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Safety and Efficacy Assessment of Different Therapeutic Regimens in Experimentally Induced Osteoarthritis Animal Model

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ABSTRACT
The aim of this study was to determine the efficacy and safety of Steroids,NSAIDs, Calcium + Vitamin $D_3$and NSAIDs + steroids in experimentally induced osteoarthritis animal model. Total of 24 male albino rabbits having age 6-8 months were included in the study. Osteoarthritis induction was accomplished by the administration of the intra-articular injection of L-cysteine and papain in the knee joint on the day first, fourth and seventh. The confirmation of osteoarthritis was done with the help of X-rays. Kellgren and Lawrence Grading scale was used to evaluate the knee joint. At the same time biochemical tests were performed before and at the end of the treatment to determine the safety of treatment procedure. All of the treatment groups were effective in reversing the osteoarthritis. Among all treatment Dexamethasone and Piroxicam were more effective and safe to cure the rabbits. This study concludes that different therapeutic regimens are effective in rabbit model. However, further studies needed to use all of the drugs in combination to observe the synergistic effect to achieve the results in less time.
Keywords*: Osteoarthritis, Rabbit, Biochemical tests, NSAIDs, Vitamin D, Efficacy, KL scale.*

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INTRODUCTION

Osteoarthritis, also called as the degenerative joint disease or degenerative disease of cartilage[1]. Osteoarthritis is one of the main causes of older person disability. But the mechanism, that how aging and osteoarthritis are related is not understood yet [2]. Osteoarthritis mainly occurs due to injury, aging and genetic basis. Alongside these additional causes may contain Alkaptonuria, Hemochromatosis, injury to ligaments, joint infection, inflammatory diseases, and diabetes [3] etc. Pathogenesis is thought to involve the central mechanism regulating the degradation of cartilage. Diffuse matrix exposure is pathological condition either due to injury to chondrocytes or local mediators. Animal studies involving osteoarthritis, mainly knee joint is used. Other joints which are used are metacarpophalangeal join (horses), tempromandibular joint (mice), discoidon domain receptor 1 DDR1 (mice) [4-6]. Imaging modalities used in humans to indicate osteoarthritis include X-ray, CT scan, ultrasound and MRI [7]. Different type of classification systems are used to grade the osteoarthritis. They may be Western Ontario and McMaster Universities Arthritis Index (WOMAC) score and Kellegren-Lawrence grading system. Various animal models i.e. rats, rabbits and mice have been studied by using radiographs [8-10]. Management of disease includes decreasing the pain, swelling, preventing the physical disability and improving the function by life style modification [11]. Treatment of osteoarthritis includes medication and therapy. Medications typically used are NSAIDs [12], steroids and antidepressants [13]. Therapy of osteoarthritis comprises physical therapy, occupational therapy, tai chi and yoga [14-17]. Additional methods include the use of cortisone injections, lubricating injections [18], and joint realignment surgery [19]. Occasionally alternative treatments such as Acupuncture[20], and viscosupplementation (a method for rheological restoration of properties of joints) are used. Glucosamine and chondroitin has also been investigated to cure osteoarthritis [21].

The objective of current study is to determine the efficacy and safety of NSAIDs, Steroids, Calcium and Vitamin D_3 in experimentally induced osteoarthritis animal model.

MATERIAL AND METHODS

Chemicals and Reagents

Normal Saline, Papain 4% solution, Nsaids tablets, Steroids tablets, Vitamin D_3 injection, Calcium tablets.

Subjects

Total twenty four male healthy albino rabbits (Oryctolaguscuniculus), aged 6-8 months were selected for study. Then rabbits were divided into four groups. The subjects were kept under normal temperature (21 ± 1 ^{0} C) and humidity (65-70 %) with light and dark cycle of 12 hours each. Daucuscarota (carrots), Muhlenbergiarigens (deer grass) and water were given as food.

Tests and Experiments

The current study was completed in two phases. During Phase I, OA was induced experimentally by procedures as specified by Bentley (1971). It was induced via 4% papain solution (0.2ml) and L-cysteine HCl (0.1ml) as an activator at first, fourth and seventh days. Afore induction of disease, biochemical tests and X-ray analysis were also performed. The development of the disease was further confirmed using X-ray imaging of anterio-posterior radiographs of knee joint. The evaluation of knee was done according to the Kellgren and Lawrence Grading Scale (KL) [22]. Knee with grade 2 or higher defined OA.

During Phase II, subjects were treated according to dosage schedule.Group A received Dexamethasone (1 mg/kg), Group B received Piroxicam (0.3 mg/kg), and Group C was administered with Calcium (5ml of 10%) and Vitamin D_3(1000 IU/kg) while Group D received Dexamethasone (1 mg/kg) and Piroxicam (0.3 mg/kg). These doses were given according to Formulary for laboratory animals[23]. Summary is shown in Table 1.

Table 1: Dosage Frequency and Strength

Group ID	Group type	Drug Used	No. of subjects	Dose per kg PO (BW)
0	Normal group	No drugs	4	_
1	Control group	Water	4	N/S.
Group A	Experimental	Dexamethasone	4	1mg/kg BD
Group B	Experimental	Piroxicam	4	0.3mg/kg BD
Group C	Experimental	Calcium and Vitamin D	4	5ml (10%) + 1000 IU/kg BD
Group D	Experimental	Piroxicam and Dexamethasone.	4	0.3 mg/kg + 1 mg/kg BD

Statistical Analysis

Data were evaluated using GraphPad Prism version 6.01.

Ethics

Research protocol was approved by the Institutional Animal Ethical Committee of Hajvery University, Lahore-Pakistan.

Results

The X-ray radiographs were examined that had been acquired using a commercially available digital detector. Images were evaluated on a medical grade flat screen monitor. Figure 1showing Knee X-ray of normal rabbit.

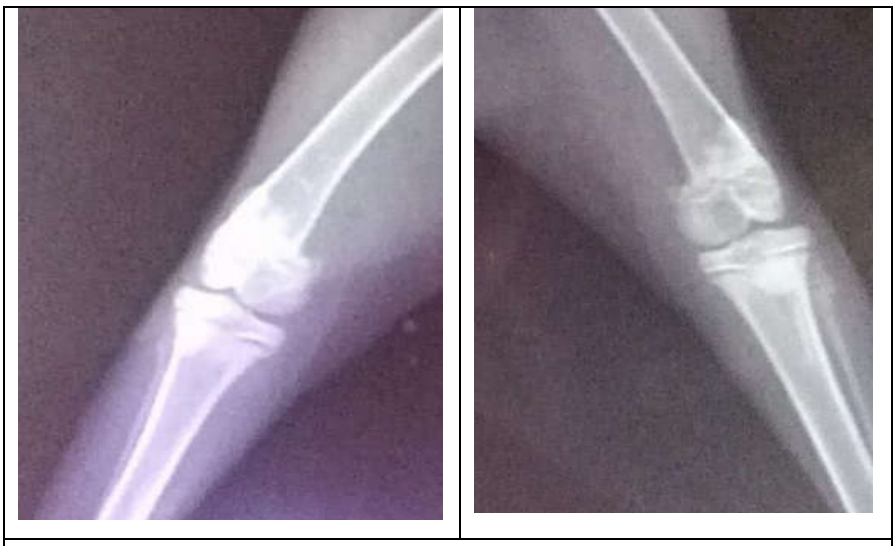


Fig 1. Shows **Normal Rabbit** X-Ray photograph of Right and left knee.

A Radiologist scored each radiographic image for the existence and severity of osteoarthritis by using a KL scale.Anterio-posterior radiographs revealed that bilateral osteoarthritis was developed in knees of rabbits. After disease induction the radiographic images showed that grade 3 osteoarthritis was developed in right knee and grade 4 was developed in left knee. These are shown in Fig 2**.**

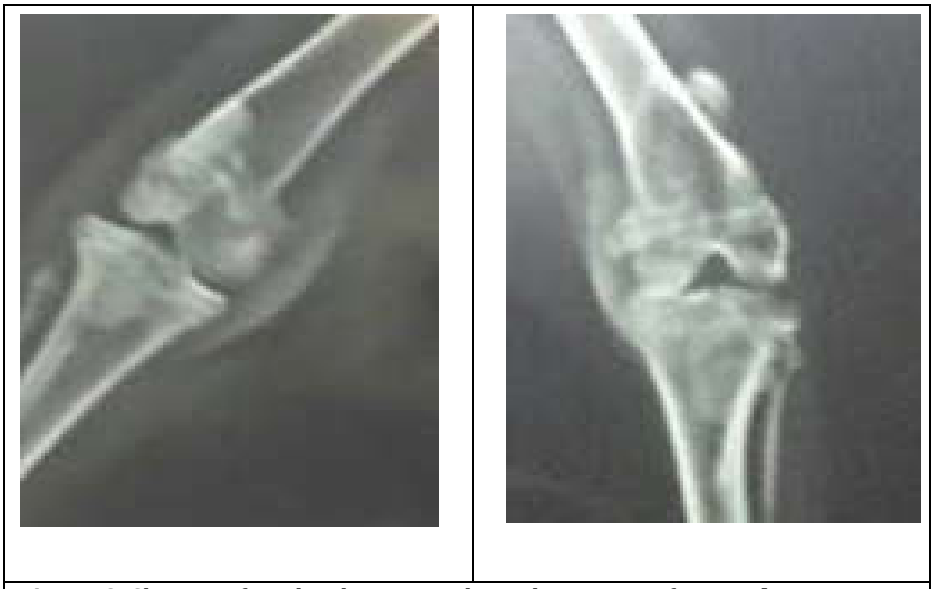


Figure 2. Shows Left and right osteoarthritic knee joint of **control group**.
Right knee: Osteophyte formation clearly visible; Joint space narrowing; Sclerosis and deformity of bone contour. According to KL scale it lies in grade 3 osteoarthritis.
Left knee shows large osteophytes, diminish joint space, Severe sclerosis and definite deformity of joint space. It lies in grade 4 KL Score.

After six weeks of treatment, radiographs depicted that osteoarthritis was reversed and returned to KL score "1" in right knee and to KL score "2" in left knee ofGroup A. While in Group B; disease progression was stopped to KL score "1" in right knee and to "1"in left knee. In Group C; disease progression was stopped to KL score "1" in right knee and KL score "1" in left knee.In Group D; disease progression was stopped to KL score "1" in right knee and KL score "2" in left knee.
A comparison of left and right knee of all these groups is shown in Fig 3, 4, 5 and 6.

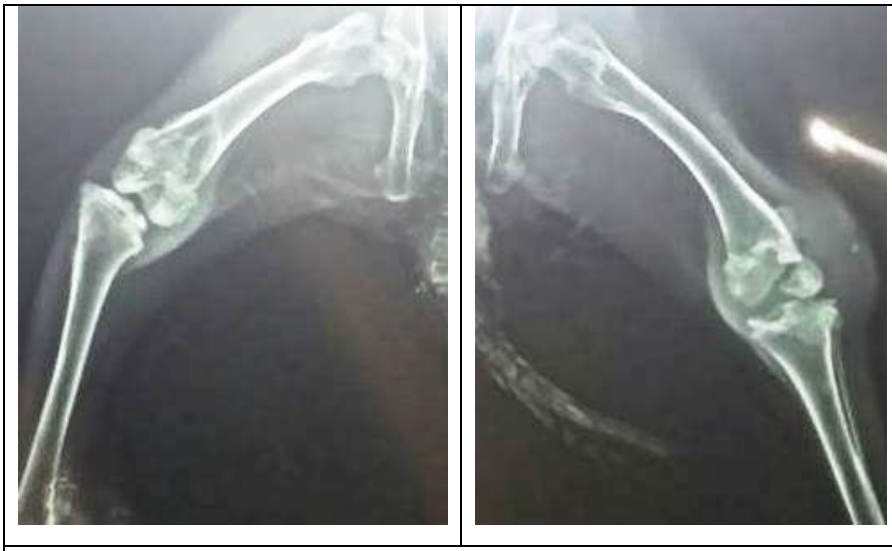


Figure 3. Shows Left and right knee joint of **Group A** after treatment.
Right knee: Improved joint space, decrease in osteophyte and sclerosis appearance and improve in bone texture. It lies in grade 1 KL score.
Left knee: Improved joint space, decrease in osteophyte and sclerosis appearance. Bone texture still needs improvement. It lies in grade 2 KL score.

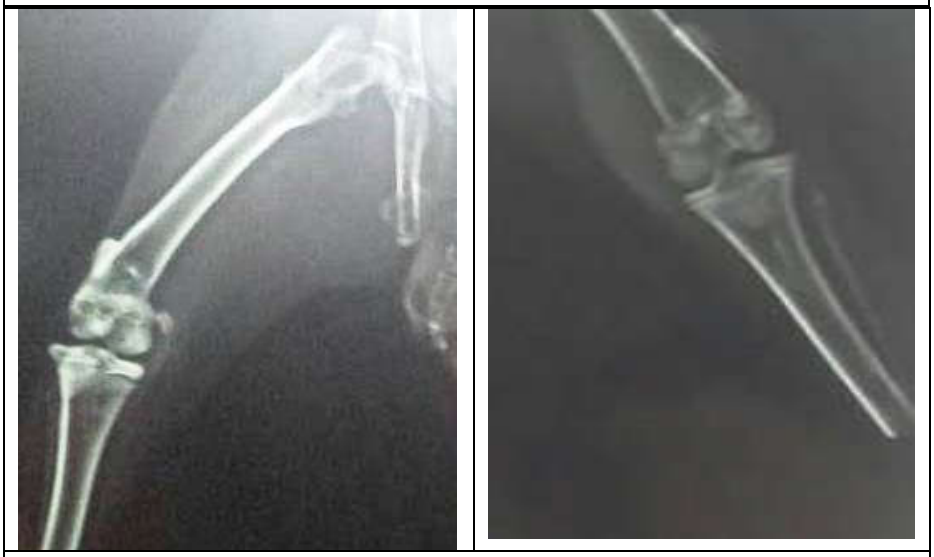


Figure 4. Shows Left and right knee joint of **Group B** after treatment.
Right knee: Improved joint space, decrease in osteophyte and sclerosis appearance and improve in bone texture. It lies in grade 1 KL score.
Left Knee: joint space still less, decrease in osteophyte and sclerosis appearance. Bone texture still needs improvement. It lies in grade 1 KL score.

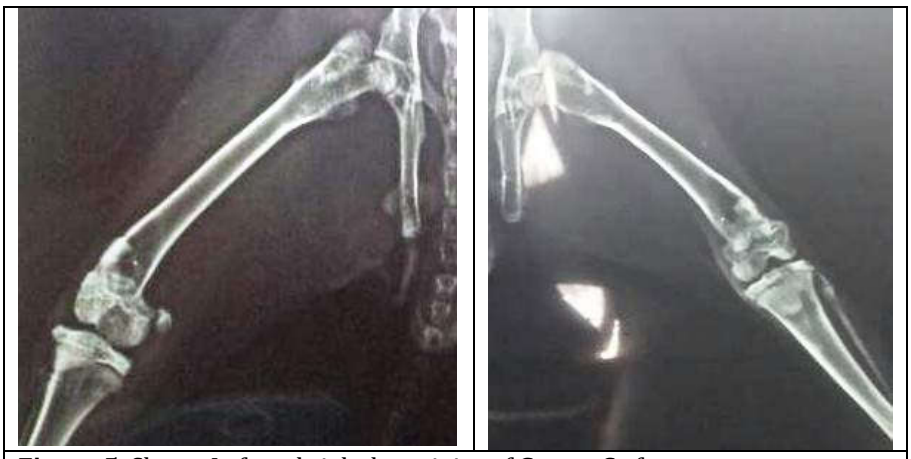


Figure 5. Shows Left and right knee joint of **Group C** after treatment.
Right Knee: Improved joint space, decrease in osteophyte and sclerosis appearance. It lies in grade 1 KL score.
Left Knee: Improved joint space, decrease in osteophyte and sclerosis appearance. Bone texture still needs improvement. It lies in grade 1 KL score.

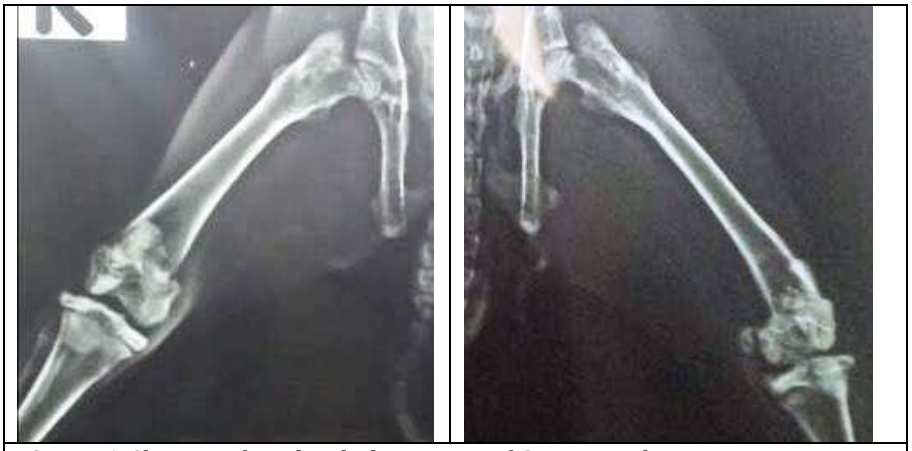


Figure 6. Shows Left and right knee joint of **Group D** after treatment.
Right Knee: Improved joint space, decrease in osteophyte and sclerosis appearance. Bone texture still needs improvement. It lies in grade 1 KL score.
Left Knee: Improved joint space, decrease in osteophyte and sclerosis appearance. Bone texture still needs improvement. It lies in grade 2 KL score.

Safety Assessment

To assess the efficacy and safety of treatment protocols, various tests were performed. These biochemical tests include liver function tests (LFTs), renal function tests (RFTs) and complete blood count (CBC).

Liver Function Tests:

LFTs include serum glutamic-pyruvic transaminase (SGPT), serum bilirubin (S. bilirubin), serum glutamic-Oxaloacetic transaminase (SGOT) and alkaline phosphatase. SGPT in normal group was (48.75 ± 0.25). While in control group it was raised to (58.75 ± 1.70). It was found that Piroxicam reduced the SGPT level (43.00 ± 0.913) then compared to Dexamethasone (44.25 ± 1.54), Calcium+ Vitamin D (46.5 ± 1.55) and Piroxicam+ Dexamethasone (55.25 ± 0.62). S. bilirubin in normal group was (0.575 ± 0.04). While in control group it was raised to (0.82 ± 0.02). However Piroxicam+ Dexamethasone reduced the S. bilirubin level (0.515 ± 0.063) then compared to Piroxicam (0.775 ± 0.025). It is witness that the Dexamethasone and Calcium+ Vitamin D increased S. bilirubin level (0.882 ± 0.064), (0.912 ± 0.059) respectively. SGOT in normal group was (49.0 ± 1.47). While in control group it was raised to (51.0 ±

1.82). The result of SGOT level in experimental groups shows that Dexamethasone reduced the SGOT level (42.25 ± 0.854) as compared to Calcium + Vitamin D (44 ± 1.08) and Piroxicam (44.5 ± 0.957). However Piroxicam +Dexamethasone raised the SGOT level (54.25 ± 0.854). Alkaline phosphatase in normal group was (88.75 ± 0.479). While in control group it was raised to (297.25 ± 3.083). It is observed that Dexamethasone reduced the alkaline phosphatase level (96.50 ± 5.008) as compared to Piroxicam (100.75 ± 2.92), Piroxicam +Dexamethasone (190.0 ± 0.40) and Calcium +Vitamin D (194.0 ± 0.40). These results are shown in form of graph in **Fig 7.**

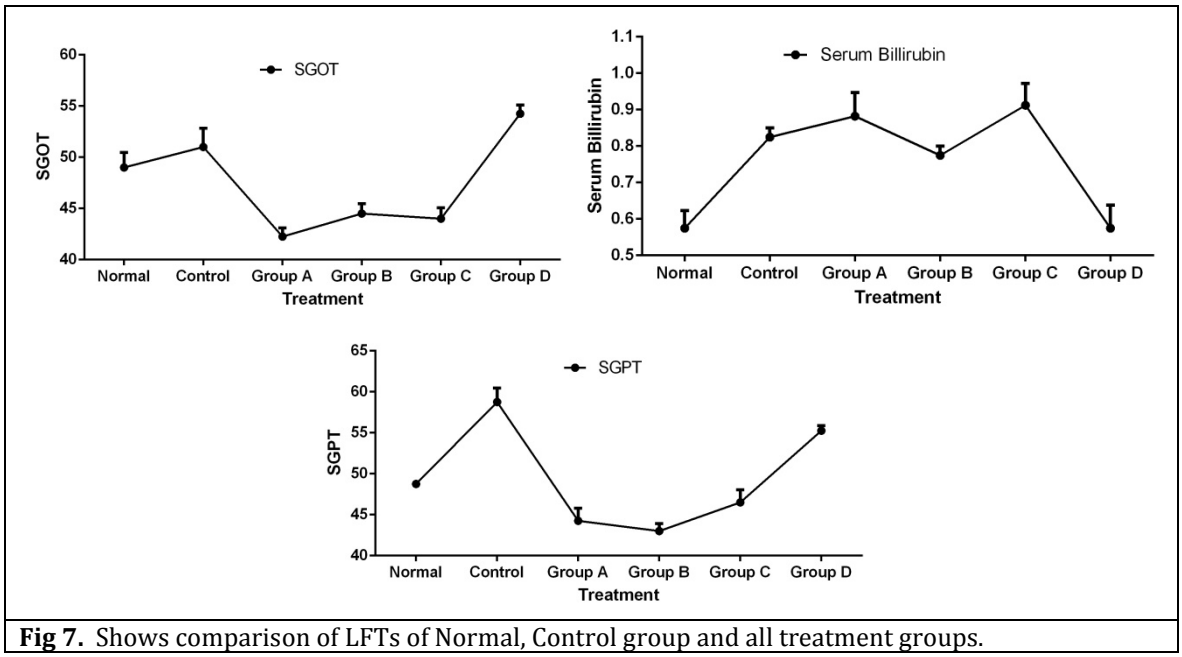
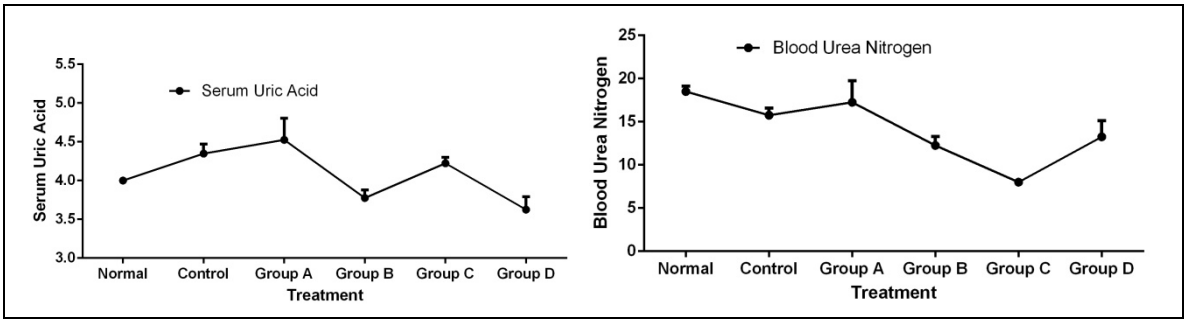


Fig 7. Shows comparison of LFTs of Normal, Control group and all treatment groups.

Renal Function Tests:

RFTs include blood urea nitrogen (BUN), blood urea (BU), serum creatinine (SC) and serum uric acid (sUA). Blood Urea in normal group was (25.0 ± 0.40). While in control group it was raised to (29.0 ± 0.40). It was observed that Blood urea (BU) level was more reduced by Calcium +Vitamin D (12.5 ±1.19) as compared to Piroxicam + Dexamethasone (22.25 ± 1.25), Dexamethasone (24.75 ± 1.31) and Piroxicam (25.0 ± 1.73). Blood Urea Nitrogen in normal group was (18.5 ± 0.64). While in control group it was decreased to (15.75 ± 0.85). It was found that Calcium + Vitamin D reduced (BUN) to (8.0 ± 0.40) as compared to Piroxicam (12.25 ± 1.03) and Piroxicam +Vitamin D (13.25 ± 1.88). However BUN was increased by Dexamethasone to (17.25 ± 2.49). Serum Creatinine in normal group was (1.0 ± 0.4). While in control group it was raised to (1.22 ± 0.18). It was observed that Serum Creatinine level was reduced more reduced in Piroxicam (0.55 ± 0.029) as compared to Calcium +Vitamin D (0.70 ± 0.16), Dexamethasone (0.825 ± 0.11) and Calcium +Vitamin D (0.92 ± 0.11). Serum Uric acid in normal group was (4.0 ± 0.0). While in control group it was raised to (4.35 ± 0.11). It was seen that Piroxicam +Dexamethasone mostly reduced serum Uric acid (3.62 ± 0.16) in comparison with Piroxicam (3.77 ± 0.10), Calcium +Vitamin D (4.22 ± 0.07). At the same time Dexamethasone raised the sUA level (4.52 ± 0.27). These results are shown in form of graph in **Fig. 8.**



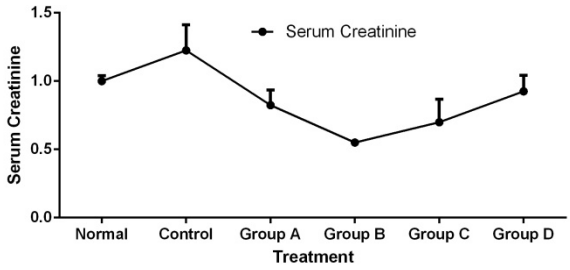


Fig 8. Shows comparison of RFTs of Normal, Control group and all treatment groups.

Complete Blood Count:

Complete blood count include White blood cells count (WBCs), Haemoglobin (Hb),Red blood corpuscles count (RBCs), Neutrophil count , Platelet count (PLT),Lymphocyte count, Monocytes count (Mon), Eosinophil count (Eosin), Basophil count , Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), and Mean corpuscular haemoglobin concentration (MCHC). MCHC in normal group was (35.5 ± 0.28). While in control group it was reduced to (30.7 ± 0.46). The MCHC concentration was further lowered by Dexamethasone to (18.40 ± 1.175), byPiroxicam +Dexamethasoneto (18.90 ± 0.372), by Piroxicam (19.700 ± 0.358) and by Calcium+VitaminD_3 to (19.875 ± 0.642). Lymphocyte in normal group was (60.0 ± 0.81). While in control group it was raised to (63.0 ± 1.08). The lymphocyte count was less in group Dexamethasone(41.000± 8.70) in comparison with Piroxicam +Dexamethasone(45.250± 3.27), Calcium +Vitamin D (52.250 ± 2.71) and Piroxicam (55.50 ± 8.68). RBCs in normal group were (6.25 ± 0.25). While in control group it was reduced to (5.07 ± 0.35). The RBCs levels were increased by Dexamethasone (5.67 ± 0.35) as compared to Calcium+VitaminD_3 (5.60 ± 0.217), Piroxicam (5.19 ± 0.07) and Piroxicam +Dexamethasone (19.875 ± 0.642).WBCs in normal group were (9.0 ± 0.70). While in control group it was reduced to (7.05 ± 0.36). The level of WBCs were increased by Dexamethasone (9.35 ± 0.10) while it was decreased in other groups of treatment Piroxicam (3.47 ± 0.16), Piroxicam +Dexamethasone (2.92 ± 0.45) and Calcium +Vitamin D (2.05 ± 0.30).Platelets in normal group were (279.5 ± 6.91). While in control group it was reduced to (179.7 ± 7.19). Platelets level was observed to increase by Dexamethasone (309.0 ± 152.01) and Piroxicam (233.7 ± 79.75). The other two groups showed the decrease in level of platelet count Calcium+VitaminD_3 (79.75 ± 5.18) and Piroxicam +Dexamethasone (72.0 ± 9.0). Haemoglobin in normal group was (10.0 ± 0.0). While in control group it was raised to (10.87 ± 0.31). The Haemoglobin (Hb) level was lowered by Piroxicam +Dexamethasone (9.22 ± 0.19), Dexamethasone (10.35 ± 0.18), Piroxicam (10.55 ± 0.15) and Calcium +Vitamin D (10.65 ± 0.18). Neutrophil in normal group was (35.75 ± 0.47). While in control group it was reduced to (33.25 ± 0.85).The Neutrophil Count were increased in Dexamethasone (53.00 ± 8.68), Piroxicam to (41.75 ± 6.11), Piroxicam +Dexamethasone to (43.50 ± 4.09) and Calcium +Vitamin Dto (38.50 ± 2.02). Monocyte in normal group was (2.75 ± 0.47). While in control group it was raised to (3.5 ± 0.28). The level of Monocytes were increased in Calcium +Vitamin D (4.0 ± 0.70) and Piroxicam +Dexamethasone (3.51 ± 0.50) while it was decreased in Dexamethasone (3.25 ± 0.25) and Piroxicam (3.0 ± 1.0). MCV in normal group was (56.5 ± 0.64). While in control group it was raised to (76.02 ± 0.38). The MCV was decreased in Piroxicam +Dexamethasone (54.07 ± 1.98) as compared to Piroxicam (54.92 ± 2.73), Dexamethasone (56.34 ± 1.35) and Calcium +Vitamin D (62.67 ± 0.99).Eosinophil in normal group was (1.5 ± 0.28). While in control group it was raised to (2.0 ± 0.0). It was seen that eosinophil level was decreased by Piroxicam to (1.75 ± 0.75). While in all other groups it was increased. Dexamethasone increased it to (2.0 ± 0.0), by Piroxicam + Dexamethasone (2.5 ± 0.5) and by Calcium + Vitamin D to (3.75 ± 0.25). In case of Basophils it was not affected at all. MCH in normal group was (56.5 ± 0.64). While in control group it was reduced to (26.8 ± 0.27). The MCH value was further decrease by Calcium + Vitamin D to (19.87 ± 0.64), Piroxicam to (19.7 ± 0.35), Piroxicam + Dexamethasone to (18.9 ± 0.37), Dexamethasone to (18.4 ± 1.17).These results are shown in form of graph in **Fig. 9.**

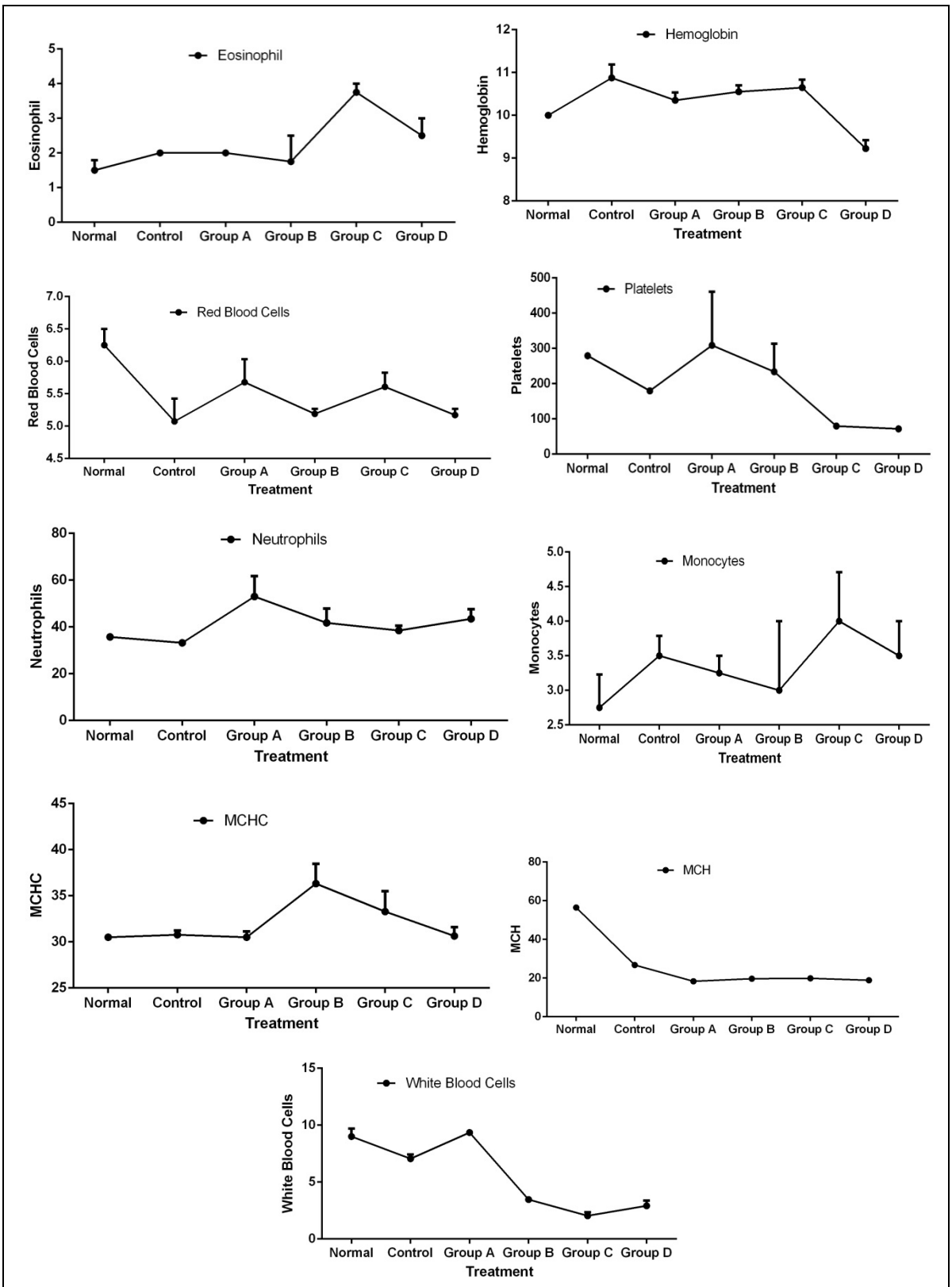


Fig 9. Shows comparison of CBC test of Normal, Control group and all treatment groups.

DISCUSSION

Since the start of human civilization, one of the most important threats to humans was the diseases[24]. Humans started to discover new drugs to encounter these diseases and one of the diseases was osteoarthritis [24-26]. Osteoarthritis is known as the degenerative joint disease with signs and symptoms of pain, swelling, stiffness, narrow joint space, osteophyte formation and spurs formation etc. Rabbits

were considered animal model for study of osteoarthritis parallel to humans [2, 27, 28]. The main objective of this study was to assess the safety and efficacy of Dexamethasone, Piroxicam, Calcium + Vitamin D, Piroxicam + Dexamethasone.

To access the efficacy of treatment X-ray photographs were performed at start of treatment and at end of six week treatment. Osteoarthritis score was calculated using the KL grading scale [22]. After six week of treatment it was observed that; disease progression was stopped to KL score "1" in right Knee and "2" in left knee in group A, disease progression was stopped to KL score "1" in right Knee and "1" in left knee in group B, disease progression was stopped to KL score "1" in right Knee and "1" in left knee in group C, disease progression was stopped to KL score "1" in right Knee and "2" in left knee in group D while disease progression was stopped to KL score "3" in right Knee and "4" in left knee in Control group. These photographic results are shown in **Fig.** These X-ray findings shows that Group B (Piroxicam), and Group C (Calcium + Vitamin D) were good to improve the tone of joint. However in case of group A (Dexamethasone) and Group D (Piroxicam + Dexamethasone) reversed the Osteoarthritis in right knee but in case of left knee they still need more progress to reverse the osteoarthritis.

For assessment of safety of treatment protocols, various biochemical tests were performed. In case of Liver Function test it was observed that Alkaline Phosphatase level was increased in control group. This level was brought to normal range by Group A and Group B. A rise in Alkaline Phosphatase indicates some liver damage [29]. Hence in this test Group A and B were effective. In case of serum Bilirubin control group increased the bilirubin level in blood and Group D effectively restored the bilirubin values to normal range. Usually low bilirubin level is of no concern but increase in bilirubin level indicates the liver is not functioning well [30]. In case of SGPT level, it is low in normal condition and increase in liver damage [31]. In this study it was observed that control group increased both SGPT and SGOT levels. Group A, B and C reduced the SGOT and SGPT levels.

In case of Renal Function Tests control group decreased the Blood Urea Nitrogen which indicates liver was damaged. The Group A was effective in restoration of BUN to normal range. Similarly increase in serum Creatinine level indicates the defect in kidney function [32]. Control group increased the creatinine level while all group A, B, C and D decreased the creatinine level. So, all of the treatments were effective to decrease the creatinine level. Similarly all treatment groups decreased the serum blood urea level. In case of serum Uric acid Group B, C and D were effective to decrease uric acid level. Increase in uric acid is related to several clinical abnormalities [33].

In case of Complete Blood Count control group shows decrease in Red Blood Cells. The Group A and C showed some improvement in RBCs level however, even then the normal level was not achieved by any of the group. On the other hand platelets were decreased in control group and only group A and B were effective to restore the Platelets. In case of Monocytes, the control group increased monocytes level. Increase in monocytes is related to inflammation [34]. The monocytes level was decreased by Group B and A. The MCHC level was well restored by Group A as compared to other. While all of the treatment and control group showed decrease in MCH level. Haemoglobin level was observed to increase in control group while group A and B was well in restoring their level. Disturbance in Haemoglobin level is related to anaemia [35]. In case of White blood cells it was seen that control group decreased the WBCs while group A restored their level to normal range as compared to other treatment groups which further decreased WBCs level.

By assessment of all above X-ray and Biochemical findings it was observed all treatment groups were effective toreverse the osteoarthritis and were safe to kidney and liver. Among all treatment groups the progress of Group A and B were highest as compared to other groups i.e. Group C and D.

CONCLUSION

This study was conducted to evaluate the safety and efficacy of different therapeutic regimens to treat osteoarthritis in rabbit model. The outcomes have showed that individual dosing of Piroxicam and Dexamethasone are effective as compared to their combined dosing. Similarly Calcium and Vitamin D also somehow recovered the osteoarthritis.

FUTURE CONSIDERATIONS

The result confirms that same treatment protocols can be used in rabbits to cure osteoarthritis. Future studies should address to consider all of the four drugs in combination to observe if they have synergistic effect to improve and bring early cure.

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We are very thankful to Dr Umer Gul for interpreting the X-ray findings.

CONFLICT OF INTEREST

We declare no conflict of interests.

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