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Research Article

Serum Cortisol Concentration Following Physical and Chemical Restrained in Nigerian Indigenous Dogs (NIDs)

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ABSTRACT
Restraint is the inhibition of an animal's free movement by moral persuasion, physical force and or administration of drugs, which is generally performed for examination, collection of samples, drug administration, manipulation or surgical intervention. In order to determine the effects of physical and chemical restraint on haematological parameters in Nigerian indigenous dogs (NIDs), the experiment was conducted in 2 stages. The dogs were divided into two equal groups consisting of ten males and ten females. After physical restraint using a muzzler, each group member was subjected to blood sample collection for haematological analyses. While in stage 2 the dogs were divided into two equal groups consisting of ten males and ten females. The male groups were subdivided into two groups of five. The first group received acepromazine while the second group received xylazine. Animals in the female group were also subdivided into two groups. The first group received acepromazine while the second group received xylazine. Blood samples (5ml) were collected for haematological analyses (serum cortisol concentration) using an ELISA kit. The serum cortisol concentrations between male (146.58±125.02) and female (247.93±74.57) NIDs differ significantly ($P < 0.05$) at the control. This study shows that physical restraint in Nigerian Indigenous Dogs has minimal effects on the serum cortisol concentration, while acepromazine and xylazine ameliorate the level of serum cortisol concentration in NIDs. This may be altered by stress events in dogs, such as capture and handling. There was an increase in the level of cortisol in females due to higher emotional responsivity.

Keywords: Acepromazine; Chemical Restraint; Muzzler; NIDs; Physical Restraint; Xylazine

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INTRODUCTION

Cortisol, a vital stress-responsive hormone, is extensively utilized as a biological marker to evaluate stress in animals subjected to various restraint methods. Significant differences in cortisol levels can result from different methods of animal restraint, providing information on the physiological stress load and assisting in the improvement of animal care procedures. The technique of capture and subsequent immobilization has a significant impact on cortisol levels, according to research conducted on non-domestic species. For example, plasma cortisol levels increased after capture, with the exception of Cape buffaloes, according to a study evaluating a variety of animal species. Notably, lower cortisol levels in these situations suggested that chemical capture techniques were generally viewed as less stressful than physical restraint (Morton *et al*., 1995).

Handling and restraint practices have a significant effect on stress indicators, such as cortisol levels, in certain instances, Physical, chemical, and psychological/non-contact methods can all be used to restrain animals in veterinary practice (Zoaka and Nwannenna, 2009). The employment of mechanical or manual methods to restrict an animal's natural voluntary movement in part or in full is known as physical restraint. Leashes, collars, dog snares (catchers), muzzles, Elizabethan collars, squeeze cages, and canine mouth speculum are a few of the equipment and tools used for physical restraint. Drugs are used to control animals in chemical restraint (Amin *et al*., 2016).

Drugs used as tranquilizers and sedatives in veterinary medicine include the phenothiazines (acepromazine, chlorpromazine, promethazine, promazine, prochlorperazine), Butyrophenones (Fluanisone, droperidol, azaperone), benzodiazepines (Diazepam, midazolam, zolazepam) and alpha-2-adrenergic agonists (xylazine, detomidine, romifidine, medetomidine) (Lemke, 2007; Pawson, 2008). These chemical sedatives may have a protective effect against hormonal changes brought on by stress while preserving regular glucose metabolism (Champagne *et al*., 2012).

Acepromazine has a gradual beginning of action (approximately 20 minutes) after intravenous or intramuscular administration, but it has a lengthy duration of action (3-6 hours). It causes mild to moderate drowsiness with no analgesic properties (Jeff, 2013).

Xylazine induces a dose dependent mild to profound degree of sedation including lateral recumbency. According to Vesal et al. (2016), it has analgesic and muscle-relaxing effects, with a quick start of action of approximately five minutes and a moderate duration of action of two hours. Xylazine administration affects haematological parameters (Sutil *et al.*, 2017). Alpha-2-adrenoceptor antagonists such yohimbine, atipamazole, and tolazoline can particularly counteract the effects of xylazine (Schwartz and Clark, 1998).

Research on other species, such the silver fox, shows that physical constraint significantly raises glucose and cortisol levels. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathoadrenal medullary (SAM) system were both significantly activated in this stress-induced hyperthermia, which was a quick reaction to handling and restraint (Moe and Bakken, 1997). These results demonstrate the rapid elicitation of physiological stress reactions, which calls for compassionate handling techniques.

Both forms of restraint have the potential to significantly impact an animal's endocrine system in real-world situations, affecting not only cortisol but also other hormone pathways. For example, research on white-tailed deer demonstrates that manual restraint generates larger amounts of adrenal hormones than pharmacologic agents (Iii *et al*., 1979). These findings highlight the necessity of using the least stressful methods of restraint in order to maintain standards for animal welfare.

Moreover, cortisol dysregulation may affect health in more general ways. Negative effects, such as immunological suppression and an increased risk of disease, have been associated with elevated cortisol levels brought on by stress or environmental causes. Cortisol, an essential glucocorticoid hormone, is crucial for stress response, immune function, and metabolism in dogs. Elevated cortisol levels are associated with stress and exercise, influencing physiological and biochemical responses, as observed in scenarios like anticipation of exercise or exposure to uncontrollable stressors (Angle *et al.*, 2009; Dess and Al, 1983

It is essential to comprehend the subtleties of cortisol responses to various restraint techniques in the fields of behavioral research and animal welfare. By reducing stress, it not only helps to improve the comfort of animals such as NIDs, but it also offers important information about the physiological effects of restraint, which informs best practices for handling and care.

MATERIALS AND METHOD

Study Area

This study was conducted in Maiduguri, Borno state, Northeastern region of Nigeria. Maiduguri is located within latitude 11.5N^0 and longitude 30.05^0 E in the Sudano-Savanna zone (Udo, 1978).

Animals

The study involved twenty (n=20) owned Nigerian indigenous dogs (NIDs) of sexes, ten (10) females and ten (10) males. The ages of the dogs were between 1-3 years. The dogs were housed at the Surgery Theater Dog Kennels of the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, University of Maiduguri. They were allowed to acclimatize for a period of two weeks before the commencement of the experiment. The dogs were fed rice, beans and some local food which was supplemented with meat.

Experimental Drugs

The drugs used are:

Acepromazine (Calmivet®, vetoquinol S. A, specialities Pharmaceutiques Veterinaires, Magny-Vernois France), Xylazine (Sedazine®, Fort Dodge Animal Health, Ford Dodge, Iowa 50501 USA).

Control

Vital parameters and blood samples were collected from all the animals before commencement of all the procedures.

Measurement of Vital Parameters

Pulse rate (beats/minute) was assessed using stethoscope placed between 2-5 intercostal spaces (1-minute full count was done).

Respiratory rate (breath/minutes) was assessed by visual observation of the thoracoabdominal movement (1-minute full count was done).

Rectal temperature (^{0}C) was measured using digital thermometer placed in the rectum for one full minute.

Physical Restraint

Restraint was carried out by starting with a soothing to the dog, loving and gentle words was were said to the dog to calm it, sudden touch and movement were avoided, hands were slowly run over the animal dog body, the muzzle was then put on to completely restraint the mouth of the dog. (J.B. Adeyanju and A.Z. Hassan 2022), these methods were repeated to each of the dog in the Group.

Chemical Restraint

This was done through the administration of acepromazine and xylazine at 0.01 and 1mg respectively.

Experimental Procedures

The dogs were divided into two (2) equal groups consisting of ten males (10) and ten (10) females. Each group member was subjected to physical restraint where temperature, heart rate and respiratory rate were taken. Blood samples (5ml) were collected after 30 minutes of the restraint (Michael *et al.*, 1997) from the cephalic vein of each dog into a plain bottle using sterile 5ml syringe and 21-gauge needles. this was allowed to clot and centrifuged at 3000 rpm for 15minutes with a clinical table centrifuge, and sera were harvested within a few hours of collection stored at -20°c in a refrigerator for biochemical analysis., Plasma cortisol concentration was determined as described by Michael *et al*., 1997. The dogs were allowed 14 days to recover from the stressed of the the physical restraint and the second experiment was conducted by dividing them into two (2) equal groups consisting of ten males (10) and ten (10) females, where temperature, heart rate and respiratory rate were taken before the procedure and after the procedure. The males' groups were subdivided into two groups consisting of five each. The first group received tranquilization (acepromazine 0.01mg/kg intravenously) while the second group received sedation (xylazine 1mg/kg intravenously). Animals in the female group were also subdivided into two groups. The first group received tranquilization (acepromazine 0.01mg/kg intravenously) while the second group received sedation (xylazine 1mg/kg intravenously). Blood samples (5ml) were collected 30 minutes of the restraint (Michael *et al.*, 1997), from the cephalic vein of each dog into a plain bottle using sterile 5ml syringe and 21-gauge needles, this was allowed to clot and centrifuged at 3000 rpm for 15minutes with a clinical table centrifuge, and sera were harvested within a few hours of collection and stored at -20°c in a refrigerator for biochemical analysis., Plasma cortisol concentration was determined as described by Michael *et al*., 1997.

Serum Cortisol Measurement

Measurement of Serum Cortisol Concentration levels were estimated by using a commercially prepared Eliza Kit (Human Diagnostic Laboratory, Wiesbaden, Germany) according to Michael *et al*., 1997. Which is,

Assay Procedure

The stored serum samples were brought out, thawed at ambient temperature in a biosafety cabinet (inoculation hood). The Eliza Kit (Human Diagnostic Laboratory, Wiesbaden, Germany) that was kept at 2°C in the refrigerator was also brought to a room temperature in a biosafety cabinet.

Reagent Preparation and Assay Protocols

All the components of the ELISA kit were brought to ambient temperature in a biosafety cabinet, Standard Working Solution (Standard Stock Solution is 240 pg/mL) was prepared as follows;

- 6 clean emptied plain (EP) tubes were provided and numbered

- 150ul of standard diluent was added to each EP tube
- 150ul of the 240 pg/mL standard stock solution was pipette and added to the first EP tube and mixed up to produce a 120 pg/ml working solution
- 60 pg/ml was pipette from the former tube into the next 2nd EP tube,
- 30 pg/ml was pipette from the 2nd EP tube to the 3rd EP tube
- 15 pg/ml was pipette from the 3rd EPtube into the 4th EP tube
- 7.5 pg/ml was pipette from the 4th EP tube into the 5th EP tube
- And the 6th EP tube was left with the Standard Diluent only.

Wash Buffer was diluted at 20ml of the concentrated buffer with 520ml of distilled water, to make up of 600ml of wash buffer.

Assay Protocols

- Diluted standard wells, Sample Wells and Blank Wells were determined, were well A1 to A12 were used for our diluted standard, well B1 to G10 are Sample wells, well G11 to H12 are for Blank wells.
- For well AI to A12, 50µl Standard solution were added
- For well B1 to G10 4µl of sample diluent were added to the wells and 1µl of sample were also added respectively.
- Well, G11 to H12 were left blank
- The plate was closed with a plate sealer, and incubated for 30mins at 37°C
- The plate sealer was uncovered, liquid was discarded and washed with washing buffer, it was drain for 30sec and washing was repeated for 5 times, and dried by pat.
- 50µl of HRP-Conjugate reagent was added to each well, neglecting the Blank wells
- The plate was closed with a plate sealer, and incubated for 10mins at 37°C
- The plate sealer was uncovered, liquid was discarded and washed with washing buffer, it was drain for 30sec and washing was repeated for 5 times, and dried by pat.
- 100µl of equal volume mixture of Substrate Reagent A and Substrate Reagent B was added to each well, again neglecting the Blank wells
- After some minutes, a blue coloration appears in the well
- 50µl of stop solution was then added to each well to stop the reaction
- The blue coloration in the well changed to yellow
- Absorbance was read at 450nm within 10 mins of stopping the reaction.

Data Analyses

Data obtained was analyzed using One Way Analysis of Variance. Graphpad Prism® was employed for the data analysis.

RESULTS

Effect of methods of restraint on vital parameters of female NIDs

Mean temperature values under both physical and chemical restraints were comparable with the control values (P<0.05). Both mean pulse; control (69.10±4.23), physical restraint (79.70±7.97), acepromazine (35.20±3.70) xylazine (52.80±7.82) and respiratory rate between the different restraint methods control (26.80±4.49), physical restraint (39.00±13.18), acepromazine (11.40±2.07) xylazine (10.20±3.35) and the control differ significantly (P<0.05). Mean pulse rate was lowest with acepromazine (35.20±3.70). Whereas, the lowest mean respiratory rate was recorded with xylazine (10.20±3.35).

Effect of methods of restraint on serum cortisol of male/female NIDs

The MSD of serum cortisol concentrations between male (146.58±125.02[a]) and female (247.93±74.57[b]) NIDs differ significantly (P < 0.05) at control. The values did not differ (P > 0.05) between physical, acepromazine and Xylazine methods of restraint among the male and female NIDs (Table 2).

Effect of methods of restraint on serum cortisol of male NIDs

There was no significant difference between the MSD of control (146.58±125.02), physical (215.01±91.44), acepromazine (175.34±109.91) and xylazine (272.05±17.14). (P<0.05). however, there is an increase in the cortisol level at Physical restraint and Xylazine as a method of chemical restraint. (Table 2)

Effect of methods of restraint on serum cortisol of female NIDs

MSD serum cortisol values were comparable among the different restraint methods; physical restraint (274.57±18.55), acepromazine (179.46±114.28), xylazine (197.90±129.65) and the baseline (247.93±74.57) and shows no significant difference (P<0.05) (Table 2).

Table 1. Vital parameters of female NIDs* subjected to physical and chemical restraint during ultrasonography

Parameters	Control	Chemical Restraint		
		Physical	Acepromazine	Xylazine
TEMP(^{0}C)	38.09±0.92^a	38.61±0.87^a	39.28±0.18^a	38.52±0.82^a
PULSE(b/min)	69.10±4.23^a	79.70±7.97^a	35.20±3.70^b	52.80±7.82^b
RESP(C/min)	26.80±4.49^a	39.00±13.18^b	11.40±2.07^c	10.20±3.35^c

Values with different superscripts within row are significantly (P<0.05) different

Table 2. Effects of serum cortisol in NIDs* subjected to physical and chemical restraint

Parameters	**Control**	**Methods of restraint**		
		Physical Restraint	**Acepromazine**	**Xylazine**
CORT (uIU)				
Male	146.58±125.02^a	215.01±91.44^a	175.34±109.91^a	272.05±17.14^a
Female	247.93±74.57^b	274.57±18.55^a	179.46±114.28^a	197.90±129.65^a

Values with different superscripts within columns are significantly (P<0.05) different

DISCUSSIONS

Respiratory value was also found to be significantly higher in dogs restraint using the physical method compared with control group and when under either acepromazine which agrees with the findings of Yohannes *et al.,* 2018 for acepromazine and xylazine respectively. The finding of the present study is in line with Imtiaz *et al.*, 2014 who in a similar study have reported significantly lower pulse rate values in dog's restraint using the same physical and chemical restraint in dogs compared to dogs under the control groups. The present study also agrees with Eduardo *et al.*, 2008 who have recorded significantly higher respiratory rate value in dogs under physical restraint compared to the control and when under either acepromazine or xylazine. These results of the present study are also in concurrence with those of Eduardo *et al.,* 2008 who evaluated the effects of acepromazine and xylazine as sedative on physiologic values in dogs and found that pulse rate and respiratory rate were significantly affected. Physical restraint produced higher mean pulse and respiratory rates compared to the control, indicating a sympathetic stimulation and stress response typically associated with manual handling. In contrast, both acepromazine and xylazine significantly lowered pulse and respiratory rates, consistent with their known pharmacological depressant effects on the cardiovascular and respiratory systems. Acepromazine, a phenothiazine tranquilizer, is known to cause vasodilation and hypotension, which may explain the lowest mean pulse rate (35.20±3.70 bpm) observed. Xylazine, an α_2-adrenergic agonist, causes marked bradycardia and respiratory depression through central nervous system depression, reflected in the lowest respiratory rate (10.20±3.35 cpm). Pulse rate was lower in dogs that received xylazine during most of the study. The mean rectal temperature did not differ significantly (P > 0.05) between the control, physical, and chemically restrained groups, this suggests that both physical and chemical restraint methods used in this study did not elicit sufficient thermogenic or thermolytic stress responses to alter body temperature significantly.

Cortisol is a sensitive indicator of physiological stress, and its concentration is influenced by both physical and psychological stimuli. In the present study, baseline cortisol levels were significantly higher (P < 0.05) in female NIDs compared to males, suggesting possible sex-related differences in stress reactivity or Hypothalamic Pituitary Adrenal (HPA) axis sensitivity, similar sex differences have been observed in other mammalian species and are often attributed to hormonal modulation by estrogen and progesterone (Juster *et al.,* 2016). The finding of the study also showed that serum cortisol values were comparable between different restraint method, this finding buttresses the report by Ali *et al.,* 2006 who have revealed that xylazine is also known to significantly improve the effects induced by stress stimuli, which might influence the values of the serum cortisol as reported in the present study. Similar finding was reported by Rashid *et al.,* 2018 in horses given different combination of acepromazine, detomidine, xylazine and ketamine. The possible explanation could be due to the dosage, used for study were not sufficient to decrease cortisol release (Maze *et al.*, 1991). The study revealed that cortisol levels are higher in females than in males. Indeed, studies on other species have previously suggested that, compared to males, females show a higher emotional responsivity (Sudnam *et al.,* 2019).

CONCLUSION

The results of this study show that, although both physical and chemical restraint techniques can be employed successfully in Nigerian Indigenous Dogs (NIDs) for clinical or diagnostic procedures, their physiological effects are very different. Elevated

respiratory and pulse rates were linked to physical constraint, suggesting a brief stress reaction probably caused by sympathetic activation. Chemical restraint with xylazine or acepromazine, on the other hand, resulted in a more stable physiological profile with lower levels of stress hormones and decreased cardiorespiratory activity. Further evidence that neither restraint method caused substantial systemic stress comes from the lack of significant variations in blood cortisol and rectal temperature.

AUTHORS CONTRIBUTION

Musa Kalim Adam and Mohammed Auwal Idris. participated in manuscript writing, data collection, data analysis, data interpretation and experimental work. Dauda Laku participated in data collection, analysis and interpretation. Zainab Bukola Yusuf, Aliyu Aliyu Haruna and Hadiza Bagobiri Aliyu participated in data collection and interpretation. Abubakar Mshelia Saidu reviewed and make corrections in the entire manuscript.

CONFLICT OF INTEREST

There is No conflict of interest in this work.

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