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CHOOSING THE RIGHT PATH: COMPARATIVE ANALYSIS OF RABBIT ARTHRITIS INDUCTION

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Abstract:
Rheumatoid arthritis (RA) is a chronic autoimmune disorder causing inflammation in the joints, leading to debilitating effects. The establishment of dependable animal models for studying RA is critical in understanding its origins and devising effective treatments. In this study, we examined four distinct methods for inducing RA in a rabbit model. Different groups of rabbits were subjected to induction using killed E. coli, formaldehyde, normal saline, and Lugol's iodine, while a control group received no induction. The rabbits underwent assessment through radiographic analysis, pre- and post-induction joint circumference measurements, palpation reports, and visual examination of joint inflammation. Our findings highlight that the killed E. coli injection method yielded the most notable RA-like symptoms compared to the other induction methods. This suggests that the killed E. coli-induced model holds promise as a dependable and efficient tool for studying RA in rabbits.

Keywords: Rheumatoid Arthritis, Killed E coli, Formaldehyde, stiffness, autoimmune Disease.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that progressively affects small to large joints and various organs, causing inflammation in joints, ligaments, cartilage, and bones. The altered synovial tissue leads to stiffness, bone destruction, and functional limitations. Symptoms include morning stiffness, fatigue, fever, weight loss, and joint stiffness, typically emerging between ages 35 and 60, with periods of remission and flare-ups. Rheumatoid arthritis can also affect children (juvenile rheumatoid arthritis). The incidence is 1-2% in the Western population and 1% worldwide.[1] Moreover, as ongoing research delves into the complex causes of rheumatoid arthritis and seeks to develop effective diagnostics and treatments, it becomes increasingly apparent that the higher prevalence of RA in women, with a 3:1 female-to-male ratio, presents a significant aspect requiring further investigation. The complex relationship between sex and RA is not fully understood. Gender medicine explores how gender differences affect physiology, pathology, and clinical aspects of diseases, considering biological, psychological, and cultural factors. Understanding sexual

dimorphism in RA susceptibility, presentation, and outcomes could pave the way for personalized approaches. While women often experience higher RA disease activity, there's evidence suggesting that men may respond better to certain therapies.[2] Expanding research efforts to refine treatment approaches for both genders holds the potential to greatly improve our comprehension and management of rheumatic diseases. Among these conditions are rheumatoid arthritis, juvenile idiopathic arthritis, seronegative spondyloarthropathies, and systemic lupus erythematosus, all of which are distinguished by inflammation related to skeletal concerns.While there are common mechanisms in skeletal remodelling, each disease uniquely affects different types of bones. Osteoclasts are important in the repair of bone loss and are affected by cytokines and growth factors . These factors directly or indirectly affect osteoclast activity by regulating molecules such as nucle ar factor kappa B ligand (RANKL) and osteoprotegerin (OPG) receptor activator.[3]

Further investigation of these mechanisms may reveal new treatment strategies to reduce bone loss f rom rheumatic diseases. It is also important to know that this disease affects many organs, including the cardiovascular, pulmonary, gastrointestinal, musculoskeletal, hematological, renal, neurological and cutaneous systems. [4]

Understanding the effects of various medications on the immune system is important for people wit h this disease because they often need more than one medication. This information is particularly im portant because early intervention is important to effectively control symptoms, improve functional capacity, and minimize damage in rheumatoid arthritis (RA). While NSAIDs and corticosteroids ca n reduce symptoms, modified-release anti-

inflammatory drugs (DMARDs), especially newer biologics, play a significant role in disease modif ication. Early treatment, including the use of biologic drugs such as TNF-α inhibitors and IL-

1 receptor antagonists, has improved disease control and slowed bone erosion. A balanced approach to medication and lifestyle is important in the treatment of RA. Methotrexate (MTX) was the first DMARD that was well studied and widely used due to its clinical benefits. Biological drugs are effe ctive in reducing damage, primarily TNF-

α inhibitors, often in combination with MTX. New biologic drugs such as denosumab are being dev eloped to expand treatment.[5]

Overall, ongoing research is exploring further options, underlining the crucial role of early intervention and a holistic approach to treating rheumatoid arthritis (RA). Meanwhile, the utility of anti-cyclic citrullinated peptide (anti-CCP) antibody testing in RA diagnosis is notable, given its high specificity, early detection capability, and ability to identify patients prone to severe disease and irreversible damage. However, its sensitivity is low, and a negative result does not exclude disease. Anti-CCP antibodies have not been found at a significant frequency in other diseases to date, and are more specific than rheumatoid factor for detecting rheumatoid arthritis. [5]

Animal models of arthritis are used to study the pathogenesis of the disease and to evaluate the pote ntial for clinical use of antiarthritic drugs. Therefore, morphological similarity to human disease and the ability of the model to predict human performance are important criteria for model selection. A mong animal models of rheumatoid arthritis (RA), those with evidence predicting human efficacy ar e particularly important. Intermediary.[6]

Animal models have conditions similar to those in humans and provide a powerful platform that inc reases the accuracy of predictions of drug efficacy in clinical situations. This demonstrates the effec tiveness of using animal models, especially models that resemble humans, as a method for rheumato id arthritis (RA) research. The importance of change. Animal models are important to reveal the bas is and mechanisms of RA**.**.[7]

In choosing a good animal model, special needs should be taken into account and its unique charact eristics and rules should be taken into account. Although collaboration with veterinarians is importa nt in animal experiments, scientists need to be familiar with experimental procedures for animal mo dels so that they can appropriately design their studies; This is especially important in areas with li mited veterinary resources. Rabbits are often chosen to test plant products because they have many advantages, but it is best to use them before testing them in large animal models. Although testing o n rabbits seems simple, there are some difficulties and pitfalls.[8]

The main aim of this study was to evaluate and compare four different methods of inducing rheumatoid arthritis in rabbits. The study aimed to determine the best way to treat arthritis in rabbits using different induction techniques. This comparison test was designed to determine which method causes the most pain and development of neck symptoms, including inflammation, soreness, and tissue damage. Ultimately, our aim is to provide an understanding of the best way to induce arthritis in rabbit models, thereby increasing the accuracy and reliability of preclinical studies aimed at improving the treatment of rheumatoid arthritis.

Literature Review

Animal models of autoimmune arthritis have become important tools for studying the pathogenic mechanisms of this disease and testing new treatments. Various mouse arthritis models have been developed using various induction methods. These include vaccinationinduced models such as proteoglycan-induced arthritis (PGIA), streptococcal cell wall arthritis, collagen induced arthritis (CIA), and antigen-induced arthritis.[9]

Additionally, the use of various druginduced or spontaneously induced animal models of fatinduced arthritis, including tumor necrosis factoralpha transgenic mice and K/BxN T cell receptor transgenic mice, has spoken of their role in shaping the important landscape. Development of modern drugs for rheumatoid arthritis (RA). Within this chapter, I explore the substantial impact of animal models on arthritis therapy, spanning from adjuvant arthritis and COX-1 inhibitors to transgenic mice and biological response modifiers. Enhanced comprehension of connective tissue disease mechanisms has significantly advanced through the examination of animal models, often revealing therapeutic targets that undergo subsequent evaluation within these models.[10]The essential connection between comprehending arthritis pathology and devising innovative therapeutic strategies, centred on the exploration of arthritis animal models, is underscored by the induction of chronic arthritis through granuloma formation, similar to FCA, by Mycobacterium tuberculosis (MTB).Although effective.[11]The use of Mycobacterium tuberculosis (MTB) requires adherence to biosafety precautions and may not entirely reproduce the autoimmune pathology observed in rheumatoid arthritis (RA). Conversely, the intra-articular injection of formaldehyde can trigger aseptic inflammation within the joint. However, the degree of inflammation and its duration may fluctuate, often leading to limited and transient chronic inflammation. As a result, this method may not fully emulate the persistent and intense inflammatory environment typically observed in certain arthritic conditions. [12]In a model simulating rheumatoid arthritis (RA) in animals, experiments were conducted in vitro on cell cultures to examine the influence of Escherichia coli lipopolysaccharide (E-LPS) on cytokine production. The findings revealed that treatment with B-LPS resulted in a notable dose-dependent reduction of cytokine production induced by E-LPS in both THP-1 monocytic cells and peripheral blood mononuclear cells (PBMCs). These results imply that B-LPS has the capacity to suppress the inflammatory response triggered by E-LPS in these cellular contexts, suggesting potential therapeutic implications for managing inflammation in rheumatoid arthritis.[13]

Employing rabbits as the animal model for arthritis induction presents numerous advantages. Unlike human bones, rabbit long bones exhibit a unique microstructure. In comparison to the secondary bone structure observed in mature human bones, rabbits demonstrate a primary vascular longitudinal tissue configuration. This arrangement comprises vascular canals of osteons running parallel to the bone's long axis, enveloping both the medullary canal and the periosteal surface. The bone material between these layers consists of dense haversian bone.

Limited literature exists on the differences between human and rabbit bone composition and density, yet some similarities have been observed, particularly in terms of bone mineral density and fracture toughness in mid-diaphyseal bone. Rabbits are preferred as experimental models due to their calm and non-aggressive nature, making them easy to handle and observe. Additionally, they are extensively bred and economically feasible compared to larger animals, with shorter vital cycles encompassing gestation, lactation, and puberty. Being classified as small animals, experimentation with rabbits falls under the jurisdiction of local ethical committees, thus bypassing the need for clearance from central ethical committees, which can be time-consuming and subject to strict

regulations. However, challenges persist in rabbit experimentation, including inadequate provision of well-equipped animal housing, a shortage of skilled handlers, limited access to appropriate medications, and a dearth of literature on rabbit care and experimental usage.[8] Moving toward the studies for induction of arthritis In multiple studies, arthritis has been induced in rabbits through intra-articular injections using agents such as normal saline, killed E. coli, Lugol's iodine, and formaldehyde. In our current research, arthritis is induced in rabbits through these four methods, and we analyse which method produces the most effective arthritic outcome.

When rabbits' knee joints were injected with distilled water or 10 percent sodium chloride, distinct effects were observed. Injecting distilled water resulted in thickening of synovial tissues and excessive fluid accumulation in the joint, while injecting normal sodium chloride led to even more severe changes. Although initial interpretations suggested similarities to osteoarthritis lesions, it's important to note that these findings may not be specific to any particular condition. Thus, caution is needed when interpreting these results as indicative of a specific disease process.[14]

An alternate method was employed, wherein bacteria for animal inoculation were cultivated in a medium containing only inorganic salts and glucose, with Escherichia coli and Bacillus subtilis being maintained in this medium through frequent transfers for over 2 years. These organisms were rendered inactive by the addition of 0.4 percent formalin, and stock suspensions were prepared using normal saline. Rabbits were then intravenously injected with a suspension of the inactivated bacteria, with smaller initial doses administered to minimize endotoxin toxicity. Blood samples were collected monthly for serum analysis, and serological methods were employed using heated sera for tests like hemagglutination, which were conducted in phosphate-buffered normal saline.[15]

An often-employed technique to induce arthritis entails the sub plantar administration of 0.1 ml of formaldehyde solution (2% v/v) into the paw of all animals. This action is designated as day 1. Subsequent to this initial induction, treatment with either vehicle or drugs continues for an additional 28 days. On the third day, a second injection of formaldehyde (0.1 ml, 2% v/v) is administered into the same paw to sustain the inflammatory response. This method facilitates the assessment of the impact of various treatments on the progression of arthritis throughout a specified timeframe.[12].

Material and Methods

Material

Killed E coli from microbiology lab DVM department, formaldehyde from microbiology lab DVM of arid agriculture department, syringes, lugol’s iodine, normal saline from microbiology department

Animal Model

Thirty male New Zealand white rabbits, weighing between 1.15 to 1.70 kg, were employed for this study. The rabbits were accommodated in a room with controlled temperature and humidity, and they were provided with a standard diet sourced from the Animal Nutrition department of NARC Islamabad. All experimental procedures adhered to the regulations outlined by the Animal Research Committee of the Faculty of Veterinary and Animal Sciences, DVM Department, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

Comparative Arthritis Induction in Rabbits

This study aims to rigorously compare four distinct methods of arthritis induction in rabbits:

Group 1: (Killed E Coli)

The group includes six male New Zealand White rabbits weighing between 1.15 and 1.7 kg. They i njected inactive E. coli (0.3 ml) into his right knee three times a week for 6 weeks. This approach in volves teaching bacteria to measure their effects on arthritis.

- **Group 2 (2% Formaldehyde Solution Injection):**

Another group of six male New Zealand white rabbits also joined the group. 2% formaldehyde solut ion (0.1 ml) was injected intra-

articularly into his right knee three times a week for 6 weeks. The purpose of using formaldehyde is to evaluate and compare as well as to stimulate arthritis.

- **Group 3 (Normal Saline Injection):**

The control group consisted of six male New Zealand White rabbits. Intra-articular saline (1 ml) was injected into his right knee three times a week for 6 weeks. This group provided a basis for comparison and allowed the development of arthritis to be assessed without an induction agent.

- **Group 4 (Lugol's Iodine Injection):**

In this group, six male New Zealand White rabbits received intra-articular injections of Lugol's iodine (1 ml) into the right knee three times a week for 6 weeks. The use of Lugol's iodine as an arthritis inducer provides a unique perspective on the health effects of iodine.

- **Group 5 (Control Group):**

The group includes six male New Zealand White rabbits weighing between 1.15 and 1.7 kg. and compare each group with this group for environmental differences, palpation reports, and visual inspection

The trial design included all different methods of arthritis induction, improving the quality of the study and making it easier to evaluate the effectiveness and impact of each method.

Parameters For Evaluation Of Arthritis

Six male New Zealand white rabbits, each weighing between 1.15 and 1.7 kg, were assigned to individual groups. The main outcome measures included:

A. **Radiographic Assessment**: X-rays of the knees were captured before and after the 6-week period to assess joint damage.

B. **Gross Examination** : To gauge the degree of arthritis or inflammation, joint swelling was evaluated by measuring the maximum diameter of the swollen joint through measurement of

I. Circumference.
II. visual inspection.
III. palpation reports.

I.Radiographic Assessment :

The radiographic assessment was done before the induction of arthritis and at last day of induction of arthritis. And arthritis in the knee right knee joint was graded as

Grade 0: Absence of pathological characteristics;
Grade 1: Incipient joint space narrowing with possible osteophytic lipping;
Grade 2: Presence of definitive osteophytes with potential joint space reduction;
Grade 3: Moderate manifestation of multiple osteophytes, evident reduction in joint space, some sclerosis, and potential bony end deformity;
Grade 4: Significant joint space reduction, severe sclerosis, and definitive bony end deformity.

II.Circumference.

Micrometer screw gauge was used for measuring the diameter of the rabbit knee joints . The difference in the circumference was noted at 1st day, 7th day, 14th day, 21th day, 28th day, 35th day, 42th day of this six week research.

III.Visual Inspection.

The visual inspection of each rabbit was done at day 1 and 42th day of the research. The visual inspection was scored at presence of following factors.

- Swelling

- Skin Joint(Redness Of Skin)
- Mobility
- Deformity.

Presence of each factor was scored at 1 and absence of each factor was scored as zero**.**

IV.Palpation Reports.

The Palpation Reports of each rabbit was done at day 1 and 42th day of the research. The palpation reports were scored at presence of following factors.

Temperature

Fluid Movement (Fluctuation)

Tenderness

Crepitus

Presence of each factor was scored at 1 and absence of each factor was scored as zero**.**

I.Statistical Analysis

Data were shown as mean ± standard error of the mean and evaluated and result were considered significant as p value is less than 0.05.

Results

The results were submitted after assessing all parameters within each group.

- Radiographic Assessment
- Circumference
- Visual inspection
- Palpation reports

1. Results on the basis of Radiographic Assessment.

Statistically, an examination was conducted comparing all groups through radiographic assessment. The analysis revealed that the p-value of the study falls below the critical threshold of 0.05, signifying statistically significant findings.

The table 1 provided displays the outcomes of a one-way ANOVA test, indicating a statistically significant contrast among all five groups in terms of radiographic analysis.

The figure 1 shows the knee joint of the rabbits where the e coli was injected and the joint become so much damaged.

To evaluate the individual groups, we initially performed a one-way ANOVA and subsequently employed Dunnett's test for post hoc multiple comparisons. Below are the findings

The table 2 post hoc presented illustrates the analysis performed among individual groups, highlighting significant findings for all groups. Specifically, the comparison between Group 4 (Lugol's Iodine) and Group 5 (control group) indicates non-significance, suggesting that rabbits administered with Lugol's Iodine do not demonstrate a significant difference in radiographic analysis compared to the control group.

Table No 1: ANOVA

radiographic

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	47.800	4	11.950	52.721	.000
Within Groups	5.667	25	.227		
Total	53.467	29			

Table 1 The Anova Test For Radiographic assessment

Table No 2:

Multiple Comparisons

Dependent Variable: radiographic
Dunnett t (>control)[a]

(I) Treatment group	(J) Treatment group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound
1	5	3.333*	.275	.000	2.71
2	5	2.500*	.275	.000	1.87
3	5	1.500*	.275	.000	.87
4	5	.333	.275	.293	-.29

*. The mean difference is significant at the 0.05 level.
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 2 Post Hoc dunnett For Radiographic Analysis

Figure 1

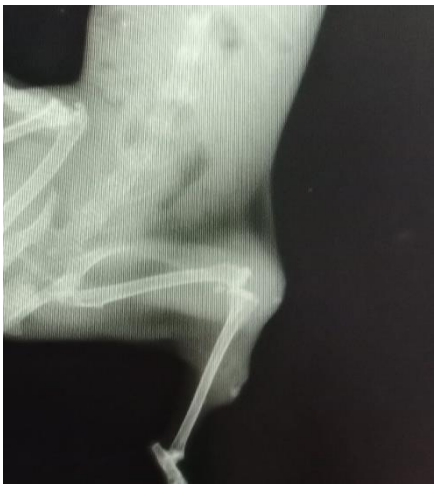

Figure 1 x-ray shows the knee joint of the rabbits where the killed e coli was injected

Graphical Presentation of Radiographic comparison

In the figure 2: The graphical representation also indicates that the treatment group 1 (killed E. coli) demonstrates the most favourable results based on radiographic analysis.

Figure 2:

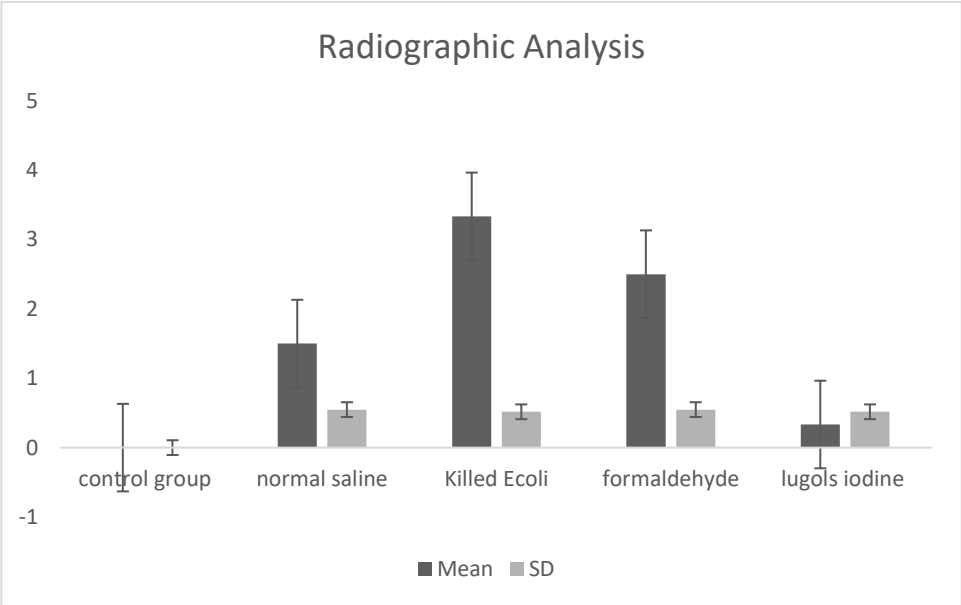

Figure 2: show the radiographic analysis .it show the mean and standard deviation in the data for the radiographic analysis between all the treatment groups

2. Results on the basis of Circumference Of Rabbits.

Statistically, an examination was conducted comparing all groups through circumference of joints of rabbits . The analysis revealed that the p-value of the study falls below the critical threshold of 0.05, signifying statistically significant findings.

The table 3 provided displays the outcomes of a one-way ANOVA test, indicating a statistically significant contrast among all five groups in terms of circumference of joints of rabbits.

To evaluate the individual groups, we initially performed a one-way ANOVA and subsequently employed Dunnett's test for post hoc multiple comparisons. Below are the findingsThe table 4 post Hoc presented illustrates the analysis performed among individual groups, highlighting significant findings for all groups.

Table 3:

ANOVA

circumference

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	980.467	4	245.117	112.439	.000
Within Groups	54.500	25	2.180		
Total	1034.967	29			

Table 3 Anova test for circumference of joints of rabbits

Table 4:

Multiple Comparisons

Dependent Variable: circumference

Dunnett t (>control)[a]

(I) Treatment group	(J) Treatment group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
					Lower Bound
1	5	15.167*	.852	.000	13.23
2	5	12.667*	.852	.000	10.73
3	5	7.500*	.852	.000	5.56
4	5	2.833*	.852	.005	.89

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 4 post Hoc Dunnett For multiple comparison

Graphical Presentation of Circumference of Joints of Rabbits

Figure 3: The graphical representation also indicates that the treatment group 1 (killed E. coli) demonstrates the most favorable results based on circumference of joints.

Figure 3:

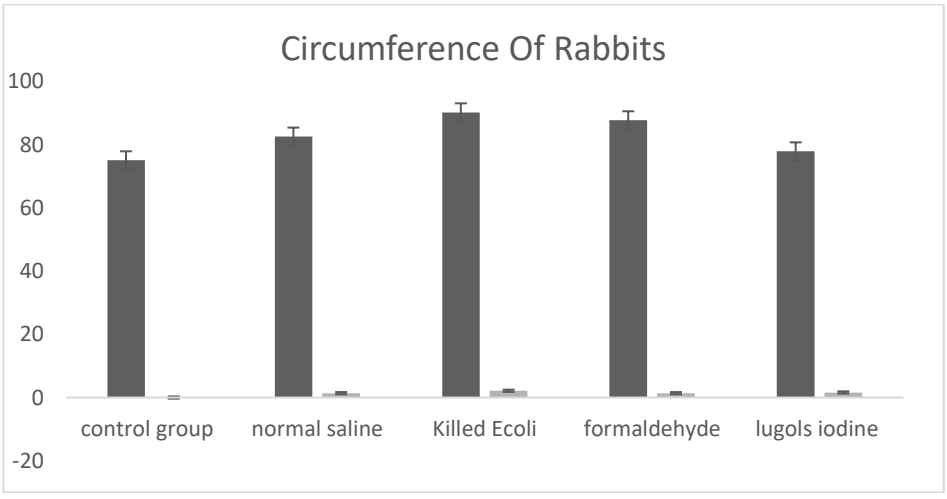

Figure 3: show the circumference of the joints .it show the mean and standard deviation in the data for the circumference of joints between all the treatment groups.

Figure 4: It clearly indicates the inflammation on the joint.

Figure 4:

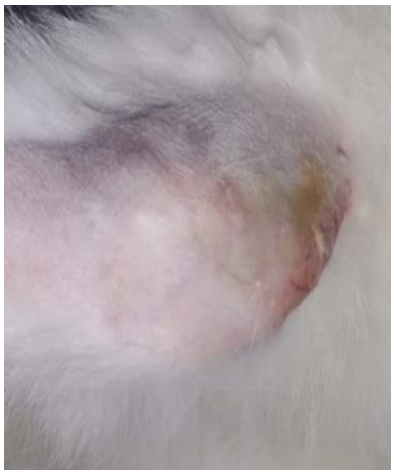

Figure 4: The figure shows the inflammation of the joint

3. Results on the basis of Visual inspection Of Rabbits.

Statistically, an examination was conducted comparing all groups through visual inspection of rabbits. The analysis revealed that the p-value of the study falls below the critical threshold of 0.05, signifying statistically significant findings.

The table 5 provided displays the outcomes of a one-way ANOVA test, indicating a statistically significant contrast among all five groups in terms of visual inspection.

To evaluate the individual groups, we initially performed a one-way ANOVA and subsequently employed Dunnett's test for post hoc multiple comparisons. Below are the findings

The table 6 post Hoc presented illustrates the analysis performed among individual groups, highlighting significant findings for all groups.

Table 5:

ANOVA

visual inspection

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	57.333	4	14.333	134.375	.000
Within Groups	2.667	25	.107		
Total	60.000	29			

Table 5 Anova Result For Visual inspection

Table 6:

Multiple Comparisons

Dependent Variable: visual inspection

Dunnett t (>control)[a]

(I) Treatment group	(J) Treatment group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
					Lower Bound
1	5	4.000*	.189	.000	3.57
2	5	3.000*	.189	.000	2.57
3	5	1.667*	.189	.000	1.24
4	5	1.333*	.189	.000	.90

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 6 post Hoc Dunnett for multiple comparsion of visual inspection

Graphical Presentation of Visual Inspection of Joints of Rabbits

Figure 5: The graphical representation also indicates that the treatment group 1 (killed E. coli) demonstrates the most favourable results based on visual inspection.

Figure 5:

Figure 5: Show the visual inspection of the joints .it show the mean and standard deviation in the data for the visual inspection of joints between all the treatment groups

The figure 6 shows the visual inspection for the redness of the joint in rabbit model

Figure 6:

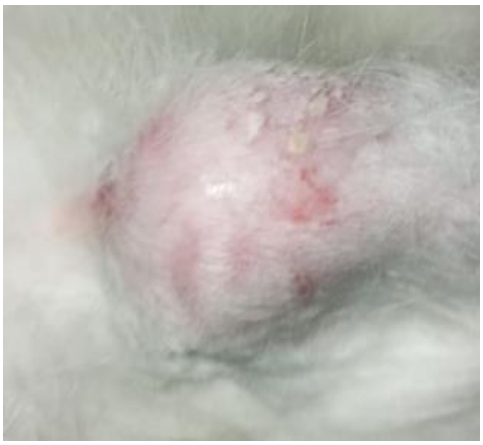

Figure 6: The figures for visual inspection shows the redness of the joint where induction of the arthritis occurs

4. Results on the basis of Palpation reports Of Joints Of Rabbits.

Statistically, an examination was conducted comparing all groups through visual inspection of rabbits. The analysis revealed that the p-value of the study falls below the critical threshold of 0.05, signifying statistically significant findings.

The table 7 provided displays the outcomes of a one-way ANOVA test, indicating a statistically significant contrast among all five groups in terms of palpation report.

To evaluate the individual groups, we initially performed a one-way ANOVA and subsequently employed Dunnett's test for post hoc multiple comparisons. Below are the findings:

The table 8 psot hoc presented illustrates the analysis performed among individual groups, highlighting significant findings for all groups. Specifically, the comparison between Group 4 (Lugol's Iodine) and Group 5 (control group) indicates non-significance, suggesting that rabbits

administered with Lugol's Iodine do not demonstrate a significant difference in palpation report compared to the control group.

Table 7:

ANOVA

palpation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	85.333	4	21.333	78.049	.000
Within Groups	6.833	25	.273		
Total	92.167	29			

Table 7 Anova results of Palpation comparison

Table 8:

Multiple Comparisons

Dependent Variable: palpation

Dunnett t (>control)[a]

(I) Treatment group	(J) Treatment group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
					Lower Bound
1	5	4.000*	.302	.000	3.31
2	5	3.500*	.302	.000	2.81
3	5	1.667*	.302	.000	.98
4	5	.000	.302	.800	-.69

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 8 post hoc dunnett for multiple comparison for palpation reports

Graphical Presentation of Palpation report of Joints of Rabbits

Figure 7 : The graphical representation also indicates that the treatment group 1 (killed E. coli) and treatment group 2 (formaldehyde induced) demonstrates the most favourable results based on palpation report.

Figure 7:

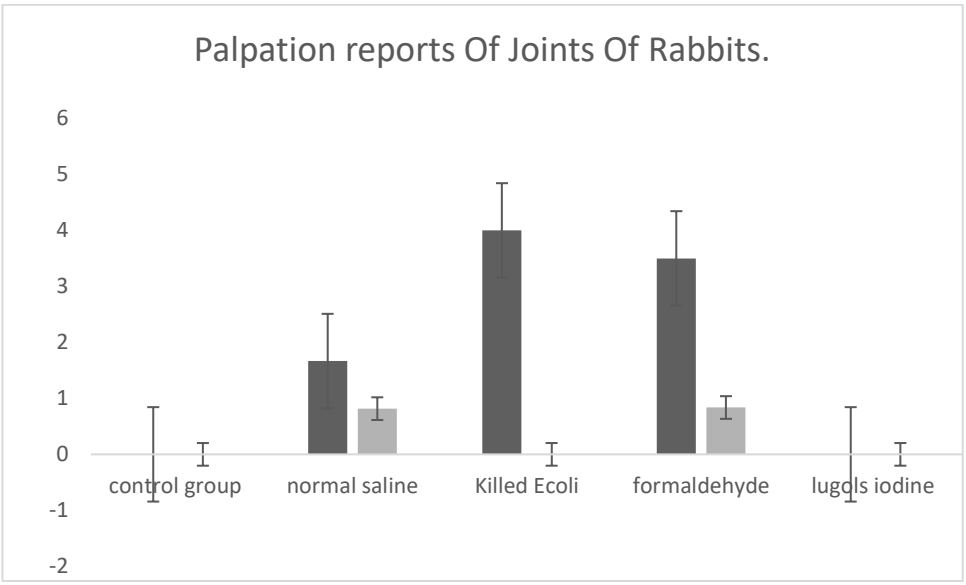

Figure 7 Show the visual inspection of the joints .it show the mean and standard deviation in the data for the visual inspection of joints between all the treatment groups

Conclusion

In conclusion, our study highlights that inducing rheumatoid arthritis (RA) in rabbits using killed E. coli injection results in more significant and consistent pathological changes in the joints compared

to other induction methods such as formaldehyde, normal saline, and Lugol's iodine. The effectiveness of induction of inactivated E. coli has been demonstrated on multiple parameters, including radiographic analysis, joint assessment, palpation, and visual assessment of joints.
These findings highlight the use of the E.coli induced model as an important tool to study the pathogenesis of RA and evaluate therapeutic interventions in preclinical studies. Future research directions should focus on uncovering the underyingmechanismsinvolved in E. coli. Escherichia coli induced arthritis in rabbits and its effects on human RA.

Discussion

Rheumatoid arthritis (RA) is a complex autoimmune disease that results in cartilage and bone erosion and joint inflammation, leading to pain and disability. The use of animal models is important for understanding the pathogenesis of RA and evaluating treatment strategies. This study aimed to compare four different methods of inducing RA in rabbits to identify a model similar to the human disease. Pathological changes in the joints include formaldehyde, physiological saline and Lugol's iodine. This is consistent with previous studies involving bacteria, specifically lipopolysaccharide (LPS) from E. coli. E. coli E. coli can cause an immune response similar to RA in humans. In addition, the size of the joints after induction indicates the presence of edema and synovial inflammation, which are characteristic of RA pathology. Palpation reports and visual tests also support the activity of inactivated E. coli that triggers the proliferation of RAlike symptoms. The pattern caused by E. coli can be determined by its ability to establish a strong and vigorous immune system in the joint, evoking similar receptor (TLR) activation and other immune responses in the body. In contrast, the induction method containing formaldehyde, saline, and Lugol's iodine will not have the immunogenicity to induce RAlike arthritis. First, the mechanism by which inactivated E. coli causes arthritis in rabbits is not fully understood, and more research on the immune system is needed. Additionally, although our study focused on short-term results, long-term studies are needed to evaluate the long-term effects of killing E. coliinduced arthritis. Comparison of the rabbit model with other RA research methods. These models are an important tool for studying the pathogenesis of RA, evaluating new treatments, and improving our understanding of the disease. Future studies should refine this model, develop translational interventions for human RA, and identify therapeutic targets for intervention.

Authors contribution:

In our research paper, this is a real team effort! Rabbiyah Shams and Khalil Ahmad began by proposing ideas for the study and deciding on the direction of the study. Later, M. Farooq Iqbal and Mazhar Ulhaq came on board to help conceptualize the design and ensure everything runs smoothly. Upon completion, M. Ul Hassan and M. A. Zafar brings great talent. Meanwhile, H. Asif is busy collecting and managing our data. While working with the documents, Ghazala Shaheen, Sultan Ayaz, Riaz ur Rehman and Syed Muhammad Ali Shah came forward to verify all the documents and make sure of everything. It was a team effort from start to finish and we all played an important role in bringing our research to life!.

Statement of Novelty:

Our study introduces a new way to induce rheumatoid arthritis (RA) in rabbits using killed E. coli bacteria, which consistently produces significant joint changes. This method proves effective through various assessments, like X-ray analysis and observation of joint inflammation. These findings emphasize the importance of this model for studying RA and testing treatments. Moving forward, we aim to explore its mechanisms and relevance to human RA."

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