDS, sodium dodecyl sulphate, is an anionic surfactant with chemical formula of $C_{12}H_{25}SO_4Na$ that has amphipatic property because of carrying 12 carbon bound to sulphate group, and can act as detergent. Since the lipase enzymes act at the water–lipid interface, presence of a detergent in the environment can affect enzyme activity.

Most of previous studies performed in this case show reduction in lipase activity in presence of SDS like results of this study. In the study of Zibaee et al. (2008) it was found that relative enzyme activity is reduce by SDS. In the investigations of Castro-Ochoa et al. (2005) on properties of lipase produced by

bactria *Bacillus thermoleovorans* CCR11 it was shown that SDS had inhibitory effect on this enzyme activity. Studies of Hiol et al. (2000) on properties of extrasellular lipase produced by bactria *Rhizopus oryzae* showed that this enzyme activity was inhibited by SDS. During study of Wang et al. (2009) on properties of lipase of *Bukholderia cepacia* ATCC25416 inhibition of this enzyme activity found in presence of SDS. The results of study of Yu et al. (2007) on properties of lipase 2 of *Yarrowia lipolitica* also indicated prohibition of enzyme activity by SDS. In the researches of Demir & Tukel (2010) on properties of lipase of *Spirulina platensis*, enzyme activation was shown in presence of SDS.

Effect of various concentrations of EDTA on enzyme activity

There were no significant differences between the effects of different concentrations of EDTA on enzyme activity (Fig. 7).

EDTA, ethylene di amin tetra acetic acid, is a chelating agent of metals that removes metal ions from compounds carrying them. thus, being insignificant of reduction made by this compound on lipase activity can be an evidence on that there is no need to presence of metal ions at the catalytic site of this enzyme.

The results of most studies in this field also indicates no effect of EDTA on enzyme activity. In the study of Hiol et al. (1999) it was found that relative enzyme activity was not affected by EDTA. This not being affected of enzyme activity by EDTA was also resulted in the study of Gaur et al. (2008). In the mentioned research of Wang et al. (2009), inhibitory effect of EDTA on enzyme activity was resulted. Yu et al. (2007) through investigation on properties of lipase 2 of *Y. lipolitica* showed that EDTA has no effect on enzyme activity. Study of Li & Zhang (2005) on properties of lipase of bactria *Geobacillus* sp.. TW1 also showed that this enzyme was stable in presence of EDTA. The results of mentioned study performed by Demir & Tukel (2010) indicated that relative enzyme activity was inhibited by EDTA.

LITERATURE CITED

Castro-Ochoa, L. D., Rodriguez-Gomez, C., Valerio-Alfaro, G. & Ros, R. O. 2005. Screening, purification and characterization of the thermoalkalophilic lipase produced by *Bacillus thermoleovorans* CCR11, Enzyme and Microbial Technology, 37: 648-654.

Cohen, A. C. 1993. Organization of digestion and preliminary characterization of salivary trypsin-like enzymes in a predaceous heteropteran, *Zelus renardii*. Journal of Insect Physiology, 39: 823-829.

Cote, A. & Shareck, F. 2008. Cloning, purification and characterization of two lipases from *Streptomyces coelicolor* A3(2). Enzyme and microbial technology, 42: 381-388.

Demir, B. S. & Tukel, S. S. 2010, Purification and characterization of lipase from *Spirolina platensis*. Journal of Molecular Catalysis B: Enzymatic, 64: 123-128.

Esmaeeli, M. 1993. Honey bee rearing, honey production and use in pollination (translation), sepehr publishing, 264 pp.

Gaur, R., Gupta, A. & Khare, S. K. 2008. Purification and characterization of lipase from solvent tolerant *Pseudomonas aeruginosa* PseA. Process Biochemistry, 43: 1040-1046.

Grillo, L. A. M., Majerowicz, D. & Gondim, K. C. 2007, Lipid metabolism in *Rhodnius prolixus* (Hemiptera: Reduviidae): Role of a midgut triacylglycerol-lipase. Insect Biochemistry and Molecular Biology, 37: 579-588.

Hasan, F., Ali Shah, A. & Hameed, A. 2009. Methods for detection and characterization of lipases. Biotechnology Advances, 27: 782-798.

Hiol, A., Jonzo, M. D., Druet, D. & Comeau, L. 1999. Production, purification and characterization of an extracellular lipase from *Mucor hiemalis f. hiemalis*. Enzyme and Microbial Technology, 25: 80-87.

Horne, I., Haritos, V. S. & Oakeshott, J. G. 2009, Comparative and functional genomics of lipases in holometabolous insects. Insect Biochemistry and Molecular Biology, 39: 547-567.

Karadzic, I., Masui, A., Zivkovic, L. I. & Fujiwara, N. 2006. Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of metalworking fluid. Journal of Bioscience and Bioengineering, 102 (2): 82-89.

Kumar, S., Kikon, K., Upadhyay, A., Kanwar, S. S. & Gupta, R. 2005. Production, purification, and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. Protein Expression and Purification, 41: 38-44.

Li, H. & Zhang, X. 2005. Characterization of thermostable lipase from thermophilic *Geobacillus sp*. TW1. Protein Expression and Purification, 42: 153-159.

Mrdakovic, M., Lazarevic, J., Peric-Mataruga, V., Ilijin, L. & Vlahovic, M. 2008. Partial characterization of a lipase from gypsy moth (*Lymantria dispar* L.) larval midgut. Folia Biologica (Krakow), 56 (1-2): 103-110.

Orscelik, O., Akpinar, M. A. & Gorgun, S. 2007. Partial purification of total body lipase from *Gryllus campestris* L. (Orthoptera: Gryllidae). Fen Bilimleri Dergisi, 28 (2): 1-10.

Poinar, G. O. 1975. Entomogenous Nematodes, Leiden, Netherlands, E. J. Brill, 316 p.

Sharma, R., Chisti, Y. & Banerjee, U. C. 2001. Production, purification, characterization and application of lipases. Biotechnology advances, 19: 627-662.

Vosoughi, G. & Nabian, S. 1995. Honey bee and its pests, predators and pathogens (translation). Tehran university publishing, 432 pp.

Wang, X., Yu, X. & Xu, Y. 2009. Homologous expression, purification and characterization of a novel high-alkaline and thermal stable lipase from *Burkholderia cepacia* ATCC 25416. Enzyme and Microbial Technology, 45: 94-102.

Yu, M., Qin, S. & Tan, T. 2007. Purification and characterization of the extracellular lipase lip2 from *Yarrowia lipolytica*. Process Biochemistry, 42: 384-391.

Zibaee, A., Bandani, A. R. & Ramzi, S. 2008, Lipase and invertase activities in midgut and salivary glands of *Chillo suppressalis* (Walker) (Lepidoptera, Pyralidae). Rice striped stem borer, ISI, 5: 180-189.

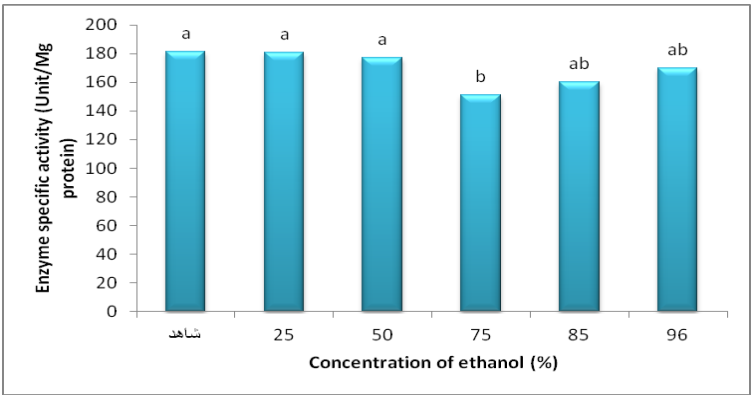

Figure 1. Change trend of effects of different concentrations of ethanol on enzyme activity and mean comparisions (different letters indicate significant differences).

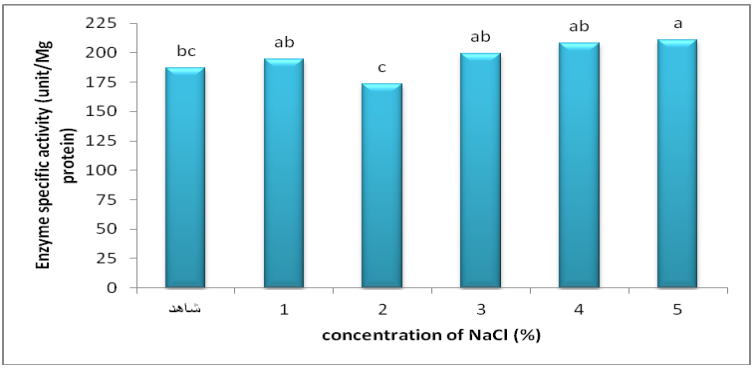

Figure 2. Trend of effects of different concentrations of NaCl on enzyme activity and mean comparisions (different letters indicate significant differences).

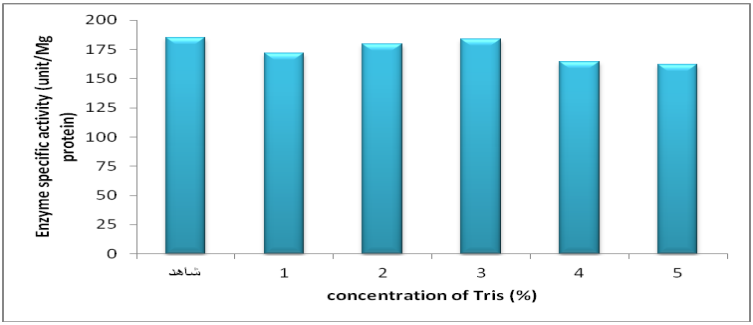

Figure 3. Trend of effects of different concentrations of Tris on enzyme activity and mean comparisions (different letters indicate significant differences).

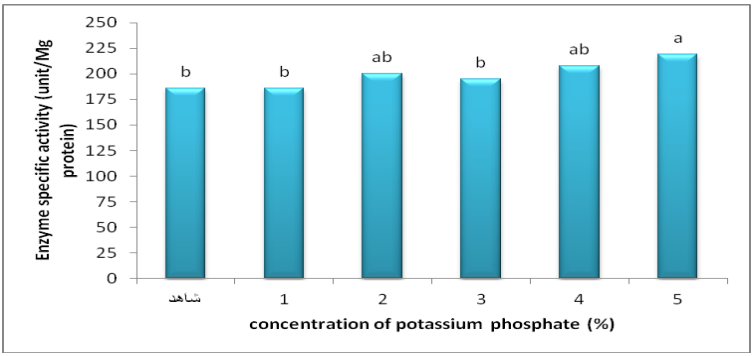

Figure 4. Trend of effects of different concentrations of potassium phosphate on enzyme activity and mean comparisions (different letters indicate significant differences).

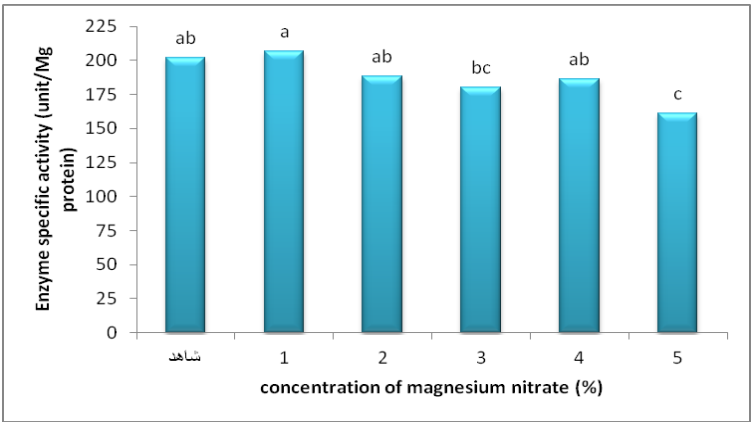

Figure 5. Trend of effects of different concentrations of magnesium nitrate on enzyme activity and mean comparisions (different letters indicate significant differences).

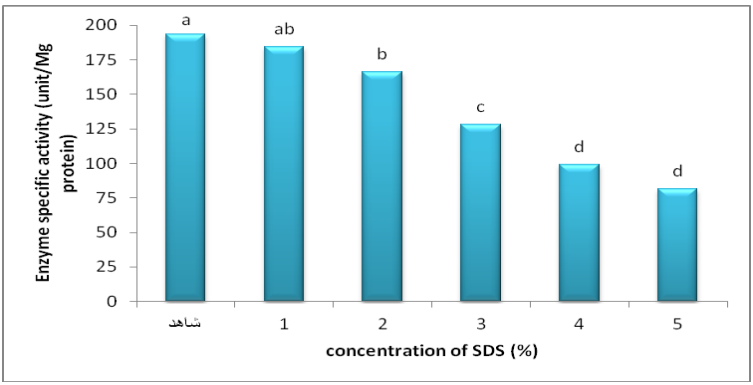

Figure 6. Trend of effects of different concentrations of SDS on enzyme activity and mean comparisions (different letters indicate significant differences).

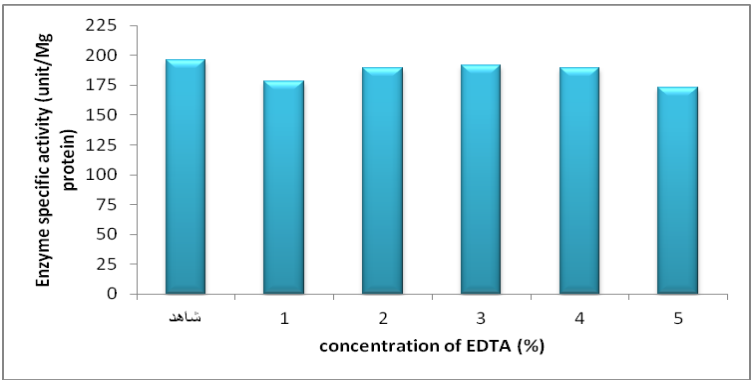

Figure 7. Trend of effects of different concentrations of EDTA on enzyme activity and mean comparisions (different letters indicate significant differences).