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Research Article

Manganese Chloride Attenuates Osteoporosis in Rats with Experimental Ulcerative Colitis

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Abstract

Osteoporosis is one of the extra intestinal manifestations of ulcerative colitis common with children living with inflammatory bowel disease (IBD). It results in abnormal growth plate morphology and is linked to early tibial epiphyseal closure rate. This study seeks to unravel the role played by manganese on bone homeostasis during ulcerative colitis. 80 male Wistar rats (3months old; 130-150g) were divided into five groups: Group 1 served as control while groups 2 to 5 were induced with colitis via rectal irrigation with1.5mLs of 6% acetic acid and treated thus: 2 & 3- 200 and 100mg/kg manganese respectively, 4- 500mg/kg sulfasalazine treatment, 5- Untreated colitis. Daily body weights, stool score, hematological, biochemical, bone study, colon macroscopic and microscopic ulcers were evaluated on days 3, 7 and 14 post- induction. Results were expressed as Mean ± SEM, analyzed using ANOVA and significant at $p<0.05$. Ulcerative colitis caused deleterious alterations in body weight, stool score, colonic weight/length ratio and macroscopic ulcer score. Manganese (200mg/kg) and Sulfasalazine reverted all the stated variables back to normal levels. Manganese treatment significantly reduced Myeloid/Erythroid Ratio unlike sulfasalazine treatment. Spleen and liver weights of manganese treated animals were decreased compared with colitis untreated. Significant increases in femur, pelvic bone and alkaline phosphatase level of high manganese treated animals was observed. Manganese treated animals had a decreased duration of tibial epiphyseal plate closure compared with other groups. It can be concluded that manganese treatment not only has ameliorative effect on colon inflammation but also modulates bone homeostasis during ulcerative colitis in young Wistar rats.

Key Words: Manganese Chloride, Ulcerative colitis, Bone homeostasis

INTRODUCTION

Inflammatory Bowel Disease (IBD) has been discovered, investigated and documented to be a chronic (progressive) disease of the (lower) gastrointestinal system leading to high morbidity and mortality (Ko *et al*., 2010, Ford *et al*., 2013). Currently, it can only be carefully managed with the use of immunosuppressive anti-inflammatory drugs which includes aminosalicylates, glucocorticoids which are aimed to help improve the quality of life of affected individuals (Irvine *et al*., 1994, Kanis *et al*., 2013). These synthetic drugs have recently been discovered to cause undesirable adverse or side effects (CCFA, 2013) in patients. Inflammation and tissue destruction marks the peak of IBD, which can be seen as a protective response pivoted via activation of (blood) inflammatory cells, particularly neutrophils and lymphocytes (Farrell *et al*., 2002; Aller *et al*., 2006) to get rid of injurious substances found within the living organism (due to the inflamed colon) as well as initiate (colon) healing (Head and Jurenka, 2003).

A lot of extra intestinal manifestations of Inflammatory Bowel Disease (IBD) has been discovered basically from the uncurbed pathological disease (Bernstein *et al*., 2001); few of these include Sweet's syndrome (Timani and Mutasim, 2006), colon cancer (Triantofillidas *et al*., 2009), renal obstruction (Ruffolo *et al*., 2004), chronic renal disease (Berstein *et al*., 2005), increased risk of osteopaenia and osteoporosis (van Staa *et al*., 2003, Piodi *et al*., 2014) as well as defects of musculoskeletal system (De Vos, 2004, Bourikas and Papadakis, 2009). Medical dictionary defines osteoporosis as the thinning of bones mostly associated with reduction in (bone) mass, as a result of bone calcium and protein depletion. Osteopenia (though not as severe as osteoporosis) is caused by depreciation of bone tissue and mineral content while osteomalacia means softening of the bones associated with resistance, abnormal metabolism and deficiency of Vitamin D (Menezes *et al*., 2006, Kuwabara *et al*., 2009, Ulitsky *et al*., 2011).

An articulated balance between osteoblastic bone formation and resorption (removal of old bones) helps in maintaining normal bone homeostasis (Raggatt and Partridge, 2010) which is mostly a constant and continuous process throughout life. In some diseased state, bone resorption far outweighs formation leading to osteopenia and if not properly handled leads to osteoporosis (Kwan *et al*., 2004; CCFA 2012). Roughly about 40 - 50% of patients diagnosed with IBD have osteopenia while about 30% have osteoporosis (Lichtenstein, 2003). It has been well documented that in osteoporotic conditions, osteoblasts are characterized with lower cell proliferation and defective functions compared with osteoblast (Perrini *et al*., 2008). Growth plates responsible for the total axial growth of long bone are severely impaired during diseased conditions with bone deformity likely to occur. Studies have also shown that colitis causes abnormal growth plate morphology (Koniaris *et al*., 1997) or impaired linear growth as seen

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mostly in about 19-31% of children with nutritional deficiencies (Hildebrand *et al*., 2006) and living with IBD (Vaisman *et al*., 2006) i.e. low-calorie consumption and not malfunction of the gut.

The tibial epiphyseal plate is well known as the main site for bone growth. Quick endochondral ossification in the growth plate occurs either in osteoporosis of the diaphyseal cortex or epiphyseal trabecular bone which is followed by a catch-up growth in which there is increased growth from release of growth inhibition (Gafni *et al*., 2002).

Trace elements are chemical non- toxic (depending on the route of administration and dose consumption) substances required in minute quantity for normal physiologic functioning. Manganese a trace element has been found to possess anti-oxidant properties, aids body metabolism and is essential for the development of strong bones but can cause damage to the brain if in excess (Emsley, 2001; Greger, 1998). This study focuses on the probable effect of manganese on osteoporosis (tibial epiphyseal plate) during ulcerative colitis healing.

MATERIALS AND METHODS

Preparation of Reagent: 10% Formalin: 10 mLs of 100% formalin was dissolved in 90mLs of distilled water.
6% acetic acid: 6mLs of 100% acetic acid was dissolved in 94mLs of distilled water.
Manganese chloride was obtained from (*Qualikems Fine Chemicals Pvt. Ltd, 15b/4, Near Old Rajinder Nagar, New Delhi*) *and* was administered through oral gavage using, (100mg/kg and 200mg/kg of Manganese chloride).

Animal model: 80 male Wistar rats (three months weighing 130-150g) were used for this study. They were purchased from the Animal House of the College of Medicine, University of Ibadan, Nigeria. The rats were housed in solid bottom polypropylene cages and kept at the Departmental Animal House at standard temperature of 26^{o}C±3^{o}C, relative humidity of about 50-70% of temperature, environmental 12 hours of light and 12 hours of dark cycle for a period of 2 weeks before (for acclimatization) and during the experiment. They were kept under pathogen-free conditions. The animals had free access to standardized rats pellet diet from Ladokun Feeds Ibadan, Nigeria throughout the research except when the rats were made to fast for 24 hours before induction of colitis. They were also provided with clean tap water *ad libitum*.

The rats were randomly divided into 5 groups of 15 rats each.
Group 1 - No colitis no treatment,
Group 2 - Manganese chloride (200 mg/kg) with colitis
Group 3 - Manganese chloride (100 mg/kg) with colitis
Group 4 - Sulfasalazine (500mg/kg) with colitis
Group 5 - Untreated Colitis

Experimental colitis
The rats were anesthetized using ketamine (0.2ml/kg) and thereafter induced with colitis using 1.5 mLs of 6% acetic acid. The acetic acid was given intra-rectally by inserting a flexible plastic catheter of 8cm long and 2mm in diameter into the colon through the anal opening. The rats were placed in an inverted position for 15 seconds after induction to prevent leaking out of the acetic acid.

Evaluation of stool
The stool of the animals induced with colitis was scored 24 hours post colitis induction using a standard scoring method.

Table 1:
Colitis Stool Scoring (Engel *et al*., 2008)

Stool score	Criteria
0	normal (well-formed fecal pellets)
1	loosely shaped, moist sticky pellets
2	amorphous, moist, sticky pellets
3	diarrhea
4	occult blood in stool

Blood collection, euthanasia and determination of biochemical parameters : On the 3rd, 7th and 14th day after induction of colitis blood was collected by ocular puncture using heparinized capillary tubes into lithium-heparinized bottle before the animals were sacrificed. Parameters examined include: Total serum protein, serum albumin, serum globulin and serum alkaline phosphatase levels using the method of Dacie and Lewis (1991).

Assessment of damage on the colon, inflammation and histopathology: On the 3rd, 7th, and 14th day after colitis induction, the animals were sacrificed and the colons of each animal was harvested, rinsed in cold phosphate buffered saline before evaluation of (colon) ulcer area and index were determined according to the method described by Morris *et al.*, (1989). The whole weight, whole length and distal 8cm (length and weight) of each colon was measured and recorded. Visible damage was scored by method described by Saleh *et al*., (2012) on a 0-10 scale system taking into consideration area of involvement and the presence or absence of ulcers as well as signs of inflammation.

Bone collection: On sacrificing the animals, the tibial bone of each animal was collected following procedures of Salami *et al.*, (2011). Briefly, soft tissue attached to the fermur, tibia and fibula such as muscles, fascia, tendons and ligaments were removed using the scapel blade. The specimen of each group was placed in a bucket of water and allowed to stand for 3 days after which the water was changed. To the changed water, potassium hydroxide (KOH) was dissolved to aid total removal of the remaining soft tissues from the bones and detergent was added to reduce fat after which the bones were boiled. The bones were allowed to dry, bleached with hydrogen peroxide (H_2O_2) and washed with Vim^{R} and brush after which they were air dried for 2-3 days.

Colon of each rats sacrificed on the different experimental days were preserved in 10% formalin and sent for histopathology studies.

Assessment of the bone samples: The right tibia and fibula were harvested; their flesh teased out and kept in 10% formalin so as to preserve it. The tibia and fibula were taken for histopathology examination in the Histopathology laboratory of the University College Hospital (UCH) Ibadan. The length of the tibial epiphyseal plate was measured for each sample at the department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan.

All the bones of each rat used for the experiment were collected, dried and weighed. The pelvic bone was also weighed separately.

RESULTS

Effect of manganese chloride on body weight of experimental animals: A steady significant increase in the body weight of animals was recorded in all the experimental groups compared with colitis - sulfasalazine treated group.

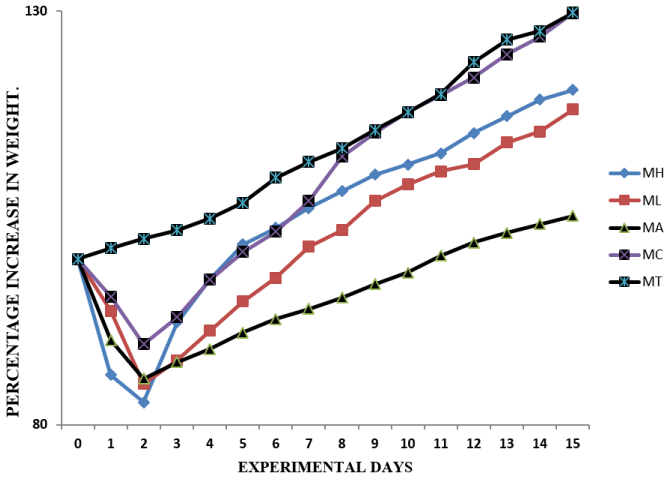

Figure 1:
Effect of manganese chloride on body weight of experimental animal MH - Colitis + high dose (200mg/kg b.w) of Manganese Chloride, ML - Colitis + low dose (100mg/kg b.w) of Manganese Chloride , MA - Colitis + (500mg/kg b.w) Sulfasalazine treatment , MC - Colitis alone, MT - No colitis no treatment

Effect of manganese chloride on stool of experimental animal: Diarrhea in all the groups induced with colitis reduced with time as healing progressed, (Table 1).

Effect of manganese chloride on ulcer scoring of experimental animal colon: The ulcer score of MA (colitis groups treated with 500mg/kg b.w Sulfasalazine) was significantly higher compared with other groups by day 3 post induction while by day 7 ulcer score of MC (colitis untreated group) was significantly higher than other experimental (treatment) groups, (Figure 2).

There were no visible ulcers observed in all the experimental groups by day 14 (heavy black arrows pointing down Figure 2).

Effect of manganese chloride on mucosal width, ulcer depth of experimental animal: There was a significant decrease in the mucosal height of the Colitis Sulphazalazine treated (MA) and Colitis untreated (MC) groups compared with other experimental and treated groups by day 3. On day 7 and 14 of treatment, the mucosal height in the colitis untreated (MC) group was significantly reduced compared with other experimental groups (Table 2).

The microscopic colon ulcer depth was significantly increased in the Colitis untreated (MC) group both on days 3 and 7 compared with all other experimental groups. There was no detectable ulcer in the Colitis Sulphazalazine treated (MA) group microscopically by day 14, (Table 2).

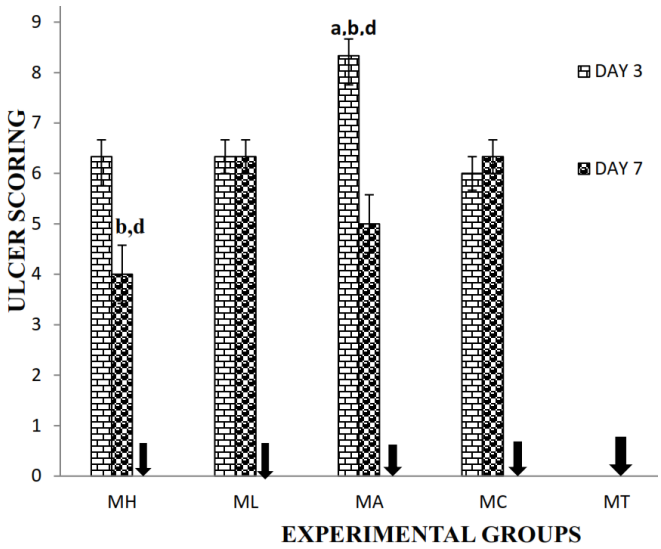

Figure 2:
Effect of Manganese Chloride on Ulcer Scores of Experimental Animal Colon
MH *- Colitis + (200mg/kg b.w) of Manganese Chloride ,* ***ML*** *- Colitis + (100mg/kg b.w) of Manganese Chloride ,* ***MA*** *- Colitis + Sulfasalazine treatment ,* ***MC*** *- Colitis alone,* ***MT*** *- No colitis no treatment*
[a] *- shows significance between* ***MH***

Effect of manganese chloride on colonic length, weight and weight/length ratio of experimental animal: The weight/length ratio of colitis untreated group (MC) was significantly higher than control - no colitis untreated group (MT) by day 3 while on days 7 and 14 there was no significance difference recorded in any of the experimental groups, (Table 3).

On all the days there was no significant difference in the colitis low (100 mg/kg b.w) manganese treated group (ML) when compared with the control- no colitis untreated (MT) group (Table 3).

Table 1:
Effect of manganese chloride on stool consistency of experimental animals

Group /Time	24 HRS	48 HRS	72 HRS	96 HRS	120 HRS	144 HRS	168 HRS
MH	2.67±0.33	2.67±0.33	1.67±0.33	1.67±0.33	1.00±0.00	0.33±0.33	0.00±0.00
ML	3.00±0.00	3.00±0.00	2.00±0.00	1.67±0.33	0.67±0.33	0.00±0.00	0.00±0.00
MA	3.00±0.00	3.00±0.00	2.00±0.00	1.33±0.33	0.33±0.33	0.33±0.33	0.00±0.00
MC	3.00±0.00	3.00±0.00	2.33±0.33	1.67±0.33	1.33±0.33	0.00±0.0	0.00±0.00
MT	0.00±0.00[abcd]	0.00±0.00[abcd]	0.00±0.00[abcd]	0.00±0.00[abd]	0.00±0.00	0.00±0.00	0.00±0.00

[a] shows significance to MH, [b] shows significance to ML, [c] shows significance to MA, [d] shows significance to MC and [e] shows significance to MT. ***MH*** *- Colitis + 200mg/kg b.w manganese chloride,* ***ML*** *- Colitis + 100mg/kg b.w of manganese chloride* ***MA*** *- Colitis + 500mg/kg b.w Sulfasalazine treatment,* ***MC*** *- Colitis alone,* ***MT*** *- No colitis no treatment*

Table 2:
Effect of Manganese Chloride on Colon Mucosal Height, Colon Ulcer Depth of Experimental Animal

Groups	Colon Mucosal width			Colon ulcer depth		
	Day 3	**Day 7**	**Day 14**	**Day 3**	**Day 7**	**Day 14**
MH	0.3 + 0.01	0.4 + 0.04	0.28 + 0.02	0.09 + 0.04	0.05 + 0.03	0.06 + 0.03
ML	0.34 + 0.02	0.33 + 0.03	0.32 + 0.04	0.22 + 0.01	0.06 + 0.02	0.03 + 0.03
MA	0.16 + 0.03[abe]	0.31 + 0.03	0.29 + 0.01	0.24 +0.12	0.17 + 0.04	0.00 + 0.00[e]
MC	0.21 + 0.01[b]	0.17 + 0.00[abe]	0.23 + 0.00[e]	0.74 + 0.08[abce]	0.22 + 0.03[abe]	0.10 + 0.02
MT	0.31 + 0.04	0.45 + 0.03	0.37 + 0.04	0.01+ 0.01	0.01 + 0.01	0.02 + 0.02

[a-] shows significance to MH [b]- shows significance to ML, [c]- shows significance to MA, [d]- shows significance to MC and [e]- shows significance to MT ***MH***- *Colitis + high dose of (200mg/kg b.w) Manganese chloride* , ***ML*** - *Colitis + low dose of (100mg/kg b.w) Manganese chloride,* ***MA***- *Colitis + (500mg/kg b.w) Sulfasalazine treatment* , ***MC***- *Colitis alone,* ***MT*** - *No colitis no treatment*

Table 3:
Effect of Manganese Chloride on Colon Length, Weight and Weight/Length Ratio of Experimental Animal

Groups /Days	**COLON LENGTH**			**COLON WEIGHT**			**COLON WEIGHT/LENGTH RATIO**		
	3	**7**	**14**	**3**	**7**	**14**	**3**	**7**	**14**
MH	17.83 ± 1.17[d]	17.50 ± 0.5	17.57 ± 0.18	1.41 ± 0.00	1.64 ± 0.00	1.28 ± 0.01	0.11±0.00	0.10±0.01	0.08±0.00
ML	16.67 ± 0.44	17.50 ± 0.06	13.37 ± 0.13[acd]	1.6 ± 0.05[a]	1.78 ±0.01[ace]	1.23 ± 0.01	0.09±0.00	0.09±0.00	0.09±0.00
MA	13.53 ± 0.29	19.30 ± 0.15[ab]	12.73 ± 0.87[ade]	1.52 ±.04	1.62 ± 0.01	1.41 ±0.00[bd]	0.10±0.02	0.08±0.00	0.08±0.00
MC	12.67 ± 1.20	15.33 ±0.17[abde]	16.13 ± 0.07	1.55 ±0.03	1.66 ± 0.03	1.24 ± 0.03	0.13±0.00[e]	0.10±0.00	0.08±0.00
MT	16.50 ± 1.00	18.50 ± 0.5	17.20 ±0.10	1.22 ± 0.03[abcd]	1.62 ± 0.04	1.28 ± 0.05	0.08±0.00	0.09±0.00	0.08±0.00

[a-] shows significance to MH [b]- shows significance to ML, [c]- shows significance to MA, [d]- shows significance to MC and [e]- shows significance to MT ***MH***- *Colitis + high dose of (200mg/kg b.w) Manganese chloride* , ***ML*** - *Colitis + low dose of (100mg/kg b.w) Manganese chloride*. ***MA***- *Colitis + (500mg/kg b.w) Sulfasalazine treatment* , ***MC***- *Colitis alone,* ***MT*** - *No colitis no treatment* .

Table 4:
Effect of manganese chloride on platelet count, red blood cell count and packed cell volume.

Groups	**Red Blood Cell Count (×10[6] µL).**			**Hemoglobin level (g/dL)**			**Packed Cell Volume (%)**			**Platelet Count million/cu mm**		
	Day 3	**Day 7**	**Day 14**	**Day 3**	**Day 7**	**Day 14**	**Day 3**	**Day 7**	**Day 14**	**Day 3**	**Day 7**	**Day 14**
MH	8.70 ±0.02[bcd]	6.98 ±0.24	7.49 ±0.12[c]	18.50 ±0.61[bcde]	14.10 ±0.32	14.53 ±0.07[bc]	48.33 ±1.67	42.00 ±0.58	40.0 ±3.51	74670 ±5925	67670 ±3283	69330 ±8667
ML	7.20 ±0.14[e]	6.47 ±0.07	6.7 ±0.07	13.57 ±0.12	13.57 ±0.12	12.77 ±0.43	43.33 ±1.76	40.00 ±0.58	37.67 ±1.20	69330 ±3712	69330 ±15450	65670 ±666.7
MA	7.45 ±0.05	6.28 ±0.13	5.55 ±0.71	14.57 ±019	12.33 ±0.07[ae]	12.10 ±0.40	45.33 ±1.20	36.00 ±0.58[ade]	34.00 ±2.31	50330 ±3930[a]	72330 ±5608	64670 ±333.3
MC	7.46 ±0.02	7.27 ±0.27	6.65 ±0.12	14.43 ±0.52	15.33 ±0.07[bc]	13.40 ±0.30	42.67 ±0.88	43.67 ±1.86	36.33 ±3.28	50670 ±5044	76670 ±11620	85670 ±6333
MT	8.33 ±0.46	7.02 ±0.34	6.6 ±0.18	17.77 ±1.17[b,d]	14.50 ±0.69	13.43 ±0.12	48.00 ±1.16	42.33 ±1.45	40.0 ±1.00	66330 ±4978	62000 ±6000	61330 ±2186

a- shows significance when compared with MH b- shows significance to ML, c- shows significance to MA, d- shows significance to MC and e- shows significance to MT. MH- Colitis + high dose of manganese chloride, ML- Colitis + low dose of manganese chloride, MA- Colitis + Sulfasalazine treatment , MC- Colitis alone , MT- No colitis no treatment

Effect of manganese chloride on Red Blood Cell count (RBC), hemoglobin level (HB), Packed Cell Volume (PCV) and platelet count.

Day 3: Red blood cell count in the colitis treated with high manganese (MH) group was significantly higher than colitis treated with low manganese (ML), colitis sulphazalazine treated (MA) and colitis untreated (MC) group while RBC in the colitis treated with low manganese (ML) group was significantly lower than no colitis untreated (MT) group. There was no significance on packed cell volume between the groups on this day. Hemoglobin level of colitis untreated (MC) group was significantly lower than all other experimental groups with colitis treated with high manganese (MH) group having the highest. Platelet count for colitis treated with high manganese (MH) group was significantly higher than colitis sulphazalazine treated (MA) group, (Table 4).

Day 7: There was no significant difference in the platelet and red blood cell counts between the groups. The packed cell volume of the colitis sulphazalazine treated (MA) group was significantly lower than the colitis treated with high manganese (MH), colitis untreated (MC) and no colitis untreated (MT) groups while the Hemoglobin level of colitis sulphazalazine treated (MA) group was the lowest on this day, (Table 4).

Day 14: The platelet count and packed cell volume of all the experimental groups were not significant different on this day. The red blood cell count of the colitis treated with high manganese (MH) group was significantly higher than the colitis sulphazalazine treated (MA) while the Hemoglobin level of the colitis treated with high manganese (MH) group was significantly higher than colitis treated with low manganese (ML) and colitis sulphazalazine treated (MA) groups, (Table 4).

Effect of manganese chloride on neutrophil count, lymphocyte count and White Blood Cell count (WBC)

The neutrophil and lymphocyte counts by day 3 of the experiment was not significant in any of the experimental groups. There was a significant increase in the WBC of the colitis treated with high manganese (MH) group compared with colitis treated with low manganese (ML) and colitis untreated (MC) groups.

However, by day 7 neutrophil count of colitis untreated (MC) group was significantly higher than other groups; colitis treated with high manganese (MH) group had significantly lower neutrophil count than colitis treated with low manganese (ML) group. The lymphocyte count of the colitis untreated (MC) group was significantly lower than all experimental other groups while that of colitis treated with high manganese (MH) was significantly higher than colitis treated with low manganese (ML) group, (Table 5).

The white blood cell count was not significant in any group on this day, (Table 5). The white blood cell count, lymphocyte count and neutrophil count were not significant in any experimental group by day 14 post colitis induction, (Table 5).

Effect of Manganese Chloride on the Neutrophil Lymphocyte Ratio (N/L) of the Experimental Animal

There was a significant increase in the N/L ratio of the colitis untreated groups (MC) by days 3 and 7 compared with all the experimental groups, (Figure 3).

The colitis treated with high dose of (200mg/kg b.w) manganese (MH) had a significantly lower N/L compared with all the other colitis treated groups by days 7 and 14, (Figure 3).

Effect of Manganese Chloride on Total Protein, Albumin Level, Globulin Level, Fibrinogen and Albumin-Globulin Ratio.

The albumin level of colitis high manganese treated (MH) was significantly higher than colitis low manganese (ML), colitis treated with sulfasalazine (MA) and colitis untreated (MC) experimental groups by day 3. On day 3 the fibrinogen level of colitis untreated (MC) group was significantly higher than colitis sulfasalazine treated (MA), colitis low manganese treated (ML) and colitis high manganese treated (MH) groups. The albumin globulin ratio of colitis untreated (MC) and MT groups were significantly lower than colitis high manganese treated (MH) and colitis low manganese (ML) groups. Total serum protein was not significant in any experimental group on this day, (Table 6).

The total serum protein and albumin level of colitis untreated (MC) group was significantly lower than all other experimental groups on day 7 post colitis induction. The Fibrinogen level was not significant by day 7 in all groups, (Table 6).

The albumin level, albumin/globulin ratio as well as total serum protein by day 14 for Colitis treated with sulfasalazine (MA) group was significantly lower than all other experimental groups. There was no significance in the fibrinogen level by day 14 post colitis induction and treatment in all the experimental groups, (Table 6).

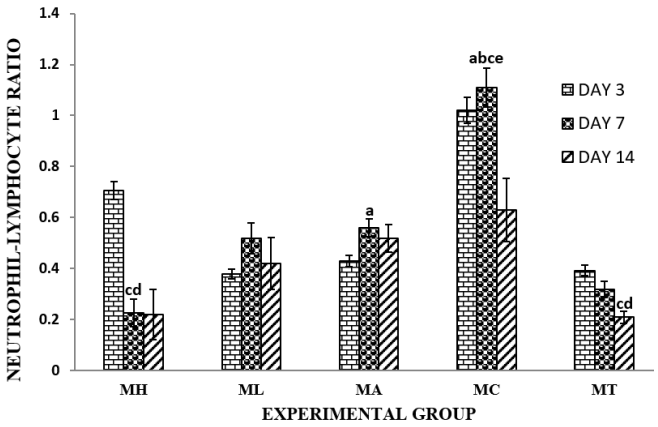

FIGURE 3:
Effect of manganese chloride on neutrophil lymphocyte ratio of experimental animal

[a] shows significance to MH, [b]- shows significance to ML, [c]- shows significance to MA, [d]- shows significance to MC and [e]- shows significance to MT

MH- *Colitis + high dose of (200mg/kg b.w) Manganese chloride,* ***ML***- *Colitis + low dose of (100mg/kg b.w) Manganese chloride,* ***MA***- *Colitis + (500mg/kg b.w) Sulfasalazine treatment,* ***MC***- *Colitis alone,* ***MT***- *No colitis no treatment*

Effect of manganese chloride on weight of spleen and liver of experimental animal

The Spleen weight of colitis untreated (MC) group was significantly higher than other experimental groups [with colitis low manganese treated (ML) group being the lowest and was significantly lower than colitis sulfasalazine treated (MA) and No colitis no treatment (MT)] on day 3. The liver weight of No colitis no treatment (MT) group was significantly lower compared with all other experimental groups by day 3.

The spleen weight of the colitis untreated group (MC) was significantly higher than other experimental groups by day 7, (Table 7).

The liver weight (on this day) of MA group was significantly increased compared with colitis high manganese treated (MH), colitis low manganese treated (ML) and No colitis no treatment (MT) groups; it was significantly higher in the colitis untreated (MC) group compared with colitis high manganese treated (MH) and No colitis no treatment (MT) also (day 7). The spleen weight for colitis untreated (MC) was significantly higher than all other experimental groups (day 7), (Table 7).

TABLE 5:
Effect of manganese chloride on white blood cell, neutrophil, lymphocyte, monocyte and eosinophil counts.

Group	White Blood Cell Count ($\times10^3$/Mm^3)			Neutrophil Count ($\times10^3$/Mm^3)			Lymphocyte Count ($\times10^3$/Mm^3)			Monocyte Level ($\times10^3$/Mm^3)			Eosinophil Level ($\times10^3$/Mm)		
	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14
MH	3433 ±467.6[bd]	3710 ±805	4833 ±384.4	40.33 ±0.88	17.67 ±3.76[b]	20.00 ±5.77	57.00 ±0.58[bcd]	80.33 ±4.10[bd]	74.00 ±4.93	1.33 ±0.33	1.67 ±0.33[bcd]	2.00 ±0.58	1.00 ±0.58	2.00±0.58	2.00±0.58
ML	1967 ±116.7	4790 ±389.3	4517 ±72.65	26.33 ±3.84	34.67 ±2.33	32.67 ±4.49	69.67 ±2.03	60.33 ±2.33	64.67 ±4.70	2.33 ±1.20	2.67 ±0.88	2.00 ±0.58	1.00 ±0.58	2.33±0.88	2.33±0.88
MA	3050 ±208.2	3920 ±409.1	4450 ±350	27.33 ±7.42	27.33 ±1.86	33.00 ±2.52	69.00 ±6.81	70.67 ±1.45	63.33 ±1.67	2.00 ±0.58	2.33 ±0.33	2.33 ±0.33	2.67 ±1.33[abe]	0.33±0.33 [abe]	1.33±0.67 [abe]
MC	1967 ±266.3	5420 ±971.2	4917 ±164.1	29.33 ±4.37	51.67 ±1.67[abce]	30.67 ±5.46	67.33 ±5.23	46.33 ±1.67[abce]	65.00 ±6.11	1.33 ±0.67	2.33 ±0.33	2.00 ±1.00	2.00 ±0.57	0.00±0.00[abe]	2.33±0.33
MT	2483 ±16.67	4820 ±337.9	4100 ±50	32.33 ±3.67	23.33 ±1.76	27.00 ±4.73	68.00 ±4.00	72.67 ±1.76	79.67 ±0.67	1.00 ±0.00	2.00 ±0.58	1.67 ±0.88	1.67 ±0.88	2.00±0.58	1.67±0.33

Table 6:
Effect of Manganese Chloride on Total Protein, Albumin Level, Globulin Level, And Albumin- Globulin Ratio.

Groups	TOTAL PROTEIN LEVEL (mg/dL)			ALBUMIN LEVEL (mg/dL)			FIBRINOGEN LEVEL (mg/dL)			GLOBULIN LEVEL (mg/dL)			ALBUMIN / GLOBULIN RATIO		
	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14
MH	5.80 ±0.15	7.50 ±0.06	7.23 ±0.12	4.43 ±0.23 [bcd]	4.60 ±0.06	4.57 ±0.12	0.1 ±0.00	0.1 ±0.00	0.3 ±0.06	2.13 ±0.03[cde]	2.77 ±0.03	2.87 ±0.03	2.07 ±0.09	1.77 ±0.09	1.53 ±0.67
ML	6.17 ±0.12	7.40 ±0.06	7.17 ±0.09	3.63 ±0.18	4.67 ±0.088	4.37 ±0.03	0.1 ±0.00	0.2 ±0.00	0.3 ±0.06	2.13 ±0.088[cde]	2.77 ±0.03	2.80 ±0.057	2.1 ±0.06	1.70 ±0.06	1.63 ±0.09
MA	6.13 ±0.07	7.27 ±0.13	6.77 ±0.03[ade]	3.60 ±0.15	4.73 ±0.09	3.67 ±0.120[abe]	0.1 ±0.00	0.17 ±0.03	0.2 ±0.06	2.67 ±0.09	2.67 ±0.09	3.17 ±0.03[ab]	1.67 ±0.13	1.70 ±0.06	1.10 ±0.06[abe]
MC	6.53 ±0.15	6.67 ±0.17[abce]	7.27 ±0.09	3.53 ±0.07	4.17 ±0.12[abce]	4.23 ±0.15	0.2 ±0.00[abc]	0.2 ±0.00	0.27 ±0.03	2.83 ±0.07	2.50 ±0.06[ab]	2.87 ±0.03	1.2 ±0.58 [ab]	1.60 ±0.06	1.37 ±0.09
MT	6.20 ±0.50	7.50 ±0.00	7.40 ±0.1	3.8 ±0.10	4.90 ±0.00	4.53 ±0.12	0.17 ±0.03	0.17 ±0.03	0.23 ±0.07	2.77 ±0.03	2.60 ±0.00	2.87 ±0.03	1.48 ±0.17 [ab]	1.80 ±0.00	1.5 ±0.06

[a] shows significance to MH, [b]- shows significance to ML, [c]- shows significance to MA, [d]-shows significance to MC and [e]- shows significance to MT.
MH- *Colitis + high dose of (200mg/kg b.w) Manganese chloride,* ***ML***- *Colitis + low dose of (100mg/kg b.w) Manganese chloride,* ***MA***- *Colitis + (500mg/kg b.w) Sulfasalazine treatment,* ***MC***- *Colitis alone,* ***MT***- *No colitis no treatment*

Table 7:
Effect of manganese chloride on weight of spleen, liver and fibrinogen level of experimental animal

Groups	Weight Of Spleen (g)			Weight Of Liver (g)		
	DAY 3	DAY 7	DAY 14	DAY 3	DAY 7	DAY 14
MH	0.56±0.00	0.68±0.03	0.67±0.02	5.41±0.11[d]	6.10±0.15	6.15±0.55[bcde]
ML	0.48±0.01[ce]	0.65±0.05	0.71±0.05	5.63±0.19	6.81±0.24	5.83±0.56
MA	0.55±0.02	0.66±0.05	0.75±0.03	5.53± 0.07	8.12±0.37[abe]	6.54±0.14
MC	0.66±0.01[abcd]	0.89±0.03[abcd]	0.82±0.05	6.37±0.27	7.87±0.07[ae]	8.43±0.4
MT	0.54.00	0.62±0.04	0.69±0.05	4.09±0.18[abcd]	6.01±0.02	5.69±0.18

[a] shows significance to MH, [b]- shows significance to ML, [c]- shows significance to MA, [d]-shows significance to MC and [e]- shows significance to MT
MH- *Colitis + high dose of manganese chloride* , ***ML***- *Colitis + low dose of manganese chloride*, ***MA***- *Colitis + Sulfasalazine treatment* , ***MC***- *Colitis alone* , ***MT***- *No colitis no treatment*

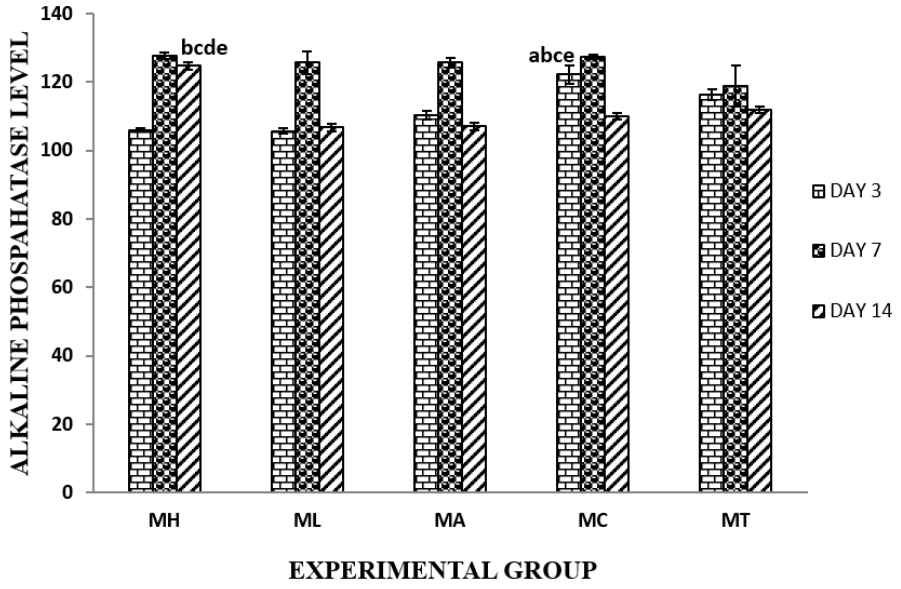

Figure 4:
Effect of Manganese Chloride on Alkaline Phosphatase Level on The Experimental Animal
[a] shows significance to MH, [b]- shows significance to ML, [c]- shows significance to MA, [d]- shows significance to MC and [e]- shows significance to MT. ***MH***- *Colitis + high dose of (200mg/kg b.w) Manganese chloride*, ***ML***- *Colitis + low dose of (100mg/kg b.w) Manganese chloride* , ***MA***- *Colitis + (500mg/kg b.w) Sulfasalazine treatment*
MC- *Colitis alone*, ***MT***- *No colitis no treatment*

Table 8:
Effect of Manganese Chloride on Weight of Pelvic Bone and Femur

Group	Weight of Pelvic Bone (g)			Weight of Femur (g)		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
MH	0.46 ±0.02	0.40 ±0.01	0.38 ±0.01	0.55 ±0.04	0.54 ±0.02	0.58 ±0.01[bc]
ML	0.39 ±0.01	0.39 ±0.02	0.39 ±0.01	0.46 ±0.01	0.46 ±0.01	0.46 ±0.01
MA	0.38 ±0.02	0.42 ±0.01	0.42 ±0.05	0.45 ±0.02	0.55 ±0.02	0.47 ±0.03
MC	0.30 ±0.03[abce]	0.40 ±0.03	0.43 ±0.03	0.54 ±0.03	0.54 ±0.04	0.62 ±0.00[bc]
MT	0.33 ±0.03	0.33 ±0.00	0.30 ±0.00	0.56 ±0.02	0.53 ±0.03	0.52 ±0.03

[a-] shows significance to MH [b]- shows significance to ML, [c]- shows significance to MA, [d]- shows significance to MC and [e]- shows significance to MT

The liver weight of colitis untreated (MC) group was significantly higher than colitis high manganese treated (MH), colitis low manganese treated (ML) and No colitis no treatment (MT) groups (Table 7).

Effect of Manganese Chloride on Alkaline Phosphatase (ALP) Level of Experimental Animal
There was a significant increase in the ALP level of the colitis untreated (MC) group compared with all other groups by day 3 post colitis induction. A significant increase was observed in the colitis high (200mg/kg b.w) manganese treated group when compared with other groups by day 14 of the experiment, (Figure 4).

Effect of Manganese Chloride on Weight of Pelvic Bone and Femur
There was an observed significant decrease in the pelvic bone weight of the colitis untreated group (MC) compared with all the other experimental groups by day 3; there was also no observable significant difference in the femur weight of other treatment groups, (Table 8).

Table 9:
Effect of manganese chloride on the mass density of tibia, length of tibia and weight of tibia

Groups	Mass density of tibia (g/cm)			Length of tibia (mm)			Weight of tibia (g)		
	DAY 3	**DAY 7**	**DAY 14**	**DAY 3**	**DAY 7**	**DAY 14**	**DAY 3**	**DAY 7**	**DAY 14**
MH	0.14± 0.02	0.16 ± 0.01	0.14 ± 0.03	31.00 ± 0.58	32.67 ± 0.33	32.00 ± 0.58	0.60 ± 0.03[bde]	0.54 ± 0.01[bd]	0.54 ± 0.03
ML	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	32.00 ± 0.58	33.33 ± 0.88	32.00 ± 0.58	0.43 ± 0.02	0.45 ± 0.01	0.46 ± 0.02
MA	0.14 ± 0.00	0.12 ± 0.00	0.13 ± 0.02	34.67 ± 0.33[abde]	33.33 ± 0.33	32.33 ± 0.33	0.54 ± 0.02	0.48 ± 0.01	0.48 ± 0.03
MC	0.12 ± 0.01	0.13 ± 0.00	0.13 ± 0.01	31.67 ± 0.33	32.00 ± 0.58	32.00 ± 0.58	0.46 ± 0.03	0.44 ± 0.02	0.50 ± 0.03
MT	0.14 ± 0.00	0.1 ± 0.00[a]	0.11 ± 0.00	31.67 ± 0.33	33.00 ± 0.58	33.00 ± 0.58	0.42 ± 0.03	0.44 ± 0.03	0.44 ± 0.03

[a] shows significance to MH [b-] shows significance to ML, [c-] shows significance to MA, [d-] shows significance to MC and [e-] shows significance to MT

***MH**- Colitis + high dose of (200mg/kg b.w) Manganese chloride, **ML**- Colitis + low dose of (100mg/kg b.w) Manganese chloride, **MA**- Colitis + (500mg/kg b.w) Sulfasalazine treatment,*
***MC**- Colitis alone, **MT**- No colitis no treatment*

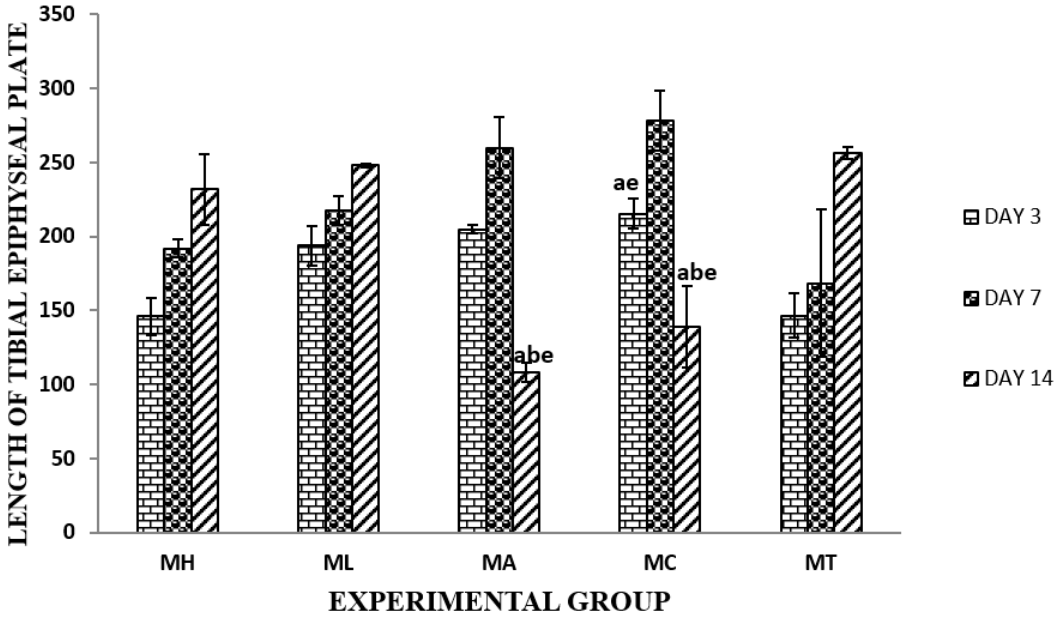

Figure 5:
Effect of manganese chloride on the length of tibial epiphyseal plate in experimental animal
[a] shows significance to MH [b-] shows significance to ML, [c-] shows significance to MA, [d-]shows significance to MC and [e-] shows significance to MT
***MH**- Colitis + high dose of (200mg/kg b.w) Manganese chloride, **ML**- Colitis + low dose of (100mg/kg b.w) Manganese chloride, **MA**- Colitis + (500mg/kg b.w) Sulfasalazine treatment ;**MC**- Colitis alone, **MT**- No colitis no treatment*

No significant difference was observed both in the weight of the pelvic bone or femur in and amongst all the experimental groups by day 7, (table 8).

There was no significant difference in the pelvic bone weight by day 14 in any of the experimental groups, (Table 8). A significant increase in the femur weight of colitis high manganese treated (MH) group was observed when compared with colitis low manganese treated (ML) and colitis sulfasalazine treated (MA) groups by day 14. Similarly, a significant increase in the femur bone of colitis untreated (MC) group was observed when compared with colitis low manganese treated (ML) and colitis sulfasalazine treated (MA) groups, (Table 8).

Effect of manganese chloride on mass density of tibia, length of tibia and weight of tibia

There was no significant difference in the bone mass density in any of the experimental groups by day 3 while a significant decrease was observed in the control – no colitis untreated group (MT) compared with all other groups by day 7 post colitis induction, (Table 9).

A significant increase was observed in the length of tibia of the colitis sulfasalazine treated group (MA) compared with all other experimental groups by day 7, (Table 9).

The weight of the tibia bone was significantly increased in the colitis high manganese treated group (MH) compared with colitis low manganese treated (ML), colitis sulfasalazine treated (MA) and no colitis untreated (MT) groups by day 3. A significant increase in the tibia bone weight of colitis high

manganese treated (MH) was observed on day 7 when compared with colitis low manganese treated (ML), colitis untreated (MC) and no colitis untreated (MT) groups, (Table 9).

There was no observed significance in the mass density, length or weight of tibia by day 14 post colitis induction, (Table 9).

TABLE 10:

Effect of Manganese Chloride on The Myeloid Erythroid Ratio of the Experimental Animal

MYELOID / ERYTHROID RATIO			
	DAY 3	**DAY 7**	**DAY 14**
MH	1.89±0.23	1.8±0.03	1.95±0.05
ML	2.05±0.18	1.42±0.21	1.69±0.06[cde]
MA	1.84±0.21	2.01±0.12	2.24±0.11
MC	2.08±0.11	2.86±0.15[abce]	2.2±0.07
MT	2.2±0.25	1.99±0.16	2.11±0.02

[a] shows significance to MH [b-] shows significance to ML, [c-] shows significance to MA, [d-]shows significance to MC and [e-] shows significance to MT ***MH****- Colitis + high dose of (200mg/kg b.w) Manganese chloride,* ***ML****- Colitis + low dose of (100mg/kg b.w) Manganese chloride,* ***MA****- Colitis + (500mg/kg b.w) Sulfasalazine treatment* ***MC****- Colitis alone,* ***MT****- No colitis no treatment.*

Effect of manganese chloride on the length of tibial epiphyseal plate of experimental group.

There was a significant increase by day 3 in the Tibial epiphyseal plate length of the Colitis untreated group (MC) compared with colitis treated with high manganese (MH) and Control - no colitis untreated (MT) groups, (Figure 5).

There was no observable significant difference by day 7 of the experiment, (Figure 5). A significant decrease was observed in the tibia epiphyseal plate length of colitis sulfasalazine treated (MA) and colitis untreated (MC) groups by day 14 of the experiment, (Figure 5).

Effect of manganese chloride on myeloid erythroid ratio of experimental animals: There was no significant difference in the myeloid erythroid ratio of all the treatment groups by day 3, (Table 10).

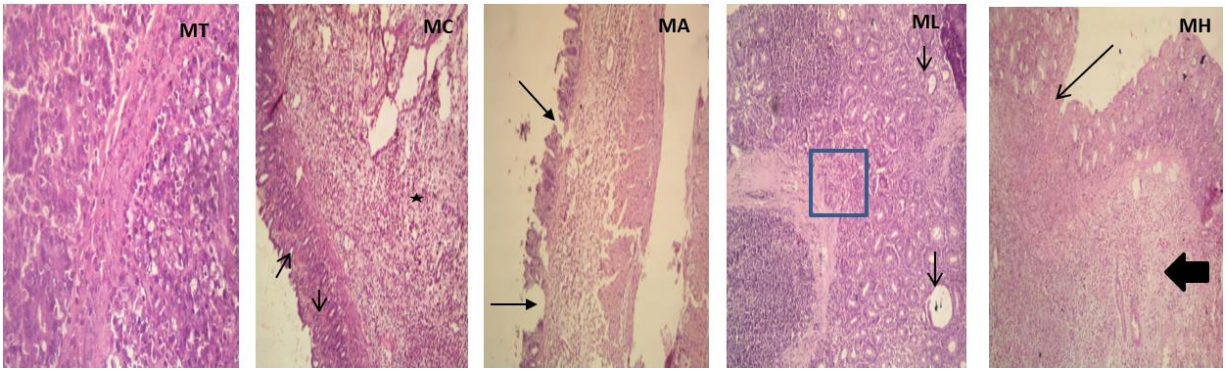

Plate 1:

Photomicrographs of Colon Sections by Day 3 Post Colitis Induction (H&E STAIN; MAG X 100) SHOWING MT (CONTROL):- There is severe extensive cryptal necrosis (right of photomicrograph). **MC (Colitis untreated group)**: There is marked widespread influx of inflammatory cells and oedema in the submucosa (star). There are moderate erosions of the surface epithelium. Note cryptal necrosis and haemorrhages between the cryptal glands (arrows). **MA (500mg/kg b.w 5ASA treated group)**: There are multiple foci of marked erosions (arrows). The submucosa (star) is oedematous and contains moderately increased amounts of polymorphonuclear leucocytes H&E X100. **ML (100mg/kg b.w Manganesse treated group)**: Left: There are a few foci (box) of cryptal necrosis with moderate influx of inflammatory cells. Note a few large cyst-like crypts (arrow) 100X. Right: Note the increased pericryptal influx of inflammatory cells and the large crypt with flattened epithelium. **MH (200mg/kg b.w Manganesse treated group)**: Ulcerated focus (thin arrow) with severe inflammatory response in the mucosa and submucosa. Note the numerous inflammatory cells and oedema in the submucosa (thick arrow).

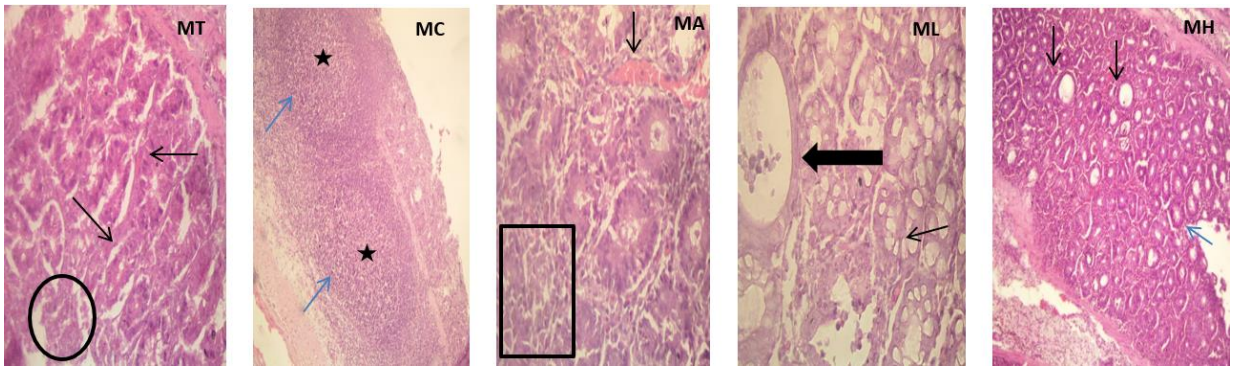

Plate 2:

Photomicrographs of colon sections by day 7 post colitis induction (H&E stain; MAG X 100) showing MT (CONTROL):- Moderately congested blood vessels (arrows) and eroded surface epithelium (circle). **MC (Colitis untreated group)**: Large lymphoid follicles (arrows) of the GALT with prominent pale-staining germinal centres (stars) suggestive of intense lymphoid proliferation and response. **MA (500mg/kg b.w 5 ASA treated group)**: Cryptal necrosis is shown in the circle while the arrow shows congested blood vessels. **ML (100mg/kg b.w Manganesse treated group):** Increased numbers of goblet cells per crypt (thin arrow). Note the large dilated cyst-like crypt (thick arrow). **MH (200mg/kg b.w Manganesse treated group):** Thick ruggae with numerous crypts and a few regenerating crypts with flattened epithelium and distended lumens (black arrows). There is mild erosion of the surface epithelium (blue arrow).

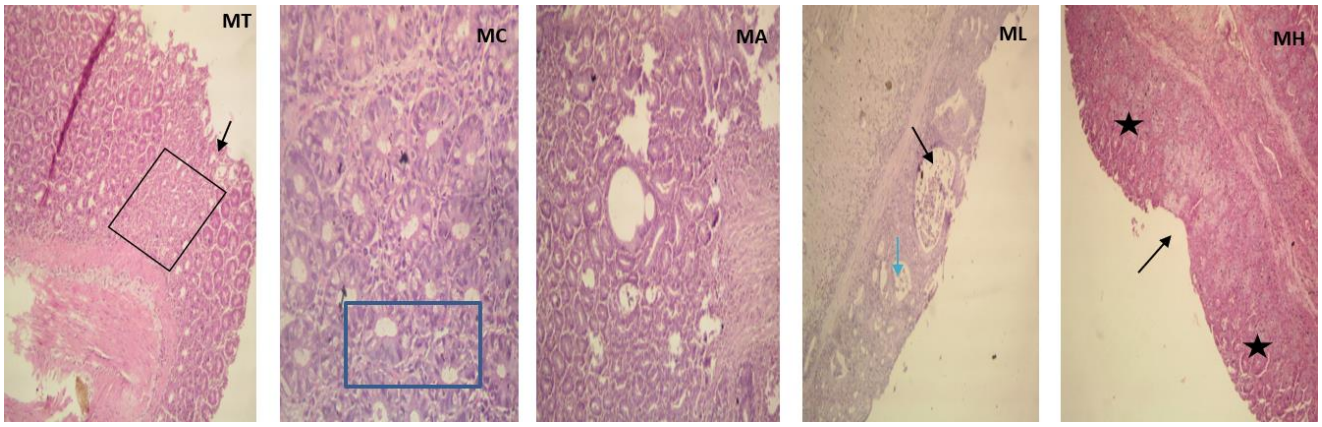

Plate 3:
Photomicrographs of colon sections by day 14 post colitis induction (H&e stain; MAG X 100) MT (Control):- There is moderate erosion of surface epithelium (arrow) and locally extensive influx of inflammatory cells in the *lamina propria* of the mucosa (box); **MC (Colitis untreated group):** There are increased numbers of goblet cells in the crypts within the box. **MA (500mg/kg b.w 5 ASA treated group)**: There are a few large dilated cryptal glands (arrows) with flattened lining epithelium suggestive of regenerating crypts. Note the widespread influx of polymorphonuclear inflammatory cells between the crypts and extending to the *lamina muscularis mucosa* (right of photomicrograph); **ML (100mg/kg b.w Manganesse treated group)**: There are numerous haphazardly-arranged large cystic crypts(arrows) with luminal debris; suggestive of healing and regeneration; **MH (200mg/kg b.w Manganesse treated group):** Depressed focus of an healed ulcer (arrow); Normal crypts (stars) surround the healed ulcer.

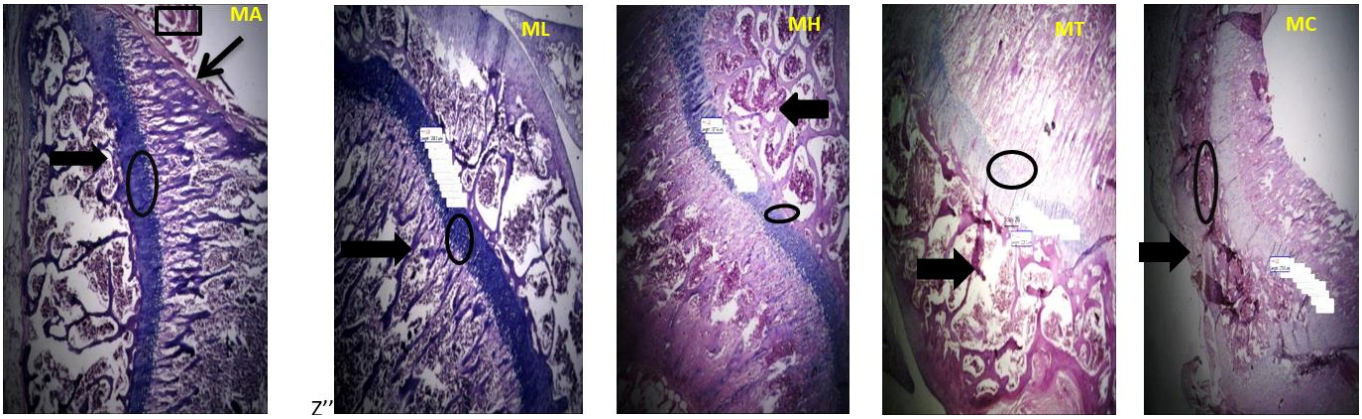

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Plate 4:
Photomicrographs of tibial epiphyseal plates sections post colitis induction (H&E STAIN; MAG X 100) for MA, ML, MH, MT AND MC GROUPS. *: thick arrows represent trabecular bone, the oval shape represents the tibial epiphyseal plate the thin arrow shows the tendon while the rectangular box represents the skeletal muscle*

The colitis untreated (MC) group exhibited a significantly high myeloid erythroid ratio compared with all other treatment groups by day 7 of treatment, (Table 10).

On day 14 of treatment, the low manganese treated (ML) group had a significantly lower myeloid erythroid ratio compared with the Colitis sulfasalazine treated (MA), colitis untreated (MC) and no colitis untreated (MT) groups, (Table 10).

Plates 1 – 3 show the histological features of the colon of the control and manganese treated rats while Plate 4 shows the tibila epiphyseal plates.

DISCUSSION

Ulcerative Colitis is known to affect other organs of the body other than the gastrointestinal tract (Ardizzonea *et al.*, 2008) one of these organs is the bone (Ardizzone *et al.*, 2000). Osteoporosis which literally means "porous bone" is one of the extra intestinal manifestations of UC characterized by decreased bone mineral density leading to fracture of the bone, disability, low quality of life of affected individual and 30% mortality within a year (WHO, 1994). Osteoporosis in UC is as a result of corticosteroid treatment, inadequate vitamin D or activity of inflammatory cytokines on the bone (Raggatt and Partridge 2010).

Loss of body weight as well as reduced growth rate velocity or rather stunted growth is an early signal of inflammatory bowel disease especially in children (Gassull and Stange 2003; Seidman *et al.*, 1991). This causes muscle wasting (Fisher, 1999; Valentini *et al.*, 2008) conditions in which the protein stores especially those in the skeletal muscle are mobilized to help in balancing between energy substrate and body demand of essential amino acids to sustain it. The loss of body weight observed in the colitis untreated group probably would have been as a result of muscle wasting or depletion of amino acid stores to help augment energy demands in the experimental animals. Manganese treatment caused a steady increase in body weight all through the course of experiment probably by acting as a macronutrient which could likely reduce the rate at which energy is demanded for from stored amino acids hence preventing muscle wasting. The increase in total protein level caused by manganese treatment alone might have been as a result of Manganese mitigating against chronic loss of mean body mass which normally occurs in UC. Fisher,(1999)

observed that chronic body mass loss is partly due to altered amino acid and protein. This observed increased body weight is contradictory to the earlier reports of Ajibade *et al.,* 2011 and Salami *et al.,* 2015.

Watery bloody stool or diarrhea as well as loss of body weight during UC are major physical visible signs attributed mainly to disruption in the gastrointestinal absorptive functions (Stein *et al*., 1998). Diarrhea or watery mucus or blood stained stool was observed within 12 hours in all groups induced with colitis. Manganese intervention reverted this observation between 48 to 72 hours (unlike the colitis untreated animals) thus improving stool consistency from day 3-5 of colitis induction. It might probably be that manganese treatment helped in attenuating some processes of ulcerative colitis which resulted in improved gastrointestinal absorptive functions thereby preventing diarrhea and body weight loss.

Colonic weight length ratio a marker of inflammation normally increases during colitis (Xing *et al.,* 2012) was also observed in this study. Manganese treatment prevented an increase in weight- length ratio of the colon as well as shortening of the colon length. Manganese has been reported to be not just an antioxidant but a selective catalytic antioxidant (Riley *et al.,* 1997) it probably specifically scavenged for free radical oxygen specie generated during colitis induction. It might well be that this catalytic antioxidative ability of manganese probably modulated the inflammatory and or oxidative responses which might have been produced during colitis induction. This might have facilitated the observed ulcerative colitis healing in the treated groups unlike in all other groups, a similar enhanced activity has also been noted by Shetlar and Shetlar (1994).

The bone marrow cells have been saddled with the task of production of blood cells once signaled. The bone marrow cells have been found to be adversely affected during inflammatory bowel disease; this may partly be as a result of negative interactions occurring between inflammatory cytokines and the processes/stages of erythropoiesis (Means and Krantz 1992; Means, 1999). This adverse interaction leads to condition referred to as anaemia of chronic disease (Cartwright, 1966); one of which is being managed during ulcerative colitis or IBD (Kandhare *et al*., 2012c). This anemia has been researched and found to occur with inhibition or gross reduction of erythropoiesis [the main target begin the Blast Forming Unit – Erythroblast (BFU-E) and Colony Forming Unit – Erythroblast (CFU-E) both located within the bone marrow] due to the adverse effect or interaction of interferon –γ (Means and Krantz 1992). Several other deleterious interaction targeted at the bone marrow include: inadequate delivery of iron to the bone marrow as a result of interaction of IL-1 and TNF-α as well as an outright inhibition of erythropoietin production (IL-1, TNF-αand IL-6) (Ballinger *et al*., 2000; Favuin *et al.,* 1992).

Anemia has also been discovered to arise or occur as a result of chronic inflammation (Dyer, 1970; Dyer *et al*., 1972) as well as normoblastic bone marrow disorder (Thomspon *et al.,* 1978) such as occurs during inflammatory bowel disease. In the course of this study, Manganese intervention prevented anemia as observed in the blood variables in a dose and duration dependent manner. This observed intervention was further confirmed in the ample decrease of Myeloid-Erythroid ratio in Manganese treated animals thus revealing its ability to attenuate the adverse interactions of inflammatory cytokines with cells of bone marrow (especially the erythroid series saddled with red blood cell production). The 5-aminosalicylates group showed a decrease in hemoglobin and packed cell volume which is similar to observations of Mintzer *et al*. (2009). Manganese intervention further decreased Neutrophil-Lymphocyte ratio (a marker of inflammation) in a dose and duration manner further buttressing its ability to reduce observed colonic inflammations during ulcerative colitis.

Hypo-albuminemia is an another condition due to either illness or anorexia, the burden of oxidative injury related to chronic inflammation leading to decreased serum total protein level (Rajinikanth *et al*., 2014). Hypo-albuminemia was observed in the colitis untreated and sulphazalazine treated groups as well as an increase in the Fibrinogen level (a chronic end stage inflammatory protein). These adverse conditions were ameliorated in the manganese treated groups probably by its ability to mop up free radicals and or modulate denaturing of plasma proteins thus preventing chronic immune activation. Manganese also prevented enlargement of spleen and liver observed during colitis.

Alkaline phosphatase (ALP) is known mostly to be associated with defective liver cells (Verma and Gorard, 2012) but is also produced by growing bones (Tobiume *et al.,* 1997; Sertbas *et al*., 2010). In growing bones, it (ALP) helps to mop up toxic phosphate ions which increase during colitis (Rajinikanth *et al.,* 2014) as well as aiding to remove pyrophosphate a known inhibitor of bone mineralization (Whyte, 2010). Alkaline phosphatase level in colitis untreated animals was significantly increased which corresponds with the increased weight of the liver revealing a defective or inflamed liver. As healing progressed manganese treatment caused an increase in the ALP but decrease in the liver weight; this increase could be as a result of increased alkaline phosphatase production by the bone chondrocytes to help mop up or reduce production of pyrophosphate thus enhancing bone mineralization.

Studies on animals have shown manganese-deficient diets in rats prevent cartilage formation and produce osteopenia which is as a result of an imbalance between osteoblastic and osteoclastic activity (Strause *et al.,* 1987). Studies have also shown that the pelvic bone and fermur (Jiang *et al.,* 2008) are usually prone to fracture during osteoporosis. Defective reduced bone weight mostly as a result of reduced bone mineral density might be complications due to adverse inflammatory cytokines spilling into the blood circulation (Kawai *et al*., 2012), corticosteroid treatments (Abitol *et al.,* 1995; Sakellariou *et al.,* 2006) or inflammatory bowel disorder (Jahnsen *et al*., 1997, Ulivieri *et al*., 2001, Khan *et al.,* 2013). Manganese treated group had increased bone weight and tibia mass density which may be as a result of increased activity of the osteoblast in bone formation which surpasses osteoclastic bone resorption. All through the study the group treated with sulfasalazine only showed increase in weight on day 7 and no change was recorded on day 14 probably due to increased osteoclastic activity on day 3 but by day 7 osteoblastic activity surpassed osteoclastic activity.

Studies have shown that children with colitis have impaired linear growth (Koniaris *et al.,*1997) which might be related to hypo-proteinaemia (Ahmed *et al.,* 2013; Vasseur *et al.,* 2010; Motil *et al.,* 1982) occurring mostly as a result of gastrointestinal protein loss. This impaired linear growth could be as a result of delayed closure of the epiphyseal plate and or

increased catabolism from inflammation (Paganelli *et al.*, 2007; Beeken *et al.,* 1972). Osteoporosis which causes an alteration in microarchitecture of the bone may be one of the factors which would delay closure of the epiphyseal plate and eventually cause retarded growth. Pollak *et al*. (1998), Schulte *et al.* (1998) have observed that an imbalance occurs between bone resorption and formation in most inflammatory bowel disease, probably Manganese treatment modulated this imbalance through enhance production of Alkaline phosphatase hence decrease in duration of closure of the tibial epiphyseal plate .

Manganese treated groups recorded improved histological parameters like a decrease in ulcer depth with accelerated mucosal healing of the ulcerated colon amongst its' (osteoporosis) bone modulatory activity.

In conclusion, the study shows that Manganese chloride mitigated against osteoporotic deficiencies and enhanced healing activities on experimentally induced ulcerative colitis in young experimental rats.

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Competing Interest.
The authors of this study have no competing interests.

REFERENCES

Abitol, V., Roux, C., Chaussade, S., Guillemant, S., Kolta, S (1995). Metabolic bone assessment in patients with inflammatory bowel disease, Gastroenterol. 108: 417-422

Ahmed, S.F., Farquharson, C., Mc Grogan, P., Russell, P.K (2013). Pathophysiology and management of abnormal growth in children with chronic inflammatory bowel disease, World Review of Nutrition and Dieteti. 106: 142-148

Ajibade, A.J., Fakunle, P.B., Fatoba, O.O., Olayemi, O.T (2011). Some effects of manganese dichloride administration on the body weight, purkinje cell population, brain and cerebellar weight of adult wistar rats. Journal of Neuroscience and Behavioural Health 3(7): 87-90

Aller, M.A., Arias, J.L., Sanchez-Patan, F., Arias, J (2006). The inflammatory response: an efficient way of life, Med. Sci. Momt., 12 RA225-234

Ardizzonea, S., Sarzi Puttini, P., Cassinotti, A., Bianchi Porro, G (2008). Extraintestinal manifestations of inflammatory bowel disease Digestive and Liver Disease, 40S:S253–S259

Ardizzone, S., Bollani, S., Bettica, P., Bevilacqua, M., Molteni, P., Bianchi Porro, G (2000). Altered bone metabolism in inflammatory bowel disease: there is a difference between Crohn's disease and ulcerative colitis, J Intern Med. 247: 63-70

Ballinger, A.B., Azooz, O., El-Haj, T., Poole, S., Farthing, M.J.G (2000). Growth failure occurs through a decrease in insulin-like growth factor 1 which is independent of under nutrition in a rat model of colitis, Gut., 46 (5): 694-700

Beeken, W.L., Busch, H.J., Sylwester, D.L (1972). Intestinal protein loss in Crohn's disease, Gastroenterol. 62: 207

Bernstein, C.N., Blanchard, J.F., Rawsthorne, P., Yu, N (2001). The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study, Am J. Gastroenterol., 96:1116-1122

Bernstein, C.N., Wajda, A., Blanchard, F (2005). The clustering of other chronic inflammatory
diseases in inflammatory bowel disease: a population-based study, Gastroenterology, 129: 827-836

Bourikas, L.A., Papadakis, K.A (2009). Musculoskeletal manifestations of inflammatory bowel disease, Inflamm Bowel Dis., 15 1915-1924.

Cartwright, G.E (1966). The anemia of chronic disorders, Semin Hematol. 3 3S: 1-75

Crohn's and Colitis Foundation of America (CCFA) (Updated October 2012) from www.ccfa.org

Crohn's and Colitis Foundation of America (CCFA) (December 2013) from www.ccfa.org

Dacie, J.V., Lewis, S.M. (1991). In: Practical Hematology 7th edition ELBS with Churchill Livingstone Longman group UK,5-82: 160-175

De Vos, M (2004). Review article: joint involvement in inflammatory bowel disease, Aliment Pharmacol. Ther., 20: 36-42

de Menezes Filho, H., de Castro, L.C., Damiani, D (2006). Hypophosphatemic rickets and osteomalacia, Arq Bras Endocrinol Metabol., 50: 802–13 doi: 10.1590/S0004-27302006000400025

Dyer, N.H. Studies on Crohn's disease M.D. Thesis, Cambridge University, 1970

Dyer, N.H., Child, J.A., Mollin, D.L., Dawson, A.M (1972). Anaemia in Crohn's disease Quarterly Journal of Medicine, New Series, 41: 419-36

Emsley, John, (2001). "Manganese". Nature's Building Blocks: An A-Z Guide to the Elements Oxford, UK: Oxford University Press.; 249–253

Engel, M., Kellerman, C., Rau, T., Burnat, G., Halin, E., Konturek, P (2008). Ulcerative colitis in AKR mice is attenuated by intraperiotoneally administered anandamide, Acta Physiol Pol. 59 673

Farrell, R.J., Peppercorn, M.A (2002). Ulcerative colitis, Lancet. 359: 331-40

Fayuin, W.C., Schneider, T.J., Goldberg, M.A (1992). Effect of inflammatory cytokines on hypoxia-induced erythropoietin production Blood, 79: 1987-94

Fisher, R.L (1999). Wasting in chronic gastrointestinal diseases, J. Nutr., 129 252S-255S

Ford, A.C., Moayyedi, P., Hanauer, S.B (2013). Ulcerative colitis. BMJ 346:f432

Gafni, I.R., McCarthy, E.F., Hatcher, T., Meyers, J.L., Inoue, N., Reddy, C., Weise, Barnes, K.M., Abad, J. Baron, V (2002). Recovery from osteoporosis through skeletal growth early bone mass acquisition has little effect on adult bone density, The FASEB Journal, 16: 736-738

Gassull, M.A., Stange E.F (2003). Nutrition and diet in inflammatory disease in: Satsangi J, Sutherland LR eds. Inflammatory bowel diseases. London, UK: Elsevier, 461-74

Greger, J.L (1998). Dietary standards for manganese: overlap between nutritional and toxicological studies, J Nutr. 128: 368S-371S

Head, K.A., Jurenka, J.S (2003): Inflammatory bowel disease Part 1: Ulcerative colitis- pathophysiology and conventional and alternative treatment options Alternative medicine review: a journal of Clinical therapeutic, 8: 247-83

Hildebrand, H., Karberg, J., Kristiansson, B (1994). Longitudinal growth in children and adolescents with inflammatory bowel disease, J Paediatr Gastroenterol Nutr., 18:165-73

Irvine, E.J., Feegan, B., Rochon, J., Archambault, A., Fredorak, R.N., Groll, A., Kinnear, D., Saibil, F., McDonald, J.W (1994). Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease,

Canadian Crohn's Relapse Prevention, Trial Study Group. Gastroenterology. 106: 287-96

Jahnsen, J., Falch, J.A., Aadland, E., Mowinckel, P (1997). Bone mineral density is reduced in patients with Crohn's disease but not in patients with ulcerative colitis: a population based study, Gut, 40: 313-319 [PMID: 9135518 DOI: 10.1136/gut.40.3.313]

Jiang, G.Z., Matsumoto, H., Hori, M., Gunji, A., Hakozaki, K., Akimoto, Y., Fujii, A (2008). Correlation among geometric, densitometric and mechanical properties in mandible and femur of osteoporotic rats, Journal of Bone and Mineral metabol. 26: 130-137

Kandhare, A.D., Raygude, K.S., Ghosh, P., Ghule, A.E., Gosari, T.P., Badole, S.L., Bodhankar, S.L (2012c). Effect of hydroalcoholic extract of Hibiscusrosa sinensis Linn. Leaves in Experimental colitis in rats, Asian Pac J Trop Biomed. 2 337-44

Kanis, J.A., McCloskey, E.V., Johansson. H., Cooper, C., Rizzoli, R., Reginster, J.Y (2013). European guidance for the diagnosis and management of osteopororsis in postmenopausal women. Osteoporos Int 24: 23-57

Khan, N., Abbas, A.M., Almukhtar, R.M., Khan, A (2013): Prevalence and predictors of low bone

mineral density in males with ulcerative colitis. J Clin Endocrinol Metab. 98: 2368-2375

Ko, I.K., Kim, B.G., Awadallah, A., Mikulan, J., Lin, P., Letterio, J.J (2010). Targeting improves MSC treatment of inflammatory bowel disease, Mol. Ther. 18 1365

Koniaris, S.G., Fisher, S.E., Rubin, C.T (1997). Experimental colitis impairs linear growth independent of nutritional factors, J. Paediatr. Gastroenterol. Nutr., 25:137–41

Koniaris, S.G., Fisher, S.E., Rubin, C.T., Chawla, A (1997). Experimental colitis impairs linear bone growth independent of nutritional factor, J. Pediatr Gastroenterol Nutr., 25: 137-41

Kawai, V.K., Stein, C.M., Perrien, D.S., Griffin, M.R (2012). Effects of antitumor necrosis factor α agents on bone, Curr Opin Rheumatol., 24: 576-585 [PMID: 22810364 DOI: 10.1097/BOR. 0b013e328356d212]

Kuwabara, A., Tanaka, K., Tsugawa, N., Nakase, H., Tsuji, H., Shide, K., Kamao, M., Chiba, T., Inagaki, N., Okano, T., Kido, S (2009). High prevalence of vitamin K and D deficiency and decreased BMD in inflammatory bowel disease. Osteoporos Int 20: 935-942

Kwan Tat, S., Padrines, M., Theoleyre, S., Heymann, D., Fortun, Y (2004). IL-6, RANKL, TNF- alpha/IL-1: interrelationin bone resorption pathophysiology, Cytokine Growth Factor Rev. 15: 49-60

Lichtenstein, G.R (2003). Evaluation of bone mineral density in inflammatory bowel disease: current safety focus, Am. J. Gastroenterol., 98: S24-S30

Means, R.T.J., Krantz, S.B (1992). Progress in understanding the pathogenesis of the anemia of chronic disease, Blood. 80: 1639-47

Means, R.T. (Jr,) (1999). Advances in the anemia of chronic disease, Int J. Hematol. 70: 7- 12

Mintzer, D.M., Billet, S.N., Chmielewki, L (2009). Drug-Induced Hematoetlogic Syndromes Advances in Hematology, Article ID 495863: 1-12, doi:10.1155/2009/495863

Morris, G.P., Beck, P.L., Herridge, M.S., Depew, W.T., Szewwczuk, M.R., Wallace, J.L (1989).

Hapten induced model of chronic inflammation and ulceration in the rat colon, Gastroenterol., 96: 795-803

Motil, K.J., Grand, R.J., Maletskos, C.J., Young, V.R (1982). The effect of disease, drug and diet on whole body protein metabolism in adolescents with Crohn disease and growth failure, Journal of Padiatrics. 101: 345-351

Paganelli, M., Albanese, C., Borrelli, O., Gvitelli, F., Canitano, N., Viola, F., Passariello, R., Cucchiara, S (2007). Inflammation is the main determinant of low bone mineral density in pediatric inflammatory bowel disease, Inflamm Bowel Dis. 13: 416-23

Perrini, S., Natalicchio, A., Laviola, L., Cignarelli, A., Melchiorre, M., De Stefano, F., Caccioppoli, C., Leonardini, A., Martemucci, S., Belsanti, G., Miccoli, S., Ciampolillo, A., Corrado, A., Cantatore, F.P., Giorgino, R., Giorgino, F (2008). Abnormalities of insulin-like growth factor-I signaling and impaired cell proliferation in osteoblasts from subjects with osteoporosis, Endocrinology. 149: 1302 – 1313

Piodi, L.P., Poloni, A., Ulivieri, F.M (2014). Managing osteoporosis in ulcerative colitis: something new? World J Gastroenterol. 20 (39): 14087-14098

Pollak, R.D., Karmeli, F., Eliakim, R., Ackerman, Z., Tabb, K., Rachmilewitz, D (1998). Femoral neck osteopenia in paitients with inflammatory bowel disease, The American Journal of Gastroenterol. 93: 1483-1490

Rajinikanth, B., Venkatachalam, V.V., Manavalan, R (2014). Investigations on the potential of Serratiopeptidase – A Proteolytic enzyme,on acetic acid induced ulcerative colitis in mice, International Journal of Pharmacy and Pharmaceutical Sciences, 6: 525 -531

Raggatt, L.J., Partridge, N.C (2010). Cellular and molecular mechanisms of bone remodeling J. Biol. Chem., 285: 25103-25108

Riley, D.P., Lennon, P.J., Neumann, W.L., Weiss, R.H (1997). Toward the rational design of superoxide dismutase mimics: mechanistic studies for the elucidation of substituent effects on the catalytic activity of macrocyclic manganese (II) complexes, J. Am. Chem. Soc. 119: 6522–6528

Ruffolo, C., Angriman, I., Scarpa, M., Polese, L., Barollo, M., Bertin, M., Pagano, D., D'Amico, D.F (2004). Minimally invasive management of Crohn's disease complicated by ureteral stenosis, Surg Laparosc Endosc. Percutan Tech., 14: 292-294

Sakellariou, G.T., Moschos, J., Berberidis, C., Mpoumponaris, A., Kadis, S., Molyvas, E., Kouklakis, G (2006). Bone density in young males with recently diagnosed inflammatory bowel Disease, Joint Bone Spine, 73 725-728 [PMID: 17126059 DOI: 10.1016/j.jbspin.2006.01.017]

Salami, S.O., Ibe, C.S., Umosen, A.D., Ajayi, I.E., Maidawa, S (2011). Comparative osteometric studies of long bones in Yankassa sheep and red Sokoto goats, Int. J. Morphol. 29: 100-104

Salami, A.T., Adeola, B.O., Iyiola, T.O., Omayone, T.P., Oluwole, F.S. and Olaleye, S.B (2015). Antoixidative action of manganese treatment in delayed healing of acetic acid induced ulceration in rat stomach. J. Afr. Ass. Physiol. Sci. 3 (2): 67-78

Saleh, I.A., Gamal, A.S., Amani, S.A.,. Abd El, R.M (2012). Antiinflammatory activity, safety and protective effects of leptadenia pyrotechnica, Haloxylon salicornicum and Ochradenus baccatus in ulcerative colitis, Phytopharmacology. 2: 58-71

Schulte, C., Dignass, A.U., Mann, K., Goebell, H (1998). Reduced bone mineral density and

unbalanced bone metabolism in patients with inflammatory bowel disease, Inflammatory Bowel Dis. 4: 268-275

Seidman, E., LeLeiko, N., Ament, M., Berman, W., Caplan, D., Evans, J., Kocoshis, S., Lake, A., Motil, K., Sutphen, J., Thomas, D (1991).Nutritional issues in pediatric inflammatory bowel disease, J. Pediatr. Gastroenterol . Nutr. 12 424–438.

Sertbas, Y., Ersoy, U., Ayter, M., Tırtıl, F.G., Kucukkaya, B (2010). Ezetimibe effect on bone mineral density and markers of bone formation and resorption, Journal of Investigative Med. 58: 295-297

Shetlar, M.R., Shetlar, C.L (1994). The role of manganese in wound healing. In Klimis-Tavantiz D.L, ed. Manganese in health and disease Boca Raton: CRC Press, Inc., 145-157

Stein, J., Ries, J., Barrett, K.E (1998). Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells, Am J Physiol, Gastrointest: Liver Physiol. 274 G203-G209

Strause, L., Saltman, P., Golwacki, J (1987). The effect of deficiencies of manganese and copper on resorption of bone particles in rats, Calcified Tissue Internat. 41:145-50

Timani, S., Mutasim, D. F (2006). Skin manifestations of inflammatory bowel disease, Clin Dermatol., 26: 265-273

Thomspon, A.B., Brust, R., Ali, M.A., Mant, M.J., Valberg, L.S (1978). Iron deficiency in inflammatory bowel disease. Diagnostic efficacy of serum ferritin Digestive Diseases, 23: 705

Tobiume, H., Kanzaki, S., Hida, S., Ono, T., Moriwake, T., Yamauchi, S., Tanaka, H., Seino, Y (1997). Serum bone alkaline phosphatase isoenzyme levels in normal children and children with growth hormone (GH) deficiency: a potential marker for bone formation and response to GH therapy, J Clin Endocrinol Metab. 82: 2056-61

Triantofillidas, J.K., Nasioulas, G., Kosmidis, P.A (2009). Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies, Anticancer Res., 29: 2727-32

Ulivieri, F.M., Piodi, L.P., Taioli, E., Lisciandrano, D., Ranzi, T., Vezzoli, M., Cermesoni, L.,

Bianchi, P (2001): Bone mineral density and body composition in ulcerative colitis: a six-year follow-up. Osteoporos int. 12: 343-348

Ulitsky, A., Ananthakrishnan, A.N., Naik, A., Skaros, S., Zadvornova, Y., Binion, D.G., Issa, M (2011). Vitamin D deficiency I patients with inflammatory bowel disease: association with disease activity and quality of life. JPEN J Parenter Enteral Nutr 35: 308-316

Vaisman, N., Dotan, I., Halack, A., Niv, E (2006). Malabsorption is a major contributor to underweight in Crohn's disease patients in remission, Nutrition, 22 (9): 855-859

Valentini, L., Schaper, L., Buning, C., Hengstermann, S., Koernicke, T., Tillinger, W., Guglielmi, F.W., Norman, K., Buhner, S., Ockenga, J., Pirlich, M., Lochs, H (2008). Malnutrition and impaired muscle strength in patients with Crohn's disease and ulcerative colitis in remission Nutrition, 24: 694–702.

Vasseur, F., Gower-Rousseau, C., Vernier- Massouille, G., Dupas, J.L., Merle, V., Merlin, B., Lerebours, E., Savoye, G., Salomez, J.L., Cortot, A., Colombel, J.F., Turck, D (2010). Nutritional status and growth in pediatric Crohn's disease; A population based study, Am J Gastroenterol. 105: 1893-900

van Staa, T.P., Cooper, C., Brusse, L.S., Leufkens, H., Javaid, M.K., Arden, N.K. (2003): Inflammatory bowel disease and the risk of fracture, Gastroenterology, 125: 1591-1597

Verma, J., Gorard, D.A (2012). Persistently elevated alkaline phosphatase, BMJ. Case Rep. 2012: bcr2012006768

Whyte, M.P (2010). Physiological role of alkaline phosphatase explored in hypophosphatasia, Annals of the New York Academy of Sci. 1192: 190–200

World Health Organisation Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis (1994). Geneva WHO Technical Report Series, 843

Xing, J.F., Sun, J.N., Sun, J.Y., You, C.Y., Dong, K., Lv, J., Dong, Y.L (2012). Protective effect

of 3,4-oxo-isopropylidene-shiklmic acid on experimental colitis induced by trinitrobenzene sulfonic acid in rats, Dig Dis Sci. 57: 2045-5.