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STUDY OF HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN AN EXPERIMENTAL MODEL OF RHEUMATOID ARTHRITIS IN ALBINO WISTAR RAT TREATED WITH ORAL INDOMETHACIN & TIZANIDINE

Pharma

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ABSTRACT

Background: Rheumatoid arthritis is an autoimmune disease characterized by painful inflammation of the joints. Small joints of the hand and foot are involved first, followed by larger joints. The pathogenesis of rheumatoid arthritis involves both type III and type IV hypersensitivity reactions. **Methods:** There were 3 groups with 6 albino Wistar rats in each. Each group was injected with CFA in one of the hind paws on a day before (day 0) treatment with Indomethacin (3mg/Kg), Tizanidine (1mg/Kg), and Tizanidine (2mg/Kg). Each drug was given PO in their respective groups once a day. Body weight was measured on days 0 and 21. On the 21st day of the study, Hb level, TLC, hepatic weight, AST, and ALT were measured. **Result:** Body weight decreased in the INDO group and increased in the TZD groups. The Hb level was lowest in the INDO group, followed by the TZD (2mg/Kg) group and the highest in the TZD (1mg/Kg) group. The TLC distribution was similar to Hb, and both parameters were within normal limits. The INDO group had the lowest liver weight, followed by the TZD (2mg/Kg) group, and the highest in the TZD (1mg/Kg) group. AST and ALT were elevated in all groups, with the highest in the INDO group and the lowest in the TZD 1 mg/Kg group. **Conclusion:** Tizanidine have a lesser side effect profile with better tolerability as compared to standard drug Indomethacin in experimental model of rheumatoid arthritis.

KEYWORDS

Rheumatoid arthritis (RA), Indomethacin (INDO), Tizanidine (TZD), Haemoglobin (Hb), TLC, AST, and ALT.

INTRODUCTION

There are several types of arthritis like crystal-induced arthritis, osteoarthritis, ankylosing spondylitis, rheumatoid arthritis, etc. Crystal-induced arthritis is caused by the deposition of uric acid crystals in joints. Osteoarthritis is a degenerative disease caused by advancing age. Large weight-bearing joints are mainly involved in osteoarthritis. Ankylosing spondylitis is a disease of the axial skeleton characterized by sacroiliitis and progressive stiffening of the spine.[1]

Rheumatoid arthritis (RA) is defined as a systemic disorder associated with chronic joint inflammation that most commonly affects peripheral joints. RA occurs more often in women between 30 to 50 years of age. There is nonsuppurative proliferative and inflammatory synovitis in RA. Pathogenesis of RA involves both type III and type IV hypersensitivity reactions. One proposed mechanism of pathogenesis of RA involves CD4+Ve T Helper cell activation leading to the release of proinflammatory mediators (e.g., tumor necrosis factor). Inflamed synovial cells express an antigen that triggers B cells to produce antibodies (rheumatoid factor).[2] Pattern of joint involvement varies, but it is generally symmetrical and affects small joints before larger ones. Symptoms usually develop in the hands and feet, followed in decreasing frequency by the wrists, ankles, elbows, and knees. RA primarily involves MCP & proximal interphalangeal joints of the hands. There is painful inflammation and stiffness of joints in RA.[3]

Treatment of rheumatoid arthritis involves non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatoid drugs (DMARDs). DMARDs include non-biologic and biologic agents. Non-biologic agents include methotrexate, azathioprine, sulfasalazine, and hydroxychloroquine. Whereas biological agent includes TNFα inhibitors (etanercept, and infliximab), anakinra, abatacept, etc. As rheumatoid arthritis is a chronic condition so long-term treatment is required, which put the patient at higher risk of side effects of the above drugs.[4] These are a spectrum of side effects that may vary from peptic ulcer to aplastic anemia, renal failure, liver failure, etc.[4,5,6,]

Tizanidine (TZD) is a α_2-selective adrenergic agonist drug. It is useful for the treatment of spasticity associated with cerebral and spinal disorders.[5,7] It also shows some properties similar to those of clonidine.[8] Along with its conventional activity TZD shows anti-inflammatory activity in various studies conducted over the last several years. These studies also show that TZD has not only anti-inflammatory but analgesic effects too.[9,10] TZD has a few side effects like dry mouth, drowsiness, night-time insomnia, hallucinations & dose-dependent elevation of hepatic enzymes. TZD seems to be safer than NSAIDs & DMARDs.[5] So, this study on hematological and biochemical parameters was conducted to find out the relative effect of Indomethacin and TZD in an experimental model of RA (CFA) in albino Wistar rats treated with oral indomethacin & tizanidine.

MATERIALS & METHODS

01-STUDY SETTING

This study was conducted in the Department of Pharmacology, Index Medical College, Malwanchal University, Indore, M.P. after getting the approval.

02-DRUGS & CHEMICALS

A-Complete Freuds Adjuvant 0.1ml (CFA: 0.05% w/v Mycobacterium butyricum mineral oil)[12,13]
B-Indomethacin/ INDO (3mg/kg)[5]
C-Tizanidine/ TZD (1mg/kg and 2mg/kg)[5]

03-ANIMALS

Male Wistar albino rats weighing 150 – 200 gm and 8 to 10 weeks of age were kept in polypropylene cages with stainless steel grill tops and bedding of clean paddy husk. The animals were housed in 3 groups under normal laboratory conditions with a normal light-dark cycle. The animals were acclimatized to the lab environment for a week before experimentation with free access to water and a standard pellet diet for laboratory animals. Then animals were weight matched and divided into 3 groups with 6 animals in each.

04-STUDY OF HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Two doses of TZD were evaluated for anti-arthritic activity in comparison to the standard drug Indomethacin. Doses of TZD evaluated as per table number 01.

TABLE-01. GROUP WISE DRUG DOSES (mg/Kg)

SN	GROUP	TREATMENT GIVEN
1	GROUP I	INDOMETHACIN (3mg/kg)
2	GROUP II	TIZANIDINE (1mg/kg)
3	GROUP III	TIZANIDINE(2mg/kg)

Arthritis was induced by using a single-dose injection of 0.1 ml of 1% Complete Freund's Adjuvant (CFA) into the plantar surface of one of the hind paws of the rat. [9-10]All the animals were maintained for 21 days with daily oral administration of indomethacin and the test drug (TZD) in their respective doses. The weight of rats was measured a day before starting the experimentation (day 0) and at the end of the study (day 21). Rats were sacrificed at the end of the study after anaesthetizing the animals as per standard guidelines, and blood was collected from the

retro-orbital fossa. Blood was sent to the laboratory for analysis of hematological and biochemical parameters. The liver was removed from the animal and weighted group-wise.

RESULTS

Table-02 and figure-01, explain the pre-and post-intervention body weight of rats. In the INDO group, there is a decline in body weight by 3.0 gm (1.76%). While the TZD 1mg/Kg group shows a weight gain of 3.9 gm (2.26%). Similarly, there is a weight gain of 4.5 gm (2.63%) in the TZD 2mg/Kg group. So, we can see here that except for the INDO group, both groups of TZD show weight gain, which in within normal physiological limits. After analysis of the data using two-tailed t-Test, the *p-value* was > 0.05 for the INDO and TZD groups, which is statistically insignificant.

Table 03 and Figure 02 explain the hematological parameters at the end of the study. The haemoglobin level in the INDO group was 11.80 ± 0.43, 13.07 ±0.43 in the TZD (1mg/Kg) group, and 12.75 ± 0.27 in the TZD (2mg/Kg) group. Here we can see that the haemoglobin level was lowest in the INDO group followed by the TZD (2 mg/kg) group and the highest in the TZD (1 mg/Kg) group. Data was analysed using one-way ANOVA and the result was insignificant (*p-value* > 0.05).

Table-03 and Figure-02 explain the total leukocyte count of all the 3 groups. The TLC (10^3/µL) of the INDO group was 3.789 ± 0.183, the TZD (1mg/Kg) group was 3.802 ± 0.151, and the TZD (2mg/Kg) group was 3.815 ± 0.133. So, we can see here that TLC is minimum in the INDO group and maximum TZD (2mg/Kg) group. The TLC level of TZD (1mg/Kg) lies in between the two. All the values lie within normal limits. Analysis of data by one-way ANOVA shows that there is no significant difference between the groups (*p-Value* > 0.05).

Similarly, table-03, figure-03, explains the post-study hepatic weight. INDO, TZD (1mg/Kg), and TZD (2mg/Kg) groups have a hepatic weight of 6.87 ± 0.24, 7.08 ± 0.26, and 6.94 ± 0.32 gm, respectively. It was lowest in INDO and highest in the TZD (2mg/Kg) group. The data was analysed using one-way ANOVA, and the result was insignificant (*p-Value > 0.05*)

Similarly, table-03 and figure-04 show post-study AST and ALT levels. AST levels in INDO, TZD (1mg/Kg), and TZD (2mg/Kg) groups were 113.55 ± 8.31, 80.67 ± 6.75, and 112.27 ± 9.40 IU/L respectively. ALT levels in all 3 groups in the same sequence were 78.98 ± 7.01, 51.10 ± 7.02, and 73.21 ± 4.70 IU/L. Thus, hepatic enzymes (AST & ALT) were elevated in all three groups, with the maximum elevation in the INDO group. Data was analysed using one-way ANOVA. The result was significant for both AST & ALT (*p-Value < 0.05*) in all groups.

DISCUSSION

All the parameters were measured in the CFA model of RA to evaluate the relative safety of INDO and TZD in albino Wistar rats.[11] INDO is a standard anti-inflammatory and analgesic drug with hepatotoxicity and other side effects. In our study, we found that INDO decreases body weight while both doses of TZD do not. [14]

Table-02. Change In Body Weight (BW) In Indomethacin (INDO) And Tizanidine (TZD) Treated Rats

SN ↓	DRUGS →	INDO (3mg/Kg)	TZD (1mg/Kg)	TZD (2mg/Kg)
01	PRE-INTERVENTION BW (gm)	170.0 ± 3.63	171.9 ± 5.43	170.9 ± 3.31
02	POST-INTERVENTION BW (gm)	167.0 ± 2.85	175.8 ± 6.07	175.4 ± 3.10
03	CHANGE IN BW DAY-0 to 21 (gm)	-3.0	+3.9	+4.5
04	% CHANGE IN BODY WEIGHT	-1.76	+2.26	+2.63
05	Two tailed t-Test (p-Value)	> 0.05	> 0.05	> 0.05

Table-03. Haematological & Biochemical Parameters In Indomethacin And Tizanidine-treated Rats

SN ↓	DRUGS →	INDO (3mg/Kg)	TZD (1mg/Kg)	TZD (2mg/Kg)	One way ANOVA (p-Value)
01	Hemoglobin (gm/dL)	11.80 ± 0.43	13.07 ± 0.43	12.75 ± 0.27	> 0.05
02	Total leukocyte count (103/µL)	3.789 ± 0.183	3.802 ± 0.151	3.815 ± 0.133	> 0.05
03	Hepatic Weight (gm)	6.87 ± 0.24	7.08 ± 0.26	6.94 ± 0.32	> 0.05
04	Aspartate transaminase (AST, IU/L)	113.55 ± 8.31	80.67 ± 6.75	112.27 ± 9.40	< 0.05
05	Alanine transaminase (ALT, IU/L)	78.98 ± 7.01	51.10 ± 7.02	73.21 ± 4.70	< 0.05

FIGURE-01. CHANGE IN BODY WEIGHT (gm)

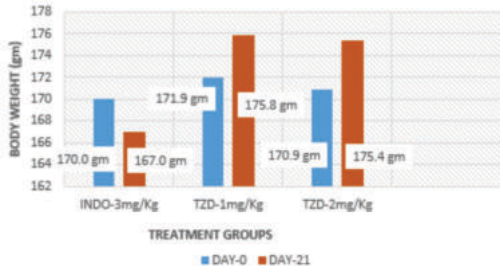


FIGURE-02. COMPARISION OF HEAMATOLOGICAL PARAMETERS (Hb & TLC)

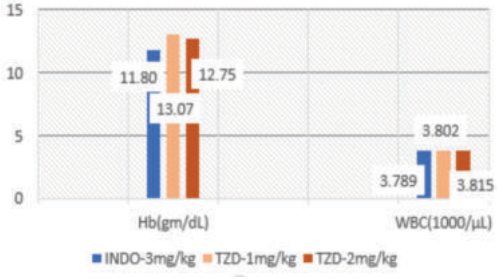


FIGURE-03. COMPARISION OF HEPATIC WEIGHT (gm)

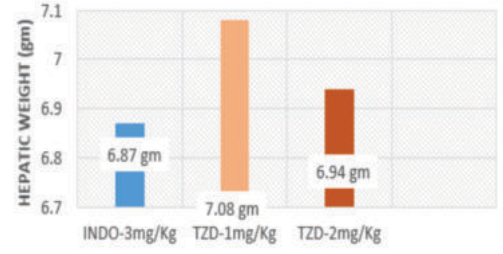
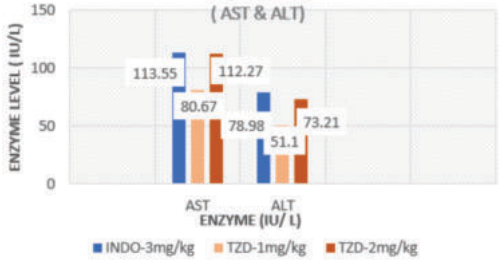


FIGURE-04. COMPARISION OF BIOCHEMICAL PARAMETERS (AST & ALT)



The level of hemoglobin decreases in chronic inflammatory diseases. Here we can see that the Hb level in every group is near to its lower normal limit. As there is no anemia in any group, both drugs inhibit the inflammation-induced anemia of chronic disease. [2] Further, the lower level of Hb in the standard drug INDO group as compared to TZD groups may be due to loss of blood from gastric ulcer. TZD has a gastroprotective effect, so there could be no blood loss, so the Hb level is more than the INDO group. [9] Although the result demonstrates that

there is no significant difference in the Hb level of different groups, TZD seems to be safer in terms of Hb level.

TLC in RA could be low because inflammatory cells (neutrophils, monocytes, and lymphocytes) migrate to the site of inflammation. Normal TLC in all the groups shows the anti-inflammatory action of standard drug INDO and test drug TZD. A lower level of TLC indirectly indicates the level of phagocytic cells in the blood. So a lower level of TLC in this study indicates anti-inflammatory activity of both drugs. [2]

Hepatotoxicity leads to hepatic cell damage with the elevation of intracellular enzymes in the blood. The weight of the liver was minimal in INDO as compared to both doses of TZD. Hepatic weight was highest in the TZD group at 1 mg/kg. So we can see here the dose-dependent hepatotoxicity of TZD. [5]

Hepatic enzymes (AST and ALT) were elevated in all the groups, so both drugs are hepatotoxic. TZD (1mg/Kg) has a minimum and the INDO group has a maximum elevation of hepatic enzymes. Here we also see that the higher dose of TZD (2 mg/Kg) group has higher AST & ALT, so here again we conclude dose dependent hepatotoxicity of TZD. [5,7]

Iqbal MP et al. (2000) [14] performed a study to see if methotrexate (MTX) and INDO-induce growth suppression in rats. Their study data indicated an additive effect of MTX and INDO on the suppression of growth in young rats, alluding to the notion that patients suffering from juvenile rheumatoid arthritis or acute lymphoblastic leukemia receiving these two drugs concomitantly over a long period might be at risk of experiencing short-term suppression of growth. This study demonstrated that growth suppression was there if both drugs were given separately, but additive growth suppression was there when they were given together.

TZD modulated the antinociceptive and anti-inflammatory effects of NSAIDs, according to Jain NK et al. (2002). [9]They found in their study that TZD and NSAID combination therapy has antinociceptive and anti-inflammatory effects with improved gastric tolerability of NSAIDs. So, they concluded that TZD and NSAID combination therapy would prove to be a novel approach for treating nociceptive and inflammatory conditions with better gastric tolerability than NSAIDs.

Patino-Camacho SI et al. (2017) [10] concluded like Jain NK in his study entitled "Low doses of tizanidine synergize the anti-nociceptive and anti-inflammatory effects of ketorolac or naproxen while reducing side effects". They suggested that, considering that tizanidine increases the anti-inflammatory and anti-nociceptive effects of naproxen or ketorolac with an increase in gastric tolerability, tizanidine could provide therapeutic advantages in the clinical treatment of inflammation and pain.

Majeed RK et al. (2018) [15] performed an acute toxicity study of indomethacin in rabbits. They found that RBC and Hb levels showed a sharp decrease while WBC increased in the INDO treated group. The levels of AST and ALT were increased in the group that was administered indomethacin.

Falzon M et al. (1985) [16] in their study titled "Comparative Effects of Indomethacin on Hepatic Enzymes, Histology and Serum Indices of Liver and Kidney Function in the Rat", they concluded that only minor hepatic damage was evident histologically in indomethacin-treated rats, and thus it was not surprising that increased serum levels of aspartate aminotransferase, a marker of cellular damage, were not observed.

CONCLUSION

In this study, we found that Tizanidine (TZD) has more body weight, hemoglobin, TLC, and hepatic weight, but less elevation of hepatic enzymes (AST and ALT) as compared to standard drug Indomethacin (INDO). So, we conclude here that Tizanidine is safer than Indomethacin for long-term use in an experimental model of rheumatoid arthritis.

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