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Original Article

Role of Vitamin E And Selenium as an Anti-Stressant in Translocation of Captive Ungulates

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ABSTRACT

Wild ungulates especially in captivity face high stress in translocation that may cause even mortality. Chemical immobilization is employed to minimize stress by using sedatives. Vitamin E and Selenium may have impact on reducing stress. **Objectives:** To estimate the impact of Vitamin E and Selenium treatment in translocation of captive ungulates. **Methods:** The study was carried out while planned translocation of 40 ungulates from different zoological gardens of four districts of Punjab to Safari Zoo, Lahore, Punjab, Pakistan was performed. The animals were divided into two equal groups i.e., A and B. Both groups were anesthetized with combination of Ketamine and Xylazine while Vitamin E and Selenium was also injected in group B after anesthesia. Blood samples were also drawn for hemato-chemical analysis in both groups. The necropsy of the dead animals was also done for internal organs and skeletal muscles examination. **Results:** In the group A, two chinkaras and two spotted deer were found dead at the time of release. The pale areas, hemorrhages and clotted blood were observed in necropsy. The values of Hemoglobin, Total Erythrocyte Count and Packed Cell Volume were moderately varied from the normal in both groups. The group A had greater lymphocyte count and lower neutrophils while in group B, lymphocyte count was lower, neutrophils were higher and Aspartate transaminase (AST), Alanine transaminase (ALT), and cortisol levels were quite higher than normal. **Conclusions:** Vitamin E and Selenium has positive impact to reduce stress level in translocation of captive ungulates.

INTRODUCTION

Ungulates are wild hoofed mammals with ruminant stomach, grooved molar teeth, belong to grand order Ungulata and have 257 identified ungulates species such as red deer, wild boar, fallow deer etc. [1]. The translocation is used in the context of wildlife conservation [2]. Translocation cause stress in the animals, hence chemical immobilization is employed to minimize stress by using xylazine hydrochloride. Globally, the most fatalities related to animal translocation are caused by capture myopathy (CM), a malignant result of stress during capture operations. It occurs when an animal's natural biological stress defenses have collapsed. Death can happen shortly after the precipitating incident, within a few minutes, hours, days, or even weeks [3]. When wild animals are captured, CM often develops. Significant symptoms of CM include a rapid rise in core temperature, muscular spasms, stiffness, lethargy, recumbency, the presence of dark crimson urine, and ultimately, death [4]. Vitamin E and selenium have strong impacts on reducing stress. Vitamin E is an antioxidant that prevents the formation of harmful

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free radicals [5]. Oxidative stress can result from a high level of free radicals as the body consumes a lot of vitamin E when under stress. Thus, taking vitamin E and selenium regularly can help to reduce stress in daily life. Selevit, a supplement that combines vitamin E and selenium, has been used in animals for several purposes such as used to treat and prevent muscular dystrophy, muscle overuse injury, neuritis, myositis, stress prevention, myasthenia, and pig liver disease [6]. Vitamin E, a powerful antioxidant, is necessary for protecting the polyunsaturated fatty acids (PUFA) found in cell membranes from oxidation. While lipid peroxides are being formed, α-tocopherol supplies hydrogen ions to the lipid peroxyl radical, which stops the oxidation cascade, prevents the oxidation of PUFAs and lipoproteins, and protects tissues by lowering free radicals. The chemical immobilization reduces motility and injury more than other approaches, it is becoming more and more common in large mammal capture. This is because the animals are operated while sedated, resulting in less capture stress. Additionally, the adverse effects of drugs vary according on the particular medication taken [7, 8]. The process culminates in the creation of lipid hydroperoxide, a radical that is less reactive.

The purpose of this study was to outline the present state of knowledge regarding role of vitamin E and selenium in reducing stress and mortality during translocation in wild ungulates. The study essentially compared ungulates that have had Injection (Inj.) Selevit treatment to those, that have not, in order to draw conclusions.

METHODS

The current study was carried out in year 2020 while planned translocation of n=40 ungulates of four species. The animals included were black buck (Antelope cervicapra), chinkara (Gazella bennettii) hog deer (Axis porcinis) and spotted deer (Axis axis). Regardless of age, size, or weight, 09 black buck, 06 spotted deer, 05 hog deer and 20 chinkara were trans-located. The Basal Declaration of Animal Research was followed to ensure animal welfare and protection of animal rights [9]. The intended study was carried out in animals which were diversely trans-located from different zoological gardens of four Districts Lahore, Kasur, Sahiwal and Bahawalpur of province Punjab to Safari Zoo, Lahore Punjab, Pakistan. The details of study sites along with their location determinants are given in table 1.

Table 1: Study Sites of Four Districts i.e., Lahore, Kasur, Sahiwal and Bahawalpur of Punjab, Pakistan along with their Location Determinants

Locations	Determinants
Safari Zoo, Lahore	31.381680243665127, 74.21825835444416
Wildlife Park, Changa Manga, Kasur	31.111006651791715, 74.00126952839986
Wildlife Park Jallo, Lahore	31.5720181211597, 74.4646045030944
Wildlife Park, Lal Suhanra, Bahawalpur	29.442806061692178, 71.98533140064954
Wildlife Park, Perowal, Sahiwal	30.692367541638585,73.08807785441839

Every animal's translocation was carried out between public sector wildlife parks and zoos in Punjab. The animals were divided into two equal groups, having 20 animals of almost same species and age in each group. The animals were transported from four captive localities to the Safari Zoo, Lahore. For transportation, the animals were sedated to reduce the chances of cardiomyopathy. Ketamine HCL (3-5 mg/kg) was administered in combination with xylazine hydrochloride (0.1 - 0.3 mg/kg) according to the estimated body weights in half of the animals as in routine practice while Inj. Selevit (Containing Vit. E and Selenium with dose rate 3-5ml/animal) was injected in other half of animals after sedation to analyze the additive effect of vitamin E and selenium. Yohimbine (0.2mg/kg) was used as reversal agent of Xylazine in both of the groups. The drugs were injected via tranquilizing gun. Pre-release blood sampling of all animals was carried out for hemato-chemical analysis. The blood was collected from left or right jugular vein in both anticoagulant added (EDTA) and Non-EDTA tubes (03 ml in each) for both of animal groups and were sent to Safari Zoo Veterinary Complex for hemato-chemical analysis i.e., Hemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Neutrophil count, lymphocyte count, Alanine Transaminase (ALT) and Aspartate Transaminase (AST). Additionally, the obtained serum samples were then sent to University Diagnostic laboratory, UVAS for serum Cortisol levels estimation. The hemato-chemical values given by Gupta *et al.*, were used as reference values irrespective of species [10]. A post-mortem examination of the animals which became dead while translocation was also carried out at post-mortem block, Department of Pathology, UVAS, Lahore to confirm the cause of death. The comprehensive examination of internal organs and skeletal muscles along with gross changes were noted. The data regarding hemato-chemical parameters were analyzed by using paired t-test. The p-values less than 0.05 were considered significant.

RESULTS

The group in which injection Selevit was not administered, two chinkara and two spotted deer were found dead at the time of release at Safari Zoo Lahore as compared to zero mortality in the group which was supplemented with injection Selevit. The pale areas were observed in the musculature of animals showing decrease in muscle myoglobin caused by capture myopathy. Hemorrhages and clotted blood were observed over the thoracic region in dead animals. The findings also revealed the widespread congestion and hemorrhages on all major organs, including the liver, heart, lungs, kidneys, and spleen.

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3.2 Hemato-chemical Parameters

The mean values of hemato-chemical parameters were compared with reference values and it was determined that mean values of Hemoglobin (Hb), Transient erythyroblastopenia (TEC), and Packed cell volume (PCV) were moderately varied from the mean values in both animal groups. The group treated with Inj. Selevit had greater lymphocyte counts while the group that was not having Selevit treatment was having lower lymphocyte values. The number of neutrophils was found to be higher in the group that had not received Inj. Selevit treatment as compared to the lower values in the group that had received. In the group without Inj. Selevit, the Alanine aminotransferase (AST), Aspartate aminotransferase (ALT), and Cortisol levels were quite high, whereas only a slight change in these values as compared to reference values was seen in the group having Selevit treatment. The graphical representation of mean values of both groups is as in Figure 1.

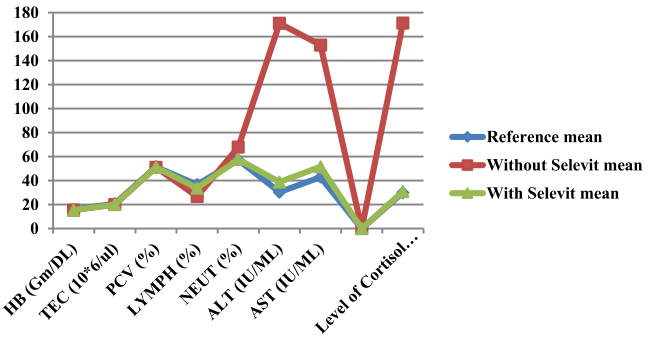

Figure 1: Graphical Representation of Hemato-chemical Variations in Both Groups i.e., Treated with Selevit and not Treated with Selevit along with Reference Mean

Statistically, all the parameters in the group treated with Inj. Selevit except Hb, TEC and PCV were found highly significant with P≤0.05 as compared to the routine protocol group i.e., without Inj. Selevit group. The details regarding hemato-chemical variations along with their p-values are given in table 2.

Table 2: Mean Values of Hemato-Chemical Parameters in Both Groups i.e., Treated with Selevit and Not Treated with Selevit along with Reference Mean and p-Values

Parameters	Reference Mean Gupta *et al.*, [10]	Without Selevit Mean	With Selevit Mean	p-value
Hemoglobin (Hb) (Gm/DL)	15.9	15.4425	15.421	p=0.61
Transient Erythyroblastopenia (TEC) (10*6/ul)	20.83	20.0945	20.0385	p=0.31
Packed Cell Volume (PCV) (%)	51.44	51.064	51.0895	p=0.84
LYMPH (%)	36.63	26.78	33.777	P≤0.05
Neutrophil (%)	57.25	67.913	57.747	P≤0.05
Alanine Aminotransferase (ALT) (IU/ML)	30.38	171.16	38.81	P≤0.05
Aspartate Aminotransferase (AST) (IU/ML)	42.88	152.9755	51.5175	P≤0.05
Cortisol Level (nmol/lit)	29.89	171.26	30.9	P≤0.05

DISCUSSION

The study was conducted to assess the effectiveness of Inj. Selevit as an anti-stressing drug in reducing the incidence of capture myopathy (CM). The mortality in animals while trans-location may be resulted by various factors such as capturing method, anesthetic drugs, sex of animal, environment, no use of anti-stressing (vitamin E and Selenium) etc. Extreme stress and physical activity causes capture myopathy, which even lead to the immediate death. On a global scale, the majority of wildlife translocation-related fatalities are attributed to capture myopathy, a cancerous result of stress during capture operations [11], and the same was observed in current study conducted at different wildlife parks of Punjab. Animals' elevated body temperatures may also bring about initial psychological stress. Physical exercise and Xylazine in the sedation combination interfere with thermoregulatory centers during chemical immobilization. During stress, the muscle enzymes' level increase that may lead to damage of tissues causing capture myopathy CM [12]. Four animals out of 40 died during the study which were only from the group which were not treated with Inj. Selevit as per routine protocol, and the results indicated that capture myopathy caused by stress in wild animals was the reason for death. These findings are in agreement with those of Ashraf *et al.*, and Paterson, who noticed the capture myopathy (CM) in spotted deer and other wild animals respectively [13, 14]. Due to stress, the blood chemistry of the group that did not get Selevit treatment revealed elevated levels of ALT and AST. These results were almost similar with research work of Prasse and Mahaffey [15]. Less fluctuation in Hb, PCV and TEC was observed in both groups during trials which were coinciding with the findings of Gupta *et al* [10]. Villar-Quiles *et al.*, also reported that the pathological results pointed to spotted deer Axis axis myopathy [16]. The study emphasizes the importance of taking mortality from capture into account when planning restocking initiatives. During gross examination, congestion, and hemorrhages were seen on the muscles as well as on the major organs like heart, kidney and lungs. These were as a result of stress-related free radical damage that was only present in the group that did not get Selevit treatment. These findings agreed with those of Basson and Hofmeyr [17]. Serum Cortisol levels were found high in group which did not get Selevit treatment. The release of this hormone causes immune suppression that makes animal more susceptible to CM and ultimately lead to death similar was observed by Awerman and Romero because an increase in circulating glucocorticoids plays a

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number of suppressive, stimulative, and preparative roles that help bring the animal back to a homeostatic state [18]. These results were keeping in similarity with the study of Donny *et al* [19]. The other very remarkable point is the antioxidant properties Se and Vitamin E observed by Xiao *et al* [20]. According to their findings, the Se and vitamin E supplementation as antioxidant agents in the health regulation of periparturient dairy cattle.

CONCLUSIONS

One of the major obstacles to restoring endangered species populations through translocation is understanding and addressing translocation-induced chronic stress. According to the findings of this study, stress is the main factor contributing to capture myopathy (CM) in wild animals during trans-location, and the risk of capture myopathy can be reduced with the use of vitamin E and Selenium combined with anesthetics.

Authors Contribution

Conceptualization: MA, NNK, MRK, AN
Methodology: MA, BNK
Formal analysis: KA
Writing-review and editing: MA, BNK, AM, MF, SA, MTM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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