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RESEARCH ARTICLE
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A NOVEL COMPARATIVE STUDY ON EFFICACY OF OZONATED OIL VERSUS SALINE ON INGLUVIES WOUND HEALING IN QUAILS (COTURNIX JAPONICA)

Sohaib Hassan[1], Naveed Hussain[2*], Zia Ullah[3], Haroon Akbar[4], Mian Saeed Sarwar[5], Amber Fatima[6], Aneela Zameer Durrani[7*], Hamad Bin Rashid[8]

[1,2*,6,8]Department of Veterinary Surgery, University of Veterinary and Animal Sciences Lahore, 54000, Pakistan
[3]Department of Small Animal Clinical Sciences, University of Veterinary and Animal Sciences Lahore, 54000, Pakistan
[4]Department of Parasitology, University of Veterinary and Animal Sciences Lahore, 54000, Pakistan
[5]Department of Clinical Studies, College of Veterinary Sciences & Animal Husbandry, Abdul Wali Khan University, Mardan, KPK, Pakistan
[7*]Department of Veterinary Medicine, University of Veterinary and Animal Sciences Lahore, 54000, Pakistan

***Corresponding author(s):** Naveed Hussain & Aneela Zameer Durrani
*Email: nhussain@uvas.edu.pk, Email: aneela@uvas.edu.pk

ABSTRACT:
Crop injuries are common in birds due to trauma, impaction or idiopathic in nature. There are many techniques to repair these injuries and to enhance the healing process. Ozone was discovered over a hundred years ago and since then it has been widely used in many areas. Studies has revealed that ozone therapy i.e. ozonated saline and ozonated oil have revealed good healing of injured tissue. Moreover, use of ozonated preparations instead of antibiotics for healing purposes help to reduce the chances of antimicrobial resistance. In our studies, a total of 18 quails were selected and allocated to 03 groups (n=06). Ingluviotomy procedure was performed done on all the birds. After closure of incision, group B and C were treated with ozonated saline and ozonated oil respectively, while group A was administered normal saline (PO) @ 0.5ml per quail, three times a week for 20 days. This study was carried out for approximately 3 weeks and therapeutic regimens were monitored on day 0, 1, 5, 10, 15 and 20 in terms of physiological parameters, hematology indexes, leakage evaluation, histological evaluation and scanning electron microscopy. At the end of trials, we noticed excellent wound healing in group C (ozonated oil) due to more retention time with superior cell proliferation and fiber organization. Healing in group B (ozonated saline) was noted good due but it lagged group C in mentioned parameters, whereas group A (control) was found inferior than group B and C. These results highlighted the therapeutic potential of ozonated preparations in avian ingluvies wound management, so we may suggest that ozonated oil is marvelous in such cure while ozonated saline is note at 2nd tier. However, further trials are needed for better understanding and routine clinical use of these regimens for avian practitioners.

Keywords: Ingluviotomy, ozonated oil, ozonated saline, wound healing, quails

1. INTRODUCTION

Digestive system of quail has esophageal out-pocketing called the crop or ingluvies, which serves as a temporary food storage and fermentation chamber. Commonly, crop is prone to injuries due to foreign bodies' ingestion including metals, feathers, stones etc., causing impaction or perforation. Moreover, septicemia

caused by leakage has negative health impact. In response to these issues, birds may undergo surgical investigation or ingluviotomy procedures. In newborns, crop rupture may occur from inappropriate gavage tube feeding. The associated clinical signs may include: regurgitation, lingering lump in crop, food retention and a delayed emptying time (1). Ingluviotomy procedure is surgical intervention performed on crop to correct numerous conditions like ruptures, impaction, or removal of foreign materials. After appropriate positioning and local anesthesia, a longitudinal incision is made on the skin and wall of the crop. After anomalies correction, single or double layer closure is for the closure (2). Inflammation, proliferation and remodeling are the overlapping stages of crop wound healing. Inflammatory phase is characterized by hemostasis and inflammation while proliferation involves epithelialization, angiogenesis, granulation and collagen deposition. Maturation or remodeling phase is the most important as here collagen deposits in an organized and well-mannered network. Ischemia, foreign bodies, edema, contamination etc. are some factors that may affect the wound healing process (3). Ozone (O_3) is a gas with three oxygen atoms and for a long time, it has been recognized as effective therapeutic agent for wound treatment with significant results in this regard and also as a powerful antibacterial agent (4). Medical ozone (O_2-O_3) is prepared with ozone generator, which exposes pure oxygen to electricity. O_2 molecule breaks down and some reunite into three to form O_3 i.e. O_2-O_3 ratio is about 95%-5% approx., respectively. The medical ozone (O_2-O_3) reveals various properties of ozone including antioxidant, anti-inflammatory, immune-regulatory etc. that may be very effective for wound healing (5). This medical ozone may further be mixed in normal saline or olive oil for wound therapy. As an adjuvant therapy, ozonated saline or oil may be appropriate fluids for irrigation and sterilization. These are justified by raised oxygen tension at wound site which promotes granulation tissue, collagen contents, tensile strength etc. that speeds up healing process (6).

Crop perforation is common in quails and very serious problem for individuals and at farms. The aim of study is to identify and compare crop rupture healing by ozonated-saline and ozonated-oil, in terms of healing efficacy and to overcome associated problems. This may be helpful for practitioners to handle crop related problems at ease with speedy recoveries. To establish these regimens as routine practice, randomized controlled trials may be in need.

2. MATERIALS AND METHODS:

2.1. Ethical Approval

The study and related treatments were conducted as per protocols, by Ethical Review Committee (Approval. No: DR/335, dated: 12-07-2023), University of Veterinary and Animal Sciences Lahore, Pakistan.

2.2. Selection of animals

The study was carried out on clinical cases, with obstruction or ruptured crop and in need of surgical intervention. The cases were treated at Pet Centre-UVAS Lahore, various quail farms and private clinics in Lahore, Pakistan.

2.3. Research design and treatment

Eighteen quails were selected, regardless of breed or sex, with age and weight about 5-6 months and 100-150 g, respectively. These were randomly assigned to three groups: A, B and C (n=06 per group) and numbered A1-A6, B1-B6, and C1-C6. In group A (control), sutures were applied on crop incision and normal saline was administered. In group B, after sutures quails were subjected to ozonated saline while in group C, after sutures ozonated oil was administered. All treatments were given per oral, 3 times a week for 20 days.

2.4. Anesthesia and procedures

Birds were administered local anesthesia i.e. lidocaine (Lidocaine®, Amson) @ 2.7-3.3 mg/kg, SC. Feathers were plucked manually and surgical site (base of the neck, over the crop) prepared aseptically to avoid any sort of contamination. Scrubbing of the surgical site was done with Povidone iodine (Pyodine scrub®, Brooke Pharma). After sterile draping, birds were positioned in a dorsal recumbency with head lifted and oesophagus covered with moist gauze to prevent drenching during procedure. Ingluviotomy surgery was performed by

cranio-caudal skin and crop incision. After successful issue management, crop was sutured by double layered suturing as per (1). The study was carried out for 3 weeks and the parameters were evaluated at day 0, 1, 5, 10, 15 and 20.

2.5. Post-operative care and management

After surgery quails were kept under observation to avoid any post-operative complication. Meloxicam (Melonac®, ICI Limited) @ 0.2mg/kg was administered to control pain and inflammation for 3 days. Normal saline, ozonated-saline and ozonated oil @ 0.5 ml (PO) were administered as per designated groups.

2.6. Parameters evaluated

2.6.1. Clinical vitals

Physical vitals including body temperature, pulse, and respiratory rates were recorded on stipulated days. Temperatures were monitored per rectum; heartbeat and respiration via heart/lung's auscultation.

2.6.2. Hematological indexes

Blood from wing vein (1ml) was collected within anticoagulant container (K_2/EDTA). Complete blood count was performed with 'Hematology Analyzer BC-2800 Vet (Mindray)' to rule potential blood abnormalities including anemia, infection, dehydration etc.

2.6.3. Leakage evaluation

Leakage from incision site was visualized and monitored by contrast radiography on stipulated days by using contrast media 'Diatrizoate Meglumine/Diatrizoate Sodium' (Gastrografin®, McKesson). Radiographs were taken by digital x-ray machine FDR smart-X (Fujifilm®).

2.6.4. Histological examination

At end of trial, small biopsy samples were collected, fixed in 10% formalin solution and send to a histopathology lab, Pathology Department, UVAS-Lahore for histological evaluation with Hematoxylin-Eosin and Masson's Trichome stains.

2.6.5. Electron microscopy

A small portion from biopsy sample was stored in biopsy jar, later dried thoroughly and mounted on slides to be dispatched to 'Laser Physics department, University of Engineering and Technology, Lahore' for SEM analysis.

2.7. Statistical Analysis

Data obtained was analyzed by statistical software GraphPad prism 6, by using 2-way ANOVA. Significance among groups was evaluated by Tuckey's test. Graphically presentation was done through GraphPad. Significant values were denoted by ($P \leq 0.05$).

Figure-1

[A]: Crop exposure after incision
[B]: Preparation of ozonated saline
[C]: Ozonated oil

3. RESULTS:

3.1. Physiological vitals

Clinical vitals revealed, an elevation in temperature above normal physiological limits (normal=73.4-78.8 ^{o}F) in each group, at day 1. Then it slightly decreased to upper normal limits at day 5, onwards gradual decline to normal base values was observed during rest study period. No significant difference (P≥0.05) among groups was observed during trials. For pulse rate, at day 1, a slight increase to upper normal range (normal=537-605) was seen in each rabbit. From day 5, it declined to lower limits and fluctuated here for rest of studies, in all groups. Statistical difference (P≤0.05) was noted between groups A and C at day 1 only. A slight increment in respiration, within normal limits (normal=30-60) was seen in groups, at day 1. Later, the values oscillated at variable levels during studies. No significant difference (P≥0.05) among groups was observed for respiratory rates (Figure-2).

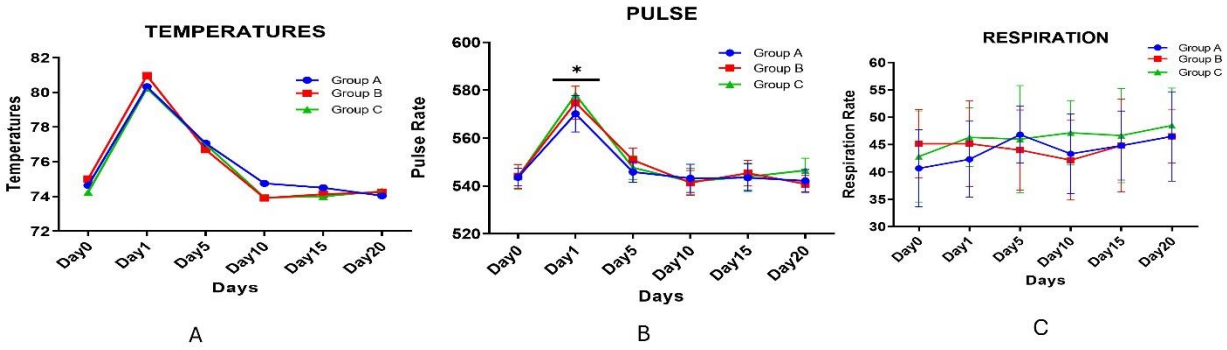

Figure-2: Time-line of changes in Clinical Vitals: (A) Temperatures, (B) Pulse, (C) Respirations.

3.2. Hematological indexes

White blood cells count was observed at lower normal ranges (normal=20-40), for each group, throughout study period. Exception was seen at day 1 and 5, when WBCs increased sufficiently above normal levels. Among groups, no significant difference (P≥0.05) was observed during trial. Platelets levels initially decreased below normal limits (normal=28.99-30.39) at day 1 and 5. Onwards, an up-trend was noted and values were at upper normal levels for remaining trial period. Statistically, no significant difference (P≥0.05) was noted between groups. Red blood cells and hemoglobin were noted fluctuating within physiological limits, throughout the trials for each group. There was no significant difference (P≥0.05) between groups for both parameters during studies (Figure-3).

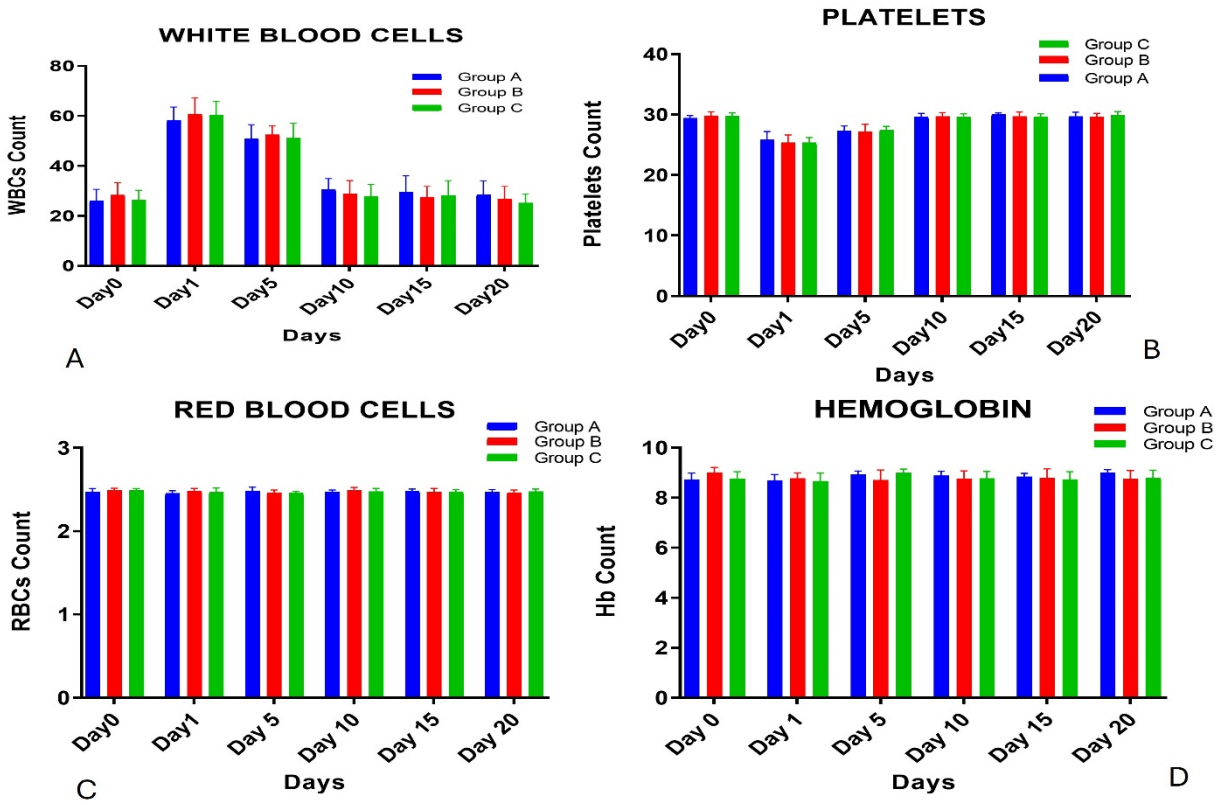

Figure-3: Time-line of changes in Hematology: (A) WBCs, (B) Platelets, (C) RBCs, (D) Hb

3.3. Leakage evaluation

Severe to moderate leakage was seen in group A quails (A1, A4 and A6), moderate to mild in group B (B4,B5) and mild in group C (C2,C4) from day 5 to variable intervals, during studies. Statistical analysis showed a significant difference (P≤0.05) at day 1, between groups A and C (Figure-4) (Figure-5).

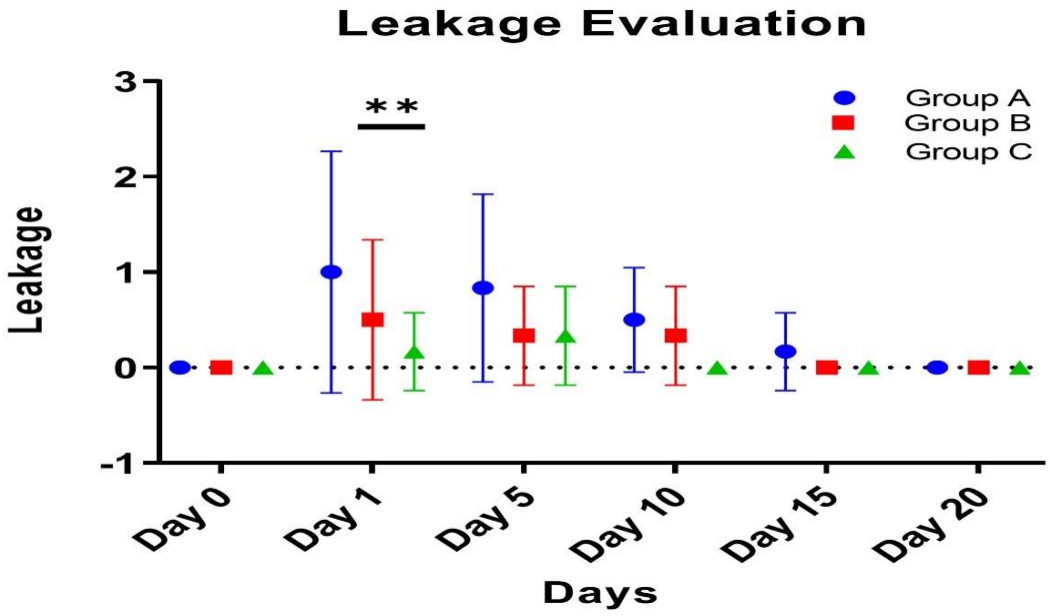

Figure-4: Time-line of changes for evaluation of leakage: Significant difference (P≤0.05) at day 1, between groups A and C.

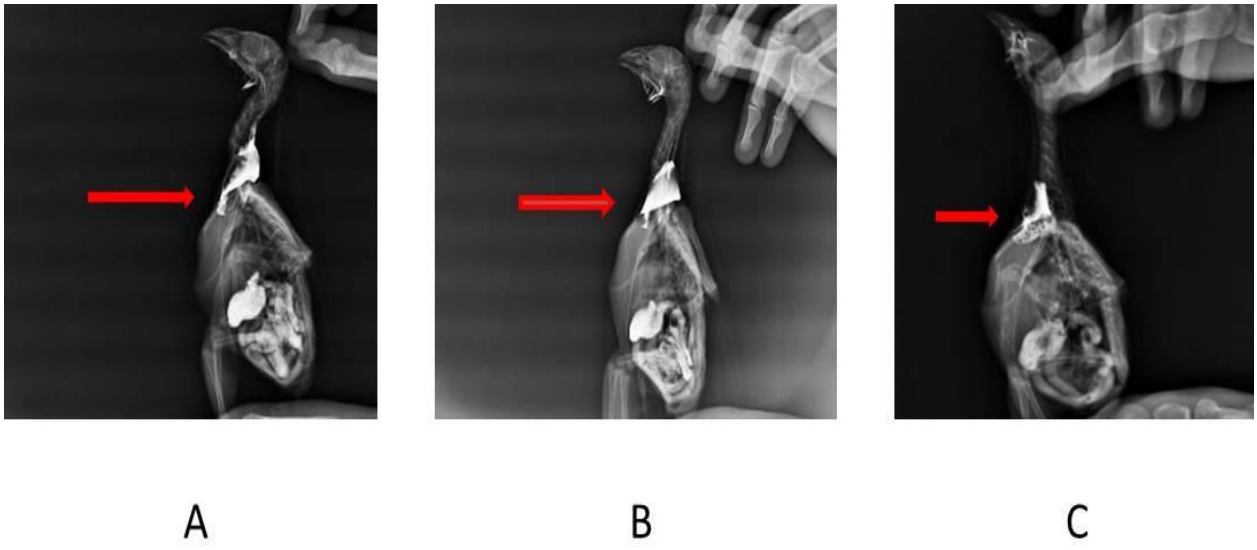

Figure-5: Radiology: Contrast radiography of three groups. Moderate, mild and no leakage in group A, B and C

3.4. Histological evaluation

At trial end, ingluvies healing was histologically evaluated by Hematoxylin-Eosin and Masson Trichome stains to observe re-epithelialization, vascularization, collagen arrangement and density in the tissue. Excellent score relating cellular organization, angiogenesis and collagen were noticed for group C (ozonated oil), followed by group B (ozonated saline and A (control), respectively (Figure-6) (Table-1).

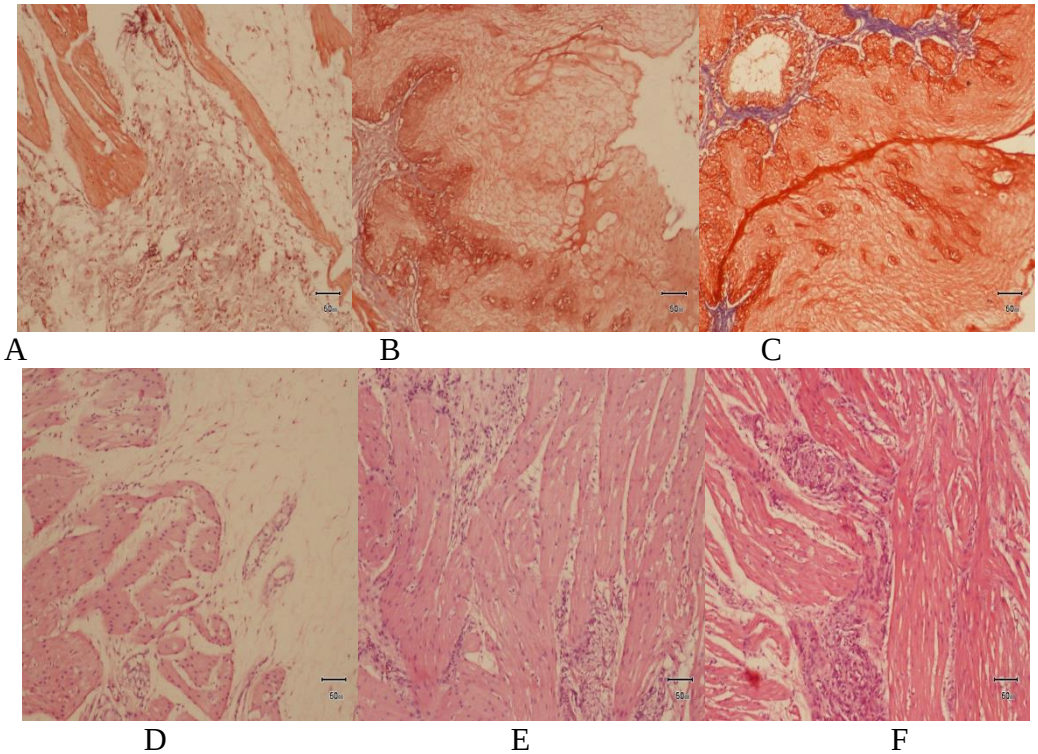

Figure-6: Histological findings-Ingluvies healing (A, B, C): H & E stain, (D, E, F): MT stain

Histology Evaluation				
Groups	**Hematoxylin & Eosin Staining**	**Masson's Trichrome Staining**		

	Inflammation	Re-epithelization	Vascularization	Collagen Array	Collagen Density	Overall Individual Score	Group Healing Points
A1	2	1	2	2	2	9	64
A2	1	2	3	2	3	11	
A3	1	3	3	3	3	13	
A4	2	1	2	2	2	9	
A5	1	2	3	2	3	11	
A6	1	3	2	3	2	11	
B1	1	4	3	3	3	14	76
B2	0	3	4	3	4	14	
B3	1	4	4	3	3	15	
B4	0	3	2	2	3	10	
B5	0	2	3	2	2	9	
B6	1	3	3	4	3	14	
C1	1	4	4	3	4	16	86
C2	0	4	3	4	3	14	
C3	0	3	4	3	4	14	
C4	0	4	3	3	3	13	
C5	1	3	3	4	3	14	
C6	0	4	4	3	4	15	

Table.1: Histological Scoring**3.5. Electron microscopy**

Scanning electron microscope (SEM) further provided high resolution details of cells within wound bed, matrix remodeling, collagen deposition, neo-vasculature and tissue integration etc. It was identified that group C (ozonated oil) showed marvelous results in healing of crop tissue, followed by group B (ozonated saline) and group A (control), respectively (Figure-7).

A B C

Figure-7: Scanning Electron Microscopy

[A]= Poor Surface wound healing (group A)
[B]= Moderate Surface wound healing (group B)
[C]= Good Surface wound healing (group C)

4. DISCUSSION

A mild rise in temperatures was noted in each group at day 1, onwards a slight decreased was seen to normal limits. This increment may be due to inflammation, stress condition and tissue injuries (7). A slight increased heartbeat and respiratory rates were noticed on day 1, and then a gradual decline to normal limits was observed for the remaining period. These parameters alterations may be associated with surgical and environmental stress, hypoxia, numerous extrinsic/intrinsic factors etc.(8).

Noticeable rise in white blood cells was observed at first week post-ingluviotomy, in all groups. The associated reason may be incisional exposure to environment, release of inflammatory cells into circulation, signals of defense mechanism, stress/inflammatory response etc. (9-11). A mild thrombocytopenia was observed at day

1 post-surgery but from day 5 and onwards, platelets count returned to normal physiological level. This initial downtrend in the count was due to pro-coagulant activity, involvement of platelets in plug formation and their transfer to injured tissue etc. (12). Red blood cells and hemoglobin remained within physiological limits throughout the trials, for each group. As ingluviotomy repairs is a minor surgery, with least bleeding or destruction of RBCs so ultimately normal RBCs and Hb levels were observed after procedures (13).

Leakage evaluation is the vital parameter that determines success of the procedure. We evaluated leakage by contrast radiography which showed its highest incidence in control group and mild to least in group B and C, respectively. Results revealed that group C (Ozonated oil) showed more medicine retention time with ability to activate healing mediators, increased expression of fibroblasts and their speedy migration to injured tissues for speedy recovery. Group B (Ozonated saline), was noted good but lagged behind due to less medicine retention within crop than ozonated-oil. Group A (control) was noted inferior than all (2, 14).

Histological evaluation and scores were done by stains i.e. Hematoxylin & Eosin and Masson's Trichome. H&E staining measured the re-epithelization, inflammation and vascularization while MT staining determined the collagen array and density in healed crop tissue. It was noted that group C (Ozonated oil) accelerated in all stages i.e. forming of neo-vessels, collagen matrix remodeling etc. resulting in faster tissue regeneration and improved wound healing than group B (ozonated saline) and group A (control). Group B and A were noted at 2^{nd} and 3^{rd} tier respectively. MT outcomes relating collagen array and strength, manifested similar results for groups as of H&E stain (15).

Scanning electron microscopy revealed crop healing in more accuracy, showing regeneration of ingluvies layer, collagen development and neo-angiogenesis where re-epithelization and collagen fibers play a pivotal role for healing. As per our observations, group C (ozonated oil) showed marvelous results in this regard; group B (Ozonated saline) manifested good healing while group A (control) revealed poor results (16, 17).

5. CONCLUSION:

Our study compares the effectiveness of ozonated oil and saline in healing of ingluvies wounds in quails (Coturnix japonica). The findings indicate that ozonated oil proves to be a superior therapeutic agent that exerted significant effects on wound closure, inflammation reduction and regeneration of tissue as compared to ozonated and norma saline. These findings may be significant for avian veterinary practice, providing potential alternative therapy for the treatment of ingluvies wounds, with least antibiotic resistance in quails. We may further suggest that future investigations regarding these regimens to refine treatment methods and examining its potential for wider use in avian wound care practices.

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Conflict of interest

Authors declare no conflict of interests.

Authors Contribution

Conceptualization: Naveed Hussain, Haroon Akbar

Research conduct and manuscript preparation: Sohaib Hassan, Naveed Hussain, Haroon Akbar

Data Analysis: Sohaib Hassan, Zia Ullah, Naveed Hussain

Supervision: Naveed Hussain, Hamad Bin Rashid, Aneela Zameer Durrani

Resources: Mian Saeed Sarwar, Amber Fatima, Zia Ullah, Hamad Bin Rashid

Writing Review: Sohaib Hassan, Zia Ullah, Amber Fatima, Mian Saeed Sarwar, Aneela Zameer Durrani,

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