# Morphological characters, antioxidant defences and oxidative stress in the lizard *Ophisops elegans* at different altitudes

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## Abstract

In the wild, animals living at different altitude environment face different stressors, accordingly generating clines for several traits and biochemical changes with altitude. The current study was conducted to examine various morphological traits and biochemical biomarkers in field population of *Ophisops elegans* at three different altitudes. Biomarkers included antioxidant enzymatic activity of glutathione peroxidase, superoxide dismutase, total antioxidant capacity (TAC) and lipid peroxidation (malondialdehyde) in bran, liver and tail tissue samples. Results show that lizards from highlands are larger than those from lowland. Lipid peroxidation in lowland lizards was significantly increased in all three brain, liver and tail tissues. Total antioxidant capacity in the liver and glutathione peroxidase activity in tail were much higher in lowland than those in highland. However, the activity of superoxide dismutase was similar among three elevations. We observed significant negative correlation between snout-vent length and lipid peroxidation in the brain and tail. Total antioxidant capacity in liver and glutathione peroxidase in

tail also recorded a negative correlation with snout-vent length. By contrast, total antioxidant capacity in liver was positively correlated with lipid peroxidation. These results clearly suggest that oxidative stress and antioxidant defenses in *Ophisops elegans* varies in response to elevation.

## 1. Introduction

Environmental stressors that affect wildlife come in many forms including hypoxia (9), extreme low or high temperature (15, 34), Environmental contaminants (17), food limitation (30)) and intense ultraviolet radiation (27). It is now well known that the cumulative number of stress events varied with the altitude and therefore, animals living at different altitudes face different oxidant environment. For animals that live at sever oxidant environment, survival requires possessing certain characteristics and behaviors that allow them to adapt to the environmental challenges. In living organisms, cells can generate harmful substances called free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS) from endogenous and exogenous sources (6). Excess of free radicals can lead to cumulative damage in proteins, lipids, and DNA (7). In order to maintain the delicate balance of free radicals generation and minimize oxidant damages, biological systems display a network of enzymatic and non-enzymatic antioxidants defenses (11, 32). Oxidative stress occurs when the productions of oxidants overwhelm antioxidants (19). In fact, oxidative stress emerges from an enhanced reactive oxygen and nitrogen species generation or from a decay of the antioxidant protective ability, being characterized by the reduced capacity of endogenous systems to fight against the oxidative attack directed towards target biomolecules (25). During recent years, researchers have taken to studying oxidative stress in

field population of lizards because it has been proposed as a major challenge for the survival of lizards living at different altitudes (28).

The snake-eyed lizard, *Ophisops elegans*, 1832 or field lizard, is widely distributed throughout the Eastern Mediterranean region and Southwestern Asia and North Africa (14). In Iran, this ground-dwelling lizard is considered as one of the most abundant lacertid lizards, mainly distributed along the Mid-Zagros area at different altitudes from 1000 to 2000 meters above sea level, m a.s.l. (1). Although some information is available on reproductive cycle (33), sexual dimorphism (22), digestive characteristics (3) and distribution pattern (21) but, there are no report of characterization of oxidative stress and antioxidant defenses in *Ophisops elegans* in Iranian Plateau. This could be essential, because it is not clear nor reported whether adaptation of this lizard to different oxidant environments is mediated by changes in oxidative damages and antioxidants status. The main goal of this study was thus, to test the hypothesis that the levels of oxidative stress and antioxidant capacity varies with altitude in *Ophisops elegans*.

## 2. Material and methods

### *2.1. Study site*

Samplings were performed in western regions of the Iranian Plateau, western slopes of the Mid-Zagros Mountain during a two weeks period of October 2016. Three sample sites were situated at different altitudes; Sarv-Abad (35° 31′ N and 46° 36′ E and 1134 m a.s.l.), Hassan-Abad (35° 24′ N and 46° 92′ E and 1995 m a.s.l) and Mahidasht (34° 14′ N and 46° 83′ E and 1907 m a.s.l.).

### *2.2. Animal sampling and procedures*

Eighteen adult male lizards (*Ophisops elegans*, six from each location) were caught by hand and transported to the nearest laboratory in each location, 20-30 Km far from the place of capture. Individuals with SVL longer or equal to 40 mm were considered adult. The lizard sex was characterized based on femoral pores which were bigger in males. This experiment and all the experimental procedures were approved by the animal ethical committee of Razi University. The animals were sacrificed with an overdose of ether and measurement was carried out on metric traits; snot-vent length, (SVL), tail length (TL), length of between forelimb and hindlimb (LHF), head length (HL), Head width (HW), length of forelimb (LFL), length of hindlimb (LHL), length of eye (LE); width of cloaca (LV) and meristic traits; number of supralabial (NSL), number of inferalabial (NIL), subdigital lamella under of forth toe (SDLT), number of ventral scales (NVS), number of dorsal scales (NDS) number of gular scales (NGS) femoral pores (FP) and subdigital lamella under of forth finger (SDLF). Metric traits were measured by using a digital caliper (accuracy 0.01 mm) and meristic traits were examined through a dissecting microscope. Then, the lizards were quickly dissected out and tissue samples; tail, brain and liver were taken and frozen in liquid nitrogen until carried to the laboratory at Razi University, where they were kept at -80 ºC for biochemical analysis.

*2.3. Biochemical analyses*

To measure cytosolic enzyme activity, total antioxidant capacity, lipid peroxidation (MDA) and soluble protein concentrations, the frozen tail, brain and liver samples were thawed and homogenized in 1.15% KCl solution. Homogenates were centrifuged at -40 °C for 15 minutes and supernatants were stored at -80°C until used for measuring of the biochemical parameters. Glutathione peroxidase (GPx)

activity was measured according to Paglia and Valentine (23), using Randox (United Kingdom). GPx was catalyzed by the oxidation of reduced glutathione in the presence of cumene hydroxyperoxide.

Tissue superoxide dismutase (SOD) activity was determined as described by Sun et al. (31). This method depends on the inhibition of nitroblue tetrazolium (NBT) reduction by xanthine-xanthine oxidase used as a superoxide generator. SOD activity was expressed as the amount of enzyme that causes 50% inhibition of the rate of NBT reduction. GPX and SOD activity was designated as unit for mg/protein of tissue.

Malondialdehyde (MDA) levels as product of lipid proxide degradation were measured using the thiobarbituric acid reactive substances (TBARS) method (18).

The soluble protein concentration was determined by Bradford protein assay reagent (2), using albumin as the standard to estimate the enzymatic specific activity.

Quantitative determination of TAC was measured in tissue supernatant using Randox Total Antioxidant Capacity kit (cat No. 2331), according to the method of Miller et al. (18).

### *2.4. Statistical analyses*

The statistical analysis was performed by one-way ANOVA. All values were expressed as means ± standard error (s.e.). Differences in morphometric characters, oxidative stress biomarker (MDA), total antioxidant capacity (TAC) and antioxidant enzymatic activity (GPX and SOD) between animals from three different sampling site analysed by Tukey's test. Pearson's correlation analyses were used to determine the correlation among oxidative stress and antioxidant biomarkers in brain, liver and tail tissues, themselves and with SVL as an

indication of body size of the lizards separately. Test values with a $P < 0.05$ were considered significantly different. All data analyses were performed using Minitab Statistical software (version 17.1, 2013).

## 3. Results

The values for morphometric characters of the lizards are presented in Table 1. Except for the SVL, no significant differences were observed in other characters. The snout-vent length (SVL) of male *O. elegans* in different sites varied from 46.0 to 52.8 mm. Lizards had larger SVL at 1907 and 1995 m a.s.l. (MA and HA) than those at 1134 m a.s.l. (SA, $P<0.05$). However, SVL was similar between lizards at MA and HA.

We found significant differences in lipid peroxidation (MDA concentration) in all three tissues (brain, liver and tail) with altitude (Fig1, a). The MDA contents of brain, liver and tail tissues in lizards at low altitude (SA) were significantly higher ($P<0.01$, $P<0.05$ and $P<0.001$ respectively) compared with those at both high altitudes (MA and HA), but MDA contents of all three tissues were similar between both high elevations (MA and HA). In the case of TAC, only value for liver tissue was significantly ($P<0.005$) different among the lizards at different altitudes (Fig1, b). In liver the level of TAC for lizards at low altitude (SA) was more than two fold higher than those for lizards at both high altitudes (MA and HA). GPX and SOD enzymes but GPX in tail tissue had similar activity in all three tissues of lizards from different altitudes (Fig1, c and d).

As shown in Table 2, MDA concentration was negatively correlated to the SVL, although only correlations in brain and tail tissues were significant ($P<0.05$ and $P<0.05$ respectively). Likewise, there was a significant negative correlation between SVL and TAC in liver tissue ($P<0.05$). By contrast GPX and SOD activity

was not significantly correlated with SVL, excepting GPX in tail tissue, which was negatively correlated with SVL ($P$<0.05). Furthermore, in tail tissue, GPX and SOD activity was positively correlated to MDA concentration ($P$<0.001 and $P$<0.05). There was no significant correlation between TAC and any enzyme activity.

## 4. Discussion

To the best of our knowledge, this is the first report on investigating oxidative stress in the lizard at in Iranian Plateau. This could be essential, because it is not clear nor reported whether adaptation of this lizard to different oxidant environments is mediated by changes in oxidative damages and antioxidants status. In the wild, animals living at different altitude environment face different stressors that can amplify the generation of reactive oxygen species (ROS) in the body causing serious damage to macromolecules of organism such as nucleic acids, proteins and lipids (16). The severity of stressors increases with elevation (4). Thus, native species who live at high altitude must possess certain adaptive characteristics and behaviors including changes in free radical metabolism that make them more tolerant to oxidant environment and provide the means to survive (13).

The current study has highlighted several important findings and indicated that SVL, as an indicator of body size, the level of oxidative stress and antioxidant statues of *Ophisops elegans* varied with elevation. Lizards at high altitude showed larger SVL. Larger body size at higher altitude has been reported in other lizards (28). Since metabolic and thus, free radical production rates are higher in smaller organisms (29), the level of oxidative stress should be higher in the lizards from

lowland. Under these circumstances Lipids are susceptible targets of oxidation because of their molecular structure abundant with reactive double bonds (26). Two of the most well studied markers of lipid peroxidation are isoprostanes (IsoPs) and malondialdehyde (12). In this study malondialdehyde levels were determined as product of lipid peroxide degradation. We found that levels of lipid peroxidation increased in all three brain, liver and tail tissues of lizards at low altitude, suggesting an increase of oxidative damage. Although, overall increasing MDA negatively correlated with SVL, however considering each elevation separately, no significant correlation observed between SVL and MDA levels. This may indicate that higher level of oxidative stress in lizards from lowland is not due to smaller body size. This result can be explained by the fact that the activities of ectotherms are greatly depend on the environmental temperature for thermoregulation (8). At low elevation, higher environmental temperature increases metabolic rate and thus, oxidative stress increases in ectotherms (10). Moreover, the increase of oxidative damage may be the result of increased free radical production or decreased antioxidant defenses induced by more contaminants at low altitude (20). Consistent with our results, previous study reported that lizards from high elevation have less oxidative stress levels than those from low elevation (28). They suggested that highland environment are less stressful than lowlands. In the current study, TAC in liver was higher in the lowland lizards and positively correlated with MDA. Oxidative stress represents an imbalance between antioxidant agents and increased free radical production. In liver proteins, lipids and DNA are among the cellular structures that are primarily affected by free radicals. The process results in structural and functional abnormalities in the liver and liver injury mediated by oxidative stress (4). Our data demonstrated a positive relationship of TAC and MDA in liver. Furthermore, we observed a significant increase in GPX activity in tail tissue of lizards at low altitude and positive relationship of both GPX and SOD

in tail tissue with MDA. These results reveal that oxidative stress response in liver and tail tissues might help the lizard to adopt a relevant strategy with respect to the stressor.

## 5. Conclusion

Consistent with our original hypothesis this study shows that the levels of oxidative stress and antioxidant capacity varies with altitude in *Ophisops elegans*. Lizards from the lowland population have more oxidative stress levels than those from high-altitude population. In addition, we found that increasing oxidative stress in the lizards was associated with a markedly increase of TAC in the liver. These results suggest that *Ophisops elegans* can develop certain adaptability to different oxidant environment.


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## Conflict of interest statement

The authors declare there is no conflict of interests to disclose.

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**Table 1.** Metric (mm) and meristic (number) characteristics for the lizard *Ophisops elegans* at different altitude (SA, Sarvabad, 1134 m a.s.l.; HA, Hassan abad, 1995 m a.s.l.; MA, Mahidasht, 1907 m a.s.l.).

| | SA | HA | MA | *P*-value |
|---|---|---|---|---|
| SVL | 48.15 ± 1.01 | 51.13 ± 0.41 | 51.19 ± 0.63 | 0.021 |
| TL | 90.16 ± 0.55 | 95.35 ± 2.14 | 94.71 ± 2.61 | 0.276 |
| LHF | 23.61 ± 0.34 | 22.18 ± 0.86 | 22.68 ± 0.76 | 0.462 |
| HL | 13.67 ± 1.34 | 14.07 ± 0.83 | 13.76 ± 0 .46 | 0.944 |
| HW | 7.07 ± 0.40 | 7.23 ± 0.28 | 7.81 ± 0.50 | 0.448 |
| LFL | 15.11 ± 0.82 | 16.75 ± 0 .86 | 15.98 ± 0 .54 | 0.371 |
| LHL | 29.13 ± 1.93 | 28.28 ± 1.37 | 29.20 ± 1.35 | 0.887 |
| LE | 2.13 ± 0 .12 | 2.03 ± 0 .14 | 2.08 ± 0 .09 | 0.845 |
| LV | 5.34 ± 0.28 | 4.77 ± .0.42 | 4.84 ± 0.23 | 0.493 |
| NSL | 7.67 ± 0.33 | 8.00 ± 0.00 | 8.00 ± 0.00 | 0.289 |
| NIL | 7.67 ± 0.33 | 8.00 ± 0.00 | 8.00 ± 0.00 | 0.289 |
| SDLT | 23.33 ± 1.45 | 22.75 ± 0.85 | 23.75 ± 1.18 | 0.814 |
| NVS | 40.67 ± 0.88 | 40.25 ± 0.85 | 40.50 ± 0.64 | 0.935 |
| NDS | 23.67 ± 0.67 | 23.75 ± 0.48 | 23.75 ± 0.48 | 0.993 |
| NGS | 18.67 ± 0.33 | 18.50 ± 0.29 | 18.5 ± 0.29 | 0.914 |
| FP | 10.33 ± 0.33 | 10.25 ± 0.25 | 10.5 ± 0.29 | 0.812 |
| SDLF | 17.33 ± 0.88 | 17.25 ± 0.0.48 | 17.25 ± 0.48 | 0.994 |

SVL, snot-vent length; TL, tail length; LHF, length of between forelimb and hindlimb; HL, head length; HW, Head width; LFL, length of forelimb ; LHL, length of hindlimb; LE, length of eye; LV, width of coloac; NSL, number of supralabial; NIL, number of inferalabial; SDLT, subdigital lamella under of forth toe; NVS, number of ventral scales, NDS, number of dorsal scales; NGS, number of gular scales; FP, femoral pores; SDLF, subdigital lamella under of forth finger. Table shows mean values and standard error (±SE) and signification value (*P*-value).

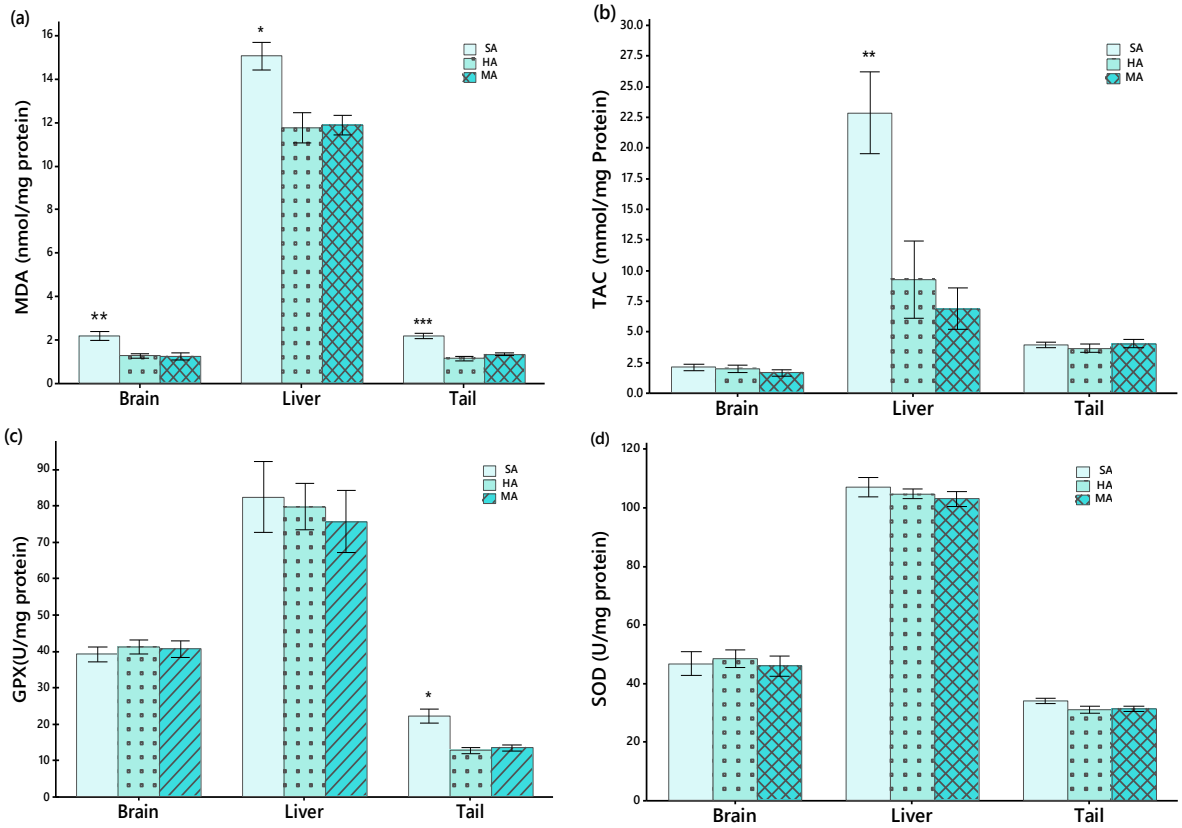


**Fig 1.** The levels of MDA, malondialdehyde **(a)**; TAC, total antioxidant capacity **(b)**; GPX, glutathione oxidase **(c)**; SOD, super oxide dismutase **(d)** in brain, liver and tail tissues for the lizard *Ophisops elegans* at different altitude (SA, Sarvabad, 1134 m a.s.l.; HA, Hassan abad, 1995 m a.s.l.; MA, Mahidasht, 1907 m a.s.l.). Figure shows mean values ± standard error (SE) and signification value, **P*<0.05; ***P*<0.01; ****P*<0.001.

**Table 2.** The correlation (Spearman's correlation) coefficients among SVL, MDA (nmol/mg protein), TAC (mmol/mg protein), GPX (U/mg protein) and SOD (U/mg protein) in brain, liver and tail tissues for the lizard *Ophisops elegans*

| | SVL | MDA | TAC | GPX |
|---|---|---|---|---|
| In brain tissue: | | | | |
| MDA | $r$ = -0.71 | | | |
| | 0.02* | | | |
| TAC | $r$ = -0.35 | $r$ = 0.04 | | |
| | 0.32 | 0.88 | | |
| GPX | $r$ = 0.63 | $r$ = -0.07 | $r$ = -0.29 | |
| | 0.05 | 0.81 | 0.30 | |
| SOD | $r$ = 0.00 | $r$ = 0.00 | $r$ = 0.47 | $r$ = 0.03 |
| | 0.99 | 0.99 | 0.09 | 0.91 |
| In liver tissue: | | | | |
| MDA | $r$ = -0.56 | | | |
| | 0.09 | | | |
| TAC | $r$ = -0.80 | $r$ = 0.71 | | |
| | 0.01* | 0.01* | | |
| GPX | $r$ = -0.14 | $r$ = -0.17 | $r$ = -0.26 | |
| | 0.70 | 0.55 | 0.40 | |
| SOD | $r$ = -0.02 | $r$ = 0.38 | $r$ = 0.09 | $r$ = 0.07 |
| | 0.52 | 0.18 | 0.77 | 0.80 |
| In tail tissue: | | | | |
| MDA | $r$ = -0.74 | | | |
| | 0.01* | | | |
| TAC | $r$ = -0.18 | $r$ = 0.26 | | |
| | 0.62 | 0.37 | | |
| GPX | $r$ = -0.67 | $r$ = 0.81 | $r$ = -0.04 | |
| | 0.03* | 0.00** | 0.89 | |
| SOD | $r$ = -0.17 | $r$ = 0.58 | $r$ = 0.27 | $r$ = 0.53 |
| | 0.64 | 0.03* | 0.34 | 0.05 |

SVL, snot-vent length; MDA, malondialdehyde; TAC, total antioxidant capacity; GPX, glutathione peroxidase; SOD, superoxide dismutase. *P* values are given below the correlation coefficients. *significant at $P<0.0$, ** significant at $P<0.01$.