Online publication ahead of print, AJMHS Vol 53, 2020

Curcumin Favors Bone Mineralization, Mitigates Lipid Peroxidation, but Causes Anemia in Normal and Castrated Rats

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Abstract

Background: Castration compromises the integrity of the skeletal and antioxidant systems. Contrarily, curcumin favors bone mineralization and antioxidant/pro-oxidant balance.

Aim: To investigate the effects of curcumin on selected biomarkers in normal and castrated rats.

Study Design: Animal experimentation.

Methods: Forty rats (N=10) were divided into the following groups, *viz*: Control (Sham castrated); Castrated (Cast); Sham Cast + Curcumin (Curcm); and Cast + Curcm. Treatments with olive oil (Vehicle) (1 ml/kg BW, *p.o.*) and curcumin (100 mg/kg BW, *p.o.*) commenced 7 days after orchidectomy and lasted for six weeks.

Results: The orchidectomized rats had significant elevations in c-terminal telopeptide of type 1 collagen (CTX-1) and total alkaline phosphatase (TALP), but significant reductions in superoxide dismutase, testosterone and packed cell volume, relative to the control group. Compared to the later, curcumin caused significant increases in estrogen, osteocalcin and parathyroid hormone in the sham castrated rats. Although the dietary supplement was observed to have no significant effect on TALP, catalase and superoxide dismutase activities in both normal and castrated states, it significantly reduced malondialdehyde, tartrate resistance acid phosphatase and CTX-1 after castration. However, curcumin precipitated anemia and increased white blood cell count in the control and castrated rats.

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Conclusion: Curcumin favored bone mineralization and mitigated lipid peroxidation in physiological and castrated states; however, it caused anemia.

Keywords: castration; rats; curcumin; bone; antioxidant; blood

INTRODUCTION

Reports on the castration of human male sex dated back to several decades. The surgical procedure has been performed for various reasons, among which include medical (e.g. treatments of prostate and testicular cancers) and occupational (e.g. servants and choir/opera singers, harem and security guards) purposes, punishment for sex offenders, religious purification and self-cleansing, and lately for sexual reassignment surgery (1, 2, 3). Orchidectomy is performed as one of the medical interventions in the management of prostate cancer, even though it results in osteoporosis and increase the risk of fracture (4, 5).

In the absence of testes, the production of testosterone is reduced to a great extent, and this has profound impact on the integrity and development of skeletal tissue (6). The effects of castration on the skeletal system, which is known to be dependent on the age at castration and duration, include kyphosis of the spine, failure of early closure of epiphyses, thinning of long bones and skull, pathological fractures, among others (7, 8). Asides from defect in bone health, testosterone affects virtually every organ in the body (9). Deficit in the hormone has been associated with defects in the antioxidant system (10). Therefore, therapeutic substances that have antioxidant properties and protects the integrity of osseous tissue could be beneficial in castrated state. Particularly, curcumin is a potent antioxidant, which influences bone formation and bone resorption processes.

Curcumin {(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione or diferuloylmethane} is a biologically active natural phytochemical phenolic compound and the primary component in turmeric - a yellow curry spice that is extracted from the perennial turmeric plant (*Curcuma longa* L.). Turmeric has been used for several decades as a dietary spice and native medicine in India for the treatment of rheumatism, anorexia, sinusitis, hepatic disorders and inflammation (11). The action of curcumin on osteoblasts (12, 13) and osteoclasts (14), which are responsible for bone formation and bone resorption (i.e. bone remodeling) respectively have also been reported. Nonetheless, the dietary supplement is considered to have an iron chelator activity (15).

As there is no report in literature on the pharmacological effects of curcumin in a bilateral model of physical orchidectomy in rats, the present study sought to investigate the effect of supplement on bone mineralization, antioxidant/pro-oxidant and hematological indices in normal and castrated rats.

MATERIALS AND METHODS

Chemicals and Drugs

Curcumin (CAS No. 458-37-7; purity: ≥99.5%) and sodium pentobarbital were purchased from Sigma - Aldrich, St. Louis, MO, USA, and Nicholas Piramal Ltd., Thane, Maharashtra, India respectively, while ketamine and xylazine were obtained from Taj Pharmaceuticals, Maharashtra, India. Olive oil was obtained from International

Agro Oil Industries, Ltd., Patparganj, Delhi, India.

Experimental Animals and Care

Forty (40) adult (12-14 weeks old) male Wistar rats weighing between 220 and 240 g were used for this study. They were acquired from trusted commercial breeders. The rats were kept in plastic cages at room temperature (25 – 27^{o} C) and photo-periodicity of 12 hrs light/12 hrs dark. After two weeks of acclimatization, the rats were randomly allotted to separate groups. They were given rodent pellet and water *ad libitum* daily, and were weighed weekly.

The experimental procedures in this study were approved (no ethical number was assigned) by the Ethical Committee of the resident university of the corresponding author and were in accordance with the specifications in the "Guide for the Care and Use of Laboratory Animals" documented by the National Academy of Sciences (16).

Experimental Design

The rats were divided into four (4) groups, which included: Control (Sham castrated); Castrated (Cast); Sham Cast + Curcumin (Curcm); and Cast + Curcm. The surgical procedures for the removal of the testes were carried out under ketamine (50 mg/kg BW, *i.p.*) and xylazine (5 mg/kg BW, *i.p.*) anesthesia (17, 18, 19). Treatments with olive oil (Vehicle, 1 ml/kg BW, *p.o.*) and curcumin (100 mg/kg BW, *p.o.*) (20, 21) started seven (7) days after the orchidectomy and lasted for six weeks.

Biochemical and hematological analyses

Twelve hours after administration on the 42nd day of the experiment, the animals were anesthetized with sodium pentobarbital (40 mg/kg, *i.m.*) (22, 23, 24) and then dissected in order to collect blood by cardiac puncture. The whole blood was collected into heparinized sample bottles which were centrifuged at 3500 revolutions per minute for 15 minutes, at – 4 ^{o}C using a cold centrifuge (Centurion Scientific Ltd., Chichester, West Sussex, UK). The supernatant plasma samples were collected into separate plain bottles and the assays were done immediately.

Diagnostic kits for the determination of parathyroid hormone (PTH), estrogen, osteocalcin, c-terminal telopeptide of type 1 collagen (CTX-1), total alkaline phosphatase (TALP), testosterone and tartrate resistance acid phosphatase (TRAP) were obtained from Fortress Diagnostics Limited, United Kingdom, while analytic kits for the determination of calcium, phosphorus, calcium to phosphorus ratio, catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were obtained from Elabscience Biotechnology Company, Ltd., Wuhan, Hubei, China. The analyses were performed according to the manufacturers' instruction. Immediately after the collection of blood, the hematological analysis was determined using an auto-analyzer machine (SFRI Blood Cell Counter, H18 Light, France).

Data Analysis

Data were analyzed using Statistical Package for Social Sciences version 18.0. Statistical evaluations of the differences between the group mean values were tested by one-way analysis of variance and then Tukey multiple comparison *post - hoc* test. The results were expressed as mean ± standard error of mean (SEM), and statistical significance was considered at $p \leq 0.05$.

RESULTS

Effects of curcumin on parathyroid hormone (PTH), estrogen, osteocalcin, c-terminal telopeptide of type 1 collagen (CTX-1), total alkaline phosphatase (TALP), testosterone, tartrate resistance acid phosphatase (TRAP), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) in normal and castrated rats

Relative to the control group, there were significant elevations in parathormone (Fig. 1a), estrogen (Fig. 1b) and osteocalcin (Fig. 1c) (p – 0.00, 0.05 and 0.01 respectively) in Sham cast + Curcm, and of CTX-1 (p – 0.02) (Fig. 2a) in Cast group, and of TALP (p – 0.00, 0.00 and 0.00 respectively) (Fig. 2b) in Cast, Sham cast + Curcm and Cast + Curcm groups; however, there were significant decreases in testosterone level (p - 0.03 and 0.02 respectively) (Fig. 2c) in Cast and Cast + Curcm groups. Compared to Cast group, a significant decrease in TRAP (Fig. 3a) and CTX-1 (p – 0.01, 0.00 respectively) was recorded in Cast + Curcm. No significant difference was noted in calcium level (Fig. 3b) and catalase activity (Fig. 3c) when comparisons were made across the groups. Significant decreases in the activity of SOD (Fig. 4a) were observed in Cast, Sham cast and Cast + Curcm (p – 0.00, 0.04 and 0.00 respectively), relative to the control (Sham cast) group. In addition, there was a significant decrease in the level of MDA (p – 0.03) (Fig. 4b) in Cast + Curcm compared to Cast group.

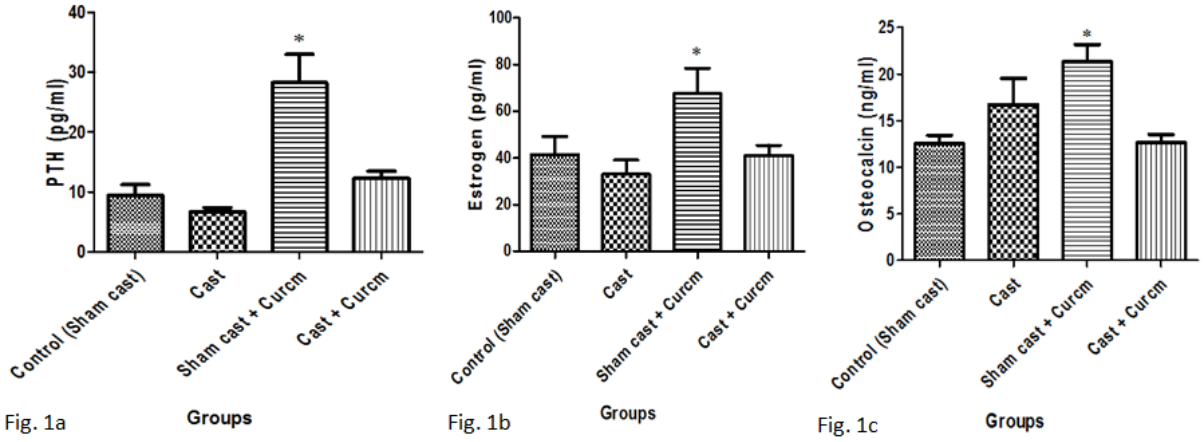

Figure 1. Effects of curcumin on parathyroid hormone (PTH) (Fig. 1a), estrogen (Fig. 1b), osteocalcin (Fig. 1c) in normal and castrated rats

Values are expressed as mean ± SEM. *p≤0.05 is significant compared to control group.

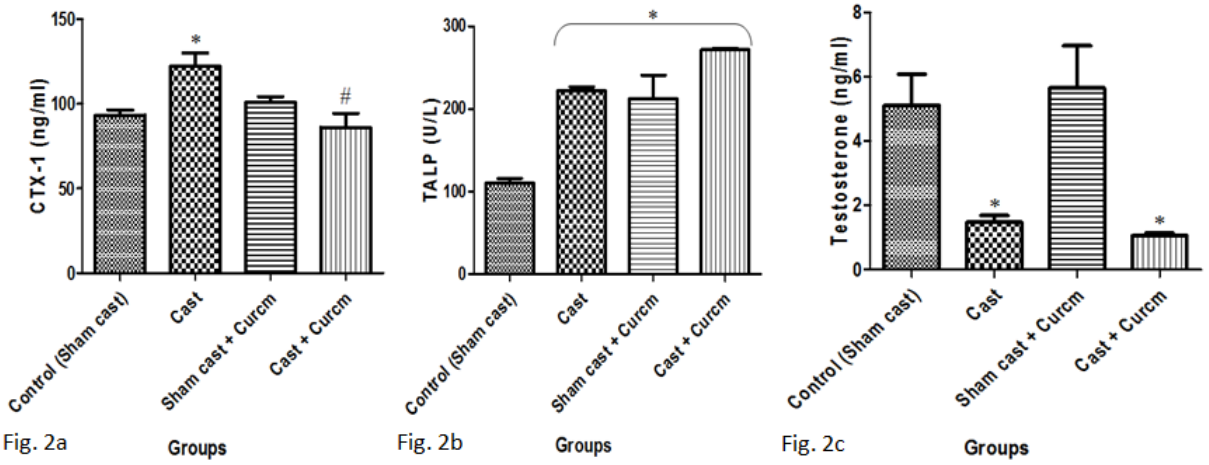

Figure 2. Effects of curcumin on c-terminal telopeptide of type 1 collagen (CTX-1) (Fig. 2a), total alkaline phosphatase (TALP) (Fig. 2b), and testosterone (Fig. 2c) in normal and castrated rats

Values are expressed as mean ± SEM. *p≤0.05 is significant compared to control group; #p≤0.05 is significant - Cast *vs* Cast + Curcm

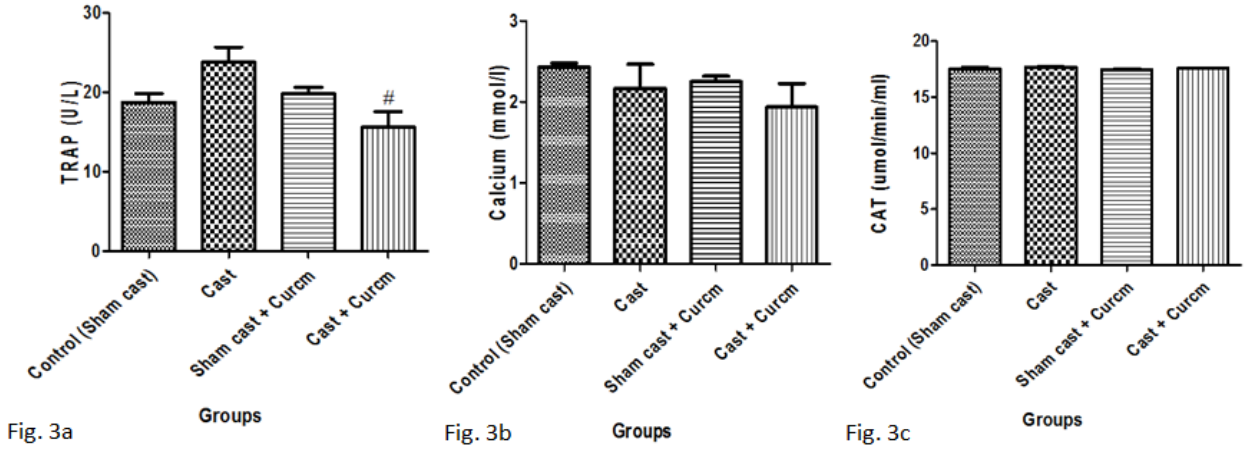

Figure 3. Effects of curcumin on tartrate resistance acid phosphatase (TRAP) (Fig. 3a), calcium (Fig. 3b) catalase (CAT) (Fig. 3c) in normal and castrated rats

Values are expressed as mean ± SEM. #p≤0.05 is significant - Cast *vs* Cast + Curcm.

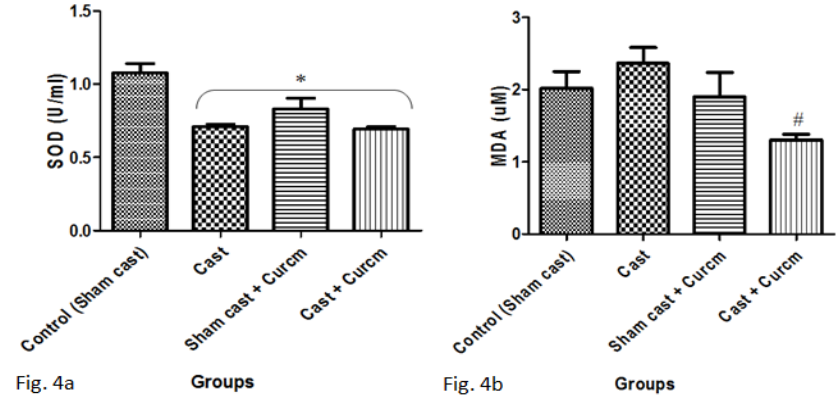

Figure 4. Effects of curcumin on superoxide dismutase (SOD) (Fig. 4a) and malondialdehyde (MDA) (Fig. 4b) in normal and castrated rats

Values are expressed as mean ± SEM. *p≤0.05 is significant compared to control group; #p≤0.05 is significant - Cast *vs* Cast + Curcm.

Effects of curcumin on hematological indices in normal and castrated rats

In comparison to the control (Sham cast) and cast groups, there were significant decreases in hemoglobin concentration (HB), packed cell volume (PCV), and red blood cell count (RBC), but a significant increase in white blood cell count (WBC) in Cast + curcm group (Table 1). Moreover, relative to control (Sham cast) group, significant decreases in PCV and RBC in Sham cast + Curcm and in PCV in Cast group were observed. However, there was a significant elevation in WBC in Sham cast + Curcum compared to the control group. No significance was recorded in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, neutrophils, lymphocytes and reticulocytes, when comparison were made across the groups (Table 1).

Table 1. Effects of curcumin on hematological indices in normal and castrated rats

Groups/ parameters	HB (g/dl)	PCV (%)	RBC (x10)	WBC ($x10^9$)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLAT ($x10^9$)	NEUT (%)	LYMP (%)	RETIC (%)
Control (Sham cast)	14.46± 0.51	46.60± 0.93	8.33± 0.10	8.26± 0.62	55.40± 0.60	17.80± 0.20	32.40± 0.24	649.00± 10.20	16.20± 2.62	82.60± 2.98	3.30± 0.27
Cast	13.92± 0.31	42.60± 1.08*	8.10± 0.01	8.38± 0.53	55.00± 0.32	18.20± 0.20	32.80± 0.37	714.20± 54.97	14.80± 2.20	85.60± 2.04	3.20± 0.19
Sham cast + Curcm	13.00± 0.16	40.60± 0.51*	7.54± 0.14*	11.12± 0.47*	53.60± 0.40	17.40± 0.40	32.20± 0.49	710.00± 54.94	19.40± 1.36	79.40± 1.75	3.02± 0.10
Cast + Curcm	12.38± 0.57*#	35.80± 0.86*#	6.71± 0.10*#	14.96± 1.04*#	53.60± 0.68	17.40± 0.24	32.20± 0.20	738.00± 49.88	14.20± 1.77	84.80± 1.80	3.42± 0.25

Values are expressed as mean ± SEM. *$p \leq 0.05$ is significant compared to control group; #$p \leq 0.05$ is significant - Cast vs Cast + Curcm.

NB: HB – hemoglobin concentration; PCV – packed cell volume; RBC – red blood cell count; WBC - white blood cell count; MCV – mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLAT – platelet count; NEUT – neutrophil count; LYMP – lymphocyte count; RETIC - reticulocyte count

DISCUSSION

The process of bone remodeling helps to preserve bone quality by maintaining equilibrium between osteoclast-dependent bone resorption and osteoblast-mediated bone formation (25). In the present study, castration was accompanied by significant increases in bone formation (TALP) and bone degradation (CTX-1) i.e. an increase in bone turnover rate - a condition that has been associated with menopause and osteoporosis (26). Few studies with contrasting reports have demonstrated the effect of curcumin on

osteoblast. Notoya *et al*. (13) reported that curcumin inhibited the proliferation of osteoblasts derived from calvaria of rats, but has no apoptotic action. Contrarily, Chan *et al*. (12) demonstrated that curcumin instigated osteoblasts apoptosis in a dose-dependent manner. However, in this study, it was observed that curcumin had no appreciable effect on osteoblast - mediated bone formation process in a castrated state, but significantly increase bone formation process in a physiological condition. Therefore, further studies should be done to clarify the effect of curcumin on osteoblastic bone formation process. We observed that curcumin significantly inhibited bone resorptive action of osteoclast after castration, possibly by causing apoptosis of the cells as reported by Ozaki *et al*. (27).

In homeostatic state, curcumin was noted to cause significant increase in PTH, asides from increasing bone formation process. PTH is released in response to reduced calcium level. The hormone suppresses urinary calcium loss, promotes bone resorption and enhances the formation of calcitriol (28). As there was no significant difference in calcium level across the group, the effect of curcumin on PTH is not a calcium-dependent process; however, this might be attributed to the direct stimulation of the parathyroid gland. Moreover, the administration of curcumin also caused significant increases in estrogen in the uncastrated rats. Traditionally, androgens and estrogens were considered as the main sex steroids influencing bone maturation and maintenance in men and women respectively. However, this concept has been questioned in the 1990's (29). Although curcumin showed no significant effect on the plasma level of testosterone in normal and castrated rats, the significant elevation in estrogen level in a physiological state affirmed that curcumin has a favorable action on bone mineral density and hence bone strength. Studies have shown that estradiol is strongly associated with indices of bone mineralization than testosterone in the male sex. Observational studies revealed that serum estradiol correlated better with bone mineral density at various sites than testosterone (30, 31). Moreover, prospective studies indicated that serum estradiol was the best predictor of both a decrease in bone density in elderly men (32, 33) and an increase in bone mass in young men (32).

It is known that curcumin protects against oxidative damage (34), which is an endogenous event that has been associated with inflammation (35, 36, 37). Moreover, the dietary supplement scavenges free radicals, and so maintains the delicate balance between endogenous antioxidant/pro-oxidant status and prevents lipid peroxidation (38). Castration causes oxidative stress (39). Afolabi and colleagues (10) observed that the removal of testes results in selective impairment of oxidative stress markers. They noted in their study that there was no significant difference in CAT activity following castration; however, there were significant alterations in MDA and SOD. These results were partly noted in our study. There was no significant difference

in CAT activity after castration, and as such, the effect of curcumin on the enzyme in this condition could not be ascertained. Contrary to Panahi *et al*. (38), curcumin showed an insignificant effect on SOD. The activity of the enzyme was significantly decreased after castration. We speculated that the effect of curcumin on the components of the antioxidant system seemed to be selective. The insignificant increase in the marker of lipid peroxidation – MDA after castration could be partly ascribed to possible intervention by endogenous adaptive mechanism or the antioxidant action of olive oil (40) which was used as a vehicle for curcumin.

Despite the favorable actions of curcumin on the antioxidant system and indices of bone mineralization in normal and castrated states, the supplement has been documented to have a dark side - iron chelating property (15). Jiao *et al*. (41) observed that cells treated with curcumin exhibited a decrease in ferritin. Therefore, the researchers submitted that the chelator activity of curcumin might be sufficient to instigate systemic iron depletion, there by initiating or escalating subclinical or clinical iron deficiency (41, 42). In agreement with Jiao and colleagues, we observed that curcumin has anemic effect. This was supported by significant decreases in PCV, HB and RBC count post-administration of the supplement. However, curcumin caused a significant increase in total WBC (43), even though it had no significant effect on the differential count. The observed effects of curcumin on PCV, HB, RBC and total WBC were noted to be more severe after castration, relative to what was observed in a physiological state. This is no doubt associated with the fact that the bone tissue is one of the major sites for the production blood cells, and is known to be compromised after castration (14). The insignificant difference in MCV, MCH and MCHC across the groups, despite the significant reductions in PCV, HB and RBC count indicated that the administration of curcumin precipitated normocytic normochromic anemia, which is known to be associated with bone marrow disorder – a condition that is implicated in castration (14).

CONCLUSION

Curcumin favored bone mineralization and mitigated lipid peroxidation in physiological and castrated state; however, it caused anemia.

Acknowledgements: LAO and WJA (concept, design, and supervision); WJA, **the co-first author** (writing, critical reviewing; literature search and statistical analysis); ODA and AJA (funding, data collection, literature search).

Conflict of Interest Disclosure: None declared.

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