Effect of vivisection on haematobiochemical parameters of experimental dogs: Towards improved animal welfare

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**ABSTRACT**

**Introduction:** Vivisection could lead to the derailment of vital body functions and hence severe health consequences. The effect of multiple vivisections on haematobiochemical parameters of dogs was investigated with a perspective to improve animal welfare.

**Methods:** Fifteen healthy Nigerian indigenous dogs that were to be used for student wet-labs were recruited for this study. The dogs were acclimatized for four weeks and then exposed to four (4) weeks (bi-weekly) vivisection. Complete physical examination and blood sampling were carried out on days 0, 14, and 28 post-vivisection. Blood samples were analysed for blood and serum biochemical profiles.

**Results:** Post-vivisection results were compared to pre-vivisection results using ANOVA. Physical examination also reveals loss of body weight, body condition score, decreased skin elasticity, sunken eyeballs, dry oral and nasal mucous membrane, as well as percent dehydration on post-vivisection parameters. Findings also revealed a decrease in haematocrit, haemoglobin, and erythrocyte count (p < 0.05), coupled with hyperproteinæmia, hyperalbuminæmia, and azotaemia (p < 0.05) which are generally accepted as indices for dehydration.

**Significance:** Conclusively, the reported anaemia and dehydration in this study could potentiate serious adverse medical effects and it was therefore recommended that the use of animals for multiple surgical procedures should be guided by standard surgical protocols with a perspective of improving animal welfare.

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**Introduction**

Vivisection or animal experimentation could be defined as “the cutting of or operation on a living animal usually for physiological or pathological investigation” (Merriam-Webster dictionary, 11th edition). This procedure which often results in suffering or even loss of life (of the animal) still played a fundamental part in the clinical training of Veterinary students (Knight, 2007; Khoo, 2018). The purpose of using animals in surgical training of students to illustrate procedures which have, in many cases, been earlier established (Fernandes and Pedroso, 2017), is in contrast to their use in research which is aimed at developing modern surgical interventional methods (Rollin, 2007). The claim that subjects are misused during vivisection is debatable, even though, it is widely known that invasive surgeries remarkably interfere with vital body functions and that harm is often sustained especially when conducting procedures on apparently healthy experimental animals (Regan, 2013). Pains, mental stress, physical impediment, and pre-mature death are various ways in which harm could be incurred (Franco, 2007; Khoo, 2018). Just like humans, animals also have a broad range of natural interests (ranging from interest in achieving a state of positive physical and physiological wellbeing to interest to be free from harm) they seek to fulfil during their lives but premature death prevents them from achieving these interests (Balluch, 2006). Although large numbers of animal are been sacrificed across the globe in an attempt to learn empirical skills or latest biological principles, worthy of note especially in the developed countries is the introduction and gradual substitution of this inhumane practice with less harmful teaching aids such as artificial stimulation, experimental models, electronic teaching, ethically-sourced cadavers, preserved specimens, non-invasive self-experimentation, and supervised clinical experiences (Gruber and Dewhurst, 2004; Pereira et al, 2017) during the last few decades.

Although the practice of animal experimentation occurs worldwide, it has provoked reactions and has been intensely debated within the scientific realm (Germain et al., 2017; Freelance, 2019). In Nigeria, even though the right of animals has been clearly defined in the criminal and penal codes, little attention has been given to animals in this regard (Hassan et al., 2005; Obi, 2014). In this present study, the effect of multiple vivisections on haematobiochemical parameters of dogs was investigated with a perspective to improve animal welfare.
Materials and Methods

Study animals and design

Fifteen (15) Nigerian indigenous dogs (identified morphologically) that were scheduled for 4 weeks (bi-weekly) surgical vivisections were recruited and used for this study. The dogs aged between 1 to 2 years were kept in the undergraduate student’s kennel of the Small Animal Unit of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria.

Upon arrival, the dogs were clinically assessed and certified to be fit for surgery; they were routinely dewormed and treated against ectoparasites. They were acclimatized for one month before the scheduled vivisection. They were fed with freshly prepared dog meal and water was provided as desired (NRC, 2006). The experimental dogs were fasted overnight before surgery. Blood samples were obtained on days 0, 14, and 28 post-vivisection.

Physical examination

A thorough physical examination was conducted on each dog before blood sample collection. Bodyweight was determined using a measuring scale (Wosu, 2002); while the body condition scores (Laflamme, 1997) and percent dehydration (Davis et al., 2013) were assessed as previously described.

Sample collection

Blood (5mls) were collected a day after each vivisection between the hours of 8 and 10 a.m. from the cephalic vein of each dog using a needle and syringe. samples (1 ml) for haematology were emptied into ethylene diamine tetra acetic acid (EDTA) containing vacutainer while the remaining (4mls) was dispensed into plain vacutainer and the serum was harvested from the clotted blood and used for serum biochemical analysis.

Haematological analysis

Packed cell volume (PCV) was evaluated as previously described (Thrall and Weiser 2002); haemoglobin (HB) was measured by spectrophotometry method (Higgins et al., 2008). Erythrocyte count (RBC) was enumerated using a haemocytometer (Thrall and Weiser 2002). Standard formulae were used to extrapolate the red cell indices (Esievo, 2017).

Serum biochemical analysis

Creatinine, urea, total protein, albumin, glucose, electrolyte profile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured using commercial test kits (Agappe, India) following manufacturer’s instruction.

Ethical Approval

This study was approved and conducted with firm adherence to the laydown guidelines of the Ahmadu Bello University Ethical committee for experimental animal use and care which were in line with approved international practices described by Wolfensohn and Lloyd (2013).

Statistical analysis

Data obtained were analysed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, U.S.A.) One-way analysis of variance (ANOVA) and Tukey’s post hoc multiple comparison tests were used to determine the differences; significance was determined at a 95% confidence interval (p < 0.05). The result was computed as mean ± SD.

Results

Physical examination findings

Physical examination of dogs at days 14 and 28 post-vivisection reveals decreased skin elasticity, dry nasal, and oral mucous membrane, sunken eyeballs while examination on day 0 post-vivisection shows no abnormal findings. Figures 1–3 show the mean (±SD) variation in body weight (BW), percent dehydration (PD), and body condition score (BCS) in the experimental dogs. The BW, PD, and BCS on day 14 and day 28 vary significantly (p < 0.05) when compared to day 0 post-vivisection.
Figure 3. Variation in a mean body condition score (±SD) of the experimental dogs

*Haematological findings*

Haematological parameters obtained on days 0, 14, 28 post-vivisections were as shown in figure 4. Significant (p < 0.05) differences were observed in PCV, HB, RBC, mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) between days 0 and 28, and also between days 14 and 28 post-vivisection.

Figure 4. Mean erythrocytic parameters of the experimental dogs

Figure 5. Mean serum metabolite concentration of the experimental dogs

*Serum biochemical changes*

Figures 5 – 7 shows the mean (± SD) serum biochemical parameters of the experimental dogs. The serum concentration of total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cr), and blood urea nitrogen/creatinine ratio (BUN/Cr) differed significantly (p < 0.05) between days 0 and 14 and also between days 0 and 28 post-vivisection (Figure 5). The mean serum electrolytes (figure 6) and the mean liver enzyme activities (Figure 7) remain unchanged.

Figure 6. Mean serum electrolyte concentration of the experimental dogs

Figure 7. Mean liver enzyme activities of the experimental dogs

*Discussion*

Decreased values of PCV, HB concentration and RBC counts on days 14 and 28 occurred as a result of anaemia which is caused by blood loss during multiple vivisections in the dogs without provision for haematinics. Lynch et al. (2015) also reported a high prevalence of hospital-acquired anaemia in dogs undergoing surgical procedures. In human subjects, the high prevalence of post-surgical anaemia has been reported (Shander et al., 2004) and the pathogenesis of this anaemia was most convincingly linked with hepcidin production as an inflammatory response to surgical procedures (Munoz et al., 2011). Hepcidin inhibits iron absorption from the small intestine and it promotes sequestration of stored iron (Henniger, 2019). The reported microcytic hypochromic anaemia was in agreement with previous works...
(Ihedioha et al., 2013; Atata et al., 2019). During blood loss, the body utilizes the stored iron in erythropoiesis and this is evident by the initial reticulocytosis (Harvey, 2008) reported in the early stage of the iron-deficiency anaemia (IDA) (Arnold and Durairaj, 2019). As blood loss becomes worse, there would be gradual depletion of the iron store and hence the reduction of myoglobin production persists (Crichton, 2009; Lopez et al., 2016). After this stage, the anaemia then becomes aggravated due to increased destruction of iron-deficient (fragile) RBC by the reticuloendothelial system (Anderson et al., 2000). The IDA reported in this study could irreversibly affect the amount and distribution of iron in the brain and this might lead to neurotransmitter and behavioural dysfunction (Beard, 2003).

The increased TP, Alb, BUN, and Cr and BUN/Cr reported on days 14 and 28 post-vivisection are consistent indicators of dehydration (Fathi and Asiaban; 2016; Lin et al., 2016). This finding coupled clinical signs of dehydration, reduced body weight and low body condition score observed in the experimental dogs were pointers to the fact that the dogs became dehydrated (Davis et al., 2013) post-vivisection. Dehydration causes rapid and severe performance problems (Popkin et al., 2010; Cheuvront et al., 2016) in dogs because of the many vital roles water plays in the maintenance of nearly all body functions. In surgical patients, there are nutritional and fluid restriction during pre-operative fasting (Holte and Kehlet, 2002). Vital body fluids (water) is also lost intra-operatively through bleeding, drainage of ascitic fluid, urination, insensible water loss, and losses into the “third spaces” (Hahn et al., 2014). Unfortunately, diet modification, anorexia, nausea, and abdominal discomfort are among the several factors that might complicate the observed dehydration post-operatively. Mild dehydration is associated with general body discomfort while moderate to severe dehydration could cause hypovolemic shock which may result in multiple organ failure and/or death (Wilson and Morley, 2008; Armstrong et al., 2016) of the patient.

In this present study, serum concentrations of sodium, calcium, chloride, potassium, phosphorus, bicarbonate, anion gap, and the activities of AST, ALT, and ALP were unchanged. The observation supports the claim that the hyperalbuminemia reported in this study was due to dehydration and not as a result of electrolyte imbalance or liver pathology.

**Conclusion**

The reported anaemia and dehydration in this study could potentiate serious adverse medical effects that could lead to multiple organ failure and/or death of the animal. It is, therefore, recommended that general guidelines in line with global best practices should be instituted for the control and treatment of vivisection induced anaemia and dehydration in dogs. We also urge veterinarians to consider the use of humane teaching methods such as artificial simulations, electronic teaching aids, “ethically-sourced cadavers”, preserved specimens, supervised clinical experiences in teaching veterinary surgical skills to students.

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**Conflict of interest**

The authors no conflict of interest.

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