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# DNA Damage, Total Antioxidant and Oxidant Status in Gunshot Wounded Wild Falcons

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**ABSTRACT**

This study was aimed to evaluate the status of total oxidant, antioxidant capacity and DNA damage caused by gunshot for wild falcons. Wounded and debilitated buzzards (buteo family) (n=20) were examined having different types of injuries caused by gunshot and other minor accidents. Buzzards were divided in to two groups, having gunshot wounds (n=10) and other kept as control group (n=10) which were having mild injuries and debilitated condition due to minor accidents.Total oxidant (TOS) and total antioxidant status (TAS) levels were determined using Rel Assay Diagnostic kit and DNA damage was determined by comet assay. There was significant ($P < 0.05$) increase in TOS and total DNA damage while non-significant ($P > 0.05$) difference was in TAS was observed in gunshot wounded falcons as compared to control group. Oxidative stress has recently been examined together with DNA damage in mammals. However this combined evaluation has never been found in wild raptors in previous literature. Therefore, this study is known to be the first report for TAS- TOS and DNA damage for wild gunshoted falcons.

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**Authors' Contribution**
**MVY and IHC designed the study and collected samples. MVY and MK examined and treated the falcons. IHC, MK and MMI executed the experimental work. IHC statistically analyzed the data. MVY and MMA wrote the article.**

**Key words**
**Buzzards, Comet assay, DNA damage, Gunshot Oxidative stress, Wild falcons**

## INTRODUCTION

Wild raptor birds are more sensitive to many aspects of physiological stress. The natural habitats of these creatures are usually sky and open spaces. Arresting and keeping them in captivity for various reasons can lead to high metabolism and stress (Coles, 2007). This increased metabolic rate and stress can change the oxidant and antioxidant status of the wild birds (McGraw, 2011). Antioxidants play a critical role on the physiology and aging process (Perez-Campo *et al*., 1993; Lopez-Torres *et al*., 1993). Besides, the lifetime of the bird is shown to be directly related to the antioxidant capacity (Ogburn *et al*., 2001; Montgomery *et al*., 2012). Antioxidants are also involved in the immune system by lysis of foreign cells (McGraw, 201; Konjufca *et al*., 2004). Free-radicals that are produced in tissue mitochondria during respiration or via immune system to fight infectious agents also damage tissues which eventually leads to cancer, atherosclerosis, and other pathologies (Graff *et al*., 1999). Measuring free radical balance could be an indicator of a future accumulation versus current production and higher metabolism of radicals may result in tissue damage.

The balance between oxidants and antioxidant status describes the level of oxidative stress, which is ultimately associated to aging, life span and fitness (Finkel and Holbrook, 2000). Environmental changes may have an adverse effect on organisms, giving rise to DNA damage by exposure to toxic substances (Dhawan *et al*., 2009).

Alkaline single cell gel electrophoresis, commonly called as Comet assay (CA), is a highly sensitive genotoxicity test used in the diagnosis of broad spectrum DNA damage in living organisms like earthworms (Ciğerci *et al*., 2016) and aquatic ecosystems (Doğu *et al*., 2015) which is simple and also considered to be a quick method (Yildiz *et al*., 2009). In this regard, by the existence of a unique wildlife, it becomes more important to work in the direction of the wilderness. Keeping in view above mentioned different factors, it is hypothesized that man related activities like gunshot may have adverse effects on the life of wild birds. Evaluation of gunshot wound injuries of wild birds in terms of oxidative stress has not been conducted so far. So, current study was carried out for the evaluation of total oxidant, antioxidant capacity and DNA damage status of gunshot wounded wild falcons.

## MATERIALS AND METHODS

Wild raptor (*Buteo* species) (n=20) were brought from the Provincial Directorate of Environment and Forestry for medical treatment to the Veterinary Hospital

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of Afyon Kocatepe University. *Buteo* species were divided in to two groups, having gunshot wounds (n=10) and other kept as control group (n=10) which only were having mild injuries and debilitated condition. Control group was made just to compare the intensity of DNA damage with the gunshot wounded flacons. General health conditions were examined and radiographical images were made for further exploration of injuries (Tables I and II). Vital parameters recorded and 2 ml blood samples were collected from ulnar cutaneous vein in to heparinized lithium vacutainers (Vacutest 4 ml, Li.Heparin 95 I.U., Kima-Arzergrande, Italy). Blood samples were stored at 4 °C for transport to the laboratory. DNA damage, total oxidant and antioxidant capacity were evaluated. Permission was obtained both from Forest Ministry of Turkey, Wildlife Conservation Department and Ethical Board of Animal Practices in Afyon Kocatepe University under the registry number: 49533702/123.

*Measurement of total oxidant and total antioxidant status*

Total oxidant (TOS) and total antioxidant status (TAS) levels were determined using Rel Assay Diagnostic kit RL0024 and RL0017 (Total Oxidant Kit, 3. Generation Antioxidant Assay Kit, Mega Tıp Korea, Gyeonggi-do, KOREA). In this assay, ferrous ion solution was mixed with hydrogen peroxide. Antioxidative effect of the samples for the potent free radical reactions were measured.

Oxidants present in the sample, oxidized the ferrous ion-o-dianisidine complex in to ferric ion. In the acidin medium, the ferric ion changes into a colored complex. The color intensity is directly proportional to the total amount of oxidant molecules present in the sample, which later was measured spectrophotometrically. TOS and TAS were analysed spectrophotometrically by reading at 530 nm and 660 nm, respectively. TOS and TAS were calculated as follows:

$$TOS = (\Delta \text{ Abs.samples} / \Delta \text{ Abs.standard2}) \times 20$$

Δ, ΔAbs.2- ΔAbs.1; Sample Absorbance, (Second Absorbance of Sample - First Absorbance of Sample); Δ Absorbance Standard 2, (Second Absorbance of Std 2 - First Absorbance of Std 2);

$$TAS = [(\Delta Abs.standard1) - \Delta Abs.sample)]/[(\Delta Abs.standard1) - (\Delta Abs.standard2)];$$

Δ, Abs.2-Abs.1; Δ Absorbance Standard1, (Second Absorbance of Std1- First Absorbance of Std1); Δ Absorbance Standard2, (Second Absorbance of Std2- First Absorbance of Std2); Δ Sample Absorbance, (Second Absorbance of Sample- First Absorbance of Sample).

*Comet assay*

Comet assay (CA) test method was used for the evaluation of DNA damage status with the following modifications (Kocyigit *et al*., 2005; Sokolovic *et al*., 2007).

Single-cell suspensions were prepared by the dilution of whole blood with phosphate buffered saline (PBS) and were utilized at once. Blood samples were processed similarly at the same time. Then, 5 µl whole blood samples were mixed with 100 µl of 0.5% low-melting agarose in PBS at 37°C. Subsequently, 80 µl of this mixture was layered onto a slide pre-coated with a thin layer of 1% normal melting point agarose (NMA), covered immediately with a coverslip and stored for 5 min at 4°C to allow the agarose to solidify. After removing the cover-slips, the slides were immersed in freshly prepared cold (4°C) lysing solution (2.5 M NaCl, 100 mM EDTA Na2; 1% Na-sarcosine, 10 mMTris–HCl, pH 10–10.5; 1% Triton X-100 with 10% DMSO being added just before use) for at least 1 h. The slides were then electrophoresed (25V/300 mA, 25 min) after they were immersed in freshly prepared alkaline electrophoresis buffer (0.3 mol/l NaOH and 1 mmol/l EDTA, pH > 13) at 4°C for unwinding (40 min). All steps were carried out under minimal illumination. After electrophoresis, the slides were neutralized (with cold $dH_2O$). Microscope slides were stained with ethidium bromide (70 µl/slide), covered with a cover-slip and analyzed using a fluorescence microscope. 100 comets (100 comets/slide) were scored visually as belonging to one of five classes (0 - undamaged, 1- mild damage, 2 – moderate damage, 3- severe damage, 4 – complete damage) using a fluorescence microscope as shown in related literature (Collins, 2004). Arbitrary Unit used to express the extent of DNA damage was calculated.

*Statistical analysis*

The data was presented as mean ± Standard deviation (SD) for parametric variables. The comparison between groups was performed by using one-way analysis of variance (ANOVA) on SPSS 15.0 version, Chicago U.S.A. for Windows software.

## RESULTS AND DISCUSSION

The data regarding the gunshot falcons and control group falcons having different types of injuries is represented in Tables I and II. Results showed that gunshot falcons were having fracture of antibrachium, humerus, tarsa, metatarsal and necrosis of corresponding extremities. Young falcons were shot more as compared to adult animals. The most of control group birds were debilitated, lightly injured, bruised and were rehabilitated soon after the treatment.

**Table I.- Species of falcons having severe injuries due to gunshot, their damaged side of extremities and reason of clinical treatment is shown.**

| Case | Species | Age | Side of extremities | Reason of clinical application |
|---|---|---|---|---|
| 1 | bb | Young | Left wing | Open fractureat distal carpus bone |
| 2 | bb | Young | Right foot | Distal metatarsal extremity necrosis |
| 3 | bb | Adult | Right Wing | Humerus fracture |
| 4 | br | Young | Right Hind Extremity | Bilateral hind extremity necrosis |
| 5 | bv | Adult | Left Wing | Fracture ofAntebrachium Bones |
| 6 | br | Adult | Left Wing | Fracture ofAntebrachium Bones |
| 7 | br | Young | Right Wing, Right Hind Extremity | Humerus, necrosis of distal femur |
| 8 | bb | Young | Right Wing , Right Hind Extremity | Open fracture of Humerus and tarsal bone |
| 9 | bb | Young | Right- Left Wing | Necrosis of antebrachi and humeral fracture |
| 10 | bb | Young | Left Wing | HumerusFracture,necrosis of antebrachium extremity |

*bb, *Buteo buteo*; br, *Buteo rufinus*; bv, *Buteo vulpinus.*

**Table II.- The control group, having mild injuries along with reason of injuries and clinical application is shown.**

| Case | Species | Age | Reason of injury | Reason of clinical application |
|---|---|---|---|---|
| 1 | br | Young | Trauma (Traffic Accident) | Debilitated |
| 2 | br | Young | Trauma (Traffic Accident) | Debilitated |
| 3 | bb | Adult | Mild Trauma and Shock | Nutritionally deficient and debilitated |
| 4 | br | Adult | Attacked by animal | Antebrachium wound, Feathers were damaged |
| 5 | br | Young | Uneducated and improper care | Feathers were damaged, light wounds |
| 6 | bb | Adult | Attacked by animal | Primary feathers were damaged, light wounds |
| 7 | bb | Adult | Uneducated-improper care | Feathers were damaged and lightly wounded |
| 8 | bb | young | Mild Trauma and Shock | Nutritionally deficient and debilitated |
| 9 | bb | young | Attacked by animal | Feathers were damaged, light wounds |
| 10 | bv | young | Uneducated-improper care | Debilitated, light bruises |

*bb, *Buteo buteo*; br, *Buteo rufinus*; bv, *Buteo vulpinus.*

**Table III.- Total DNA damage, total oxidative status (TOS) and total antioxidative status (TAS) in control and gunshot falcons.**

| Groups | N | TOS (µmol $H_2O_2$ equivalent/L) ±SD | TAS (mmol trolox equivalent/L) ±SD | DNA damage (arbitrary units) ±SD |
|---|---|---|---|---|
| Control | 10 | 2.69±1.05 | 3.11±1.06 | 9.40±3.13 |
| Gunshot falcons | 10 | 4.91±2.61 | 4.64±3.04 | 25.90±8.21 |
| *P* | | **0.035** | 0.393 | **0.00** |

The results of TOS, TAS and DNA damage are shown in Table III. There were significant (*P<0.05*) increase in TOS and total DNA damage in gunshot falcons while non-significant (*P>0.05*) difference in TAS was observed compared to control group.

These birds are birds of prey and most of hunters shot them for fun or for keeping them in captivity. As, most of the falcons were young and had various types of fracture both with the necrosis of some extremities. This could be associated with the fact that young birds are less active and inexperienced as compared to adult birds, so easy to locate by the hunter's eye ( Desmarchelier, 2010).

Increased in the level of oxidative stress and DNA damage was observed in gunshot birds as compared to control group. This clearly shows the oxidative stress related to damage in all birds due to gunshot wounds and its complications. Even nesting birds may get stressed from poachers or the presence of humans which worsen the environment. When animals are gunshot at the same time they fall on the ground from long height which lead to an extra trauma and may got fractured. These all factors were found to lead to increase in the metabolic

rate and oxidative stress (Cohen *et al*., 2007).

Many studies have been reported to understand the mechanisms determining maximum longevity in mammals along with different factors like metabolic rate (Austad, 1997; Barja and Asunción , 2000). But dearth of literature has been found for wild birds regarding this, especially in respect to gunshot wild birds. This report although is not a clear picture of the aging process in wild birds but, still open a small imagination of the impact of gunshot wounds for overall lifespan of these precious animals.

Metabolic rate increases in response to diseases and injuries, so it produces abundance of reactive oxygen species (ROS) as a normal by-product. These ROS cause oxidative damage to biological molecules and the accumulated damage, in turn, results in the breakdown of homeostatic regulatory systems, eventually causing an animal's death and consequently diminishing the characteristic maximum longevity of the particular species. The “oxidative stress theory of aging” is currently the most widely accepted explanation of an animal’s maximum lifespan, and can be divided into its functional components (Montgomery *et al*., 2012). This mechanism suggests that gunshot wounded falcons may lead to the short life span by inducing different diseases like neurodegenerative disorders, atherosclerosis, arthritis etc. (Saravanan and Pugalendi, 2005).

## CONCLUSION

This recent study showed the effect of gunshot wounds in wild falcons on the DNA damage, oxidative stress and total antioxidant capacity of animals. Increased oxidative stress is a suggestive of ill effects on the overall health and life span of falcons. This study emphasizes to promote the educational programs, law enforcement, and other mitigating measures to protect these birds of prey to optimize the health and production of wild bird’s populations.

*Conflict of interest statement*

Authors have declared no conflict of interest.

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