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Research Article

The effect of *Ormocarpum sennoides* DC on bone histomorphometry in steroid induced osteoporotic rats

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ABSTRACT

Ormocarpum sennoides DC is a leguminous shrub, which extremely efficacious in mending bone fracture and strengthening tendon and bone. Present study was conducted to investigate the anti-osteoporotic potential of ethanol extract of *Ormocarpum sennoides* DC on steroid induced bone loss in Wister albino rats; Materials and method: The 42 female wister albino rats weighing 150-200 g were randomly assigned into control group and six steroid sub groups, which received 10mg/kg body weight, subcutaneous dose of methylprednisolone acetate (MPA) on alternate days for 6 weeks and followed by daily oral administration of ethanol extract of *Ormocarpum sennoides* DC(EOS) for a period of 45 days, the group are as follows Control, MPA, MPA with 100mg EOS, MPA with 200mg EOS, MPA with 2mg Alendronate, Pre-100mg, Pre-200mg groups. Various parameters such as Femur weight, Length, Histopathological, Biochemical, CT scan analysis were studied; Results: The EOS improved bone quality, attenuates bone resorption, restored bone density, trabecular thickness, serum ALP, and calcium levels decreased due to reduced osteoblastic activity and decreased intestinal absorption of calcium. In EOS treated groups showed elevated levels due to increased bone forming activity. Serum phosphorus levels increased in MPA group and decreased in EOS treated group. CT scan analysis showed increases bone mineral density in EOS treated group with the corresponding increase in femur weight and length. It is evident from our study that EOS reverse the catabolic glucocorticoid effect and thus proving its anti-osteoporotic potential.

Keywords: Alendronate; bone mineral density, traditional Indian medicine; methylprednisolone; osteoporosis

INTRODUCTION

Osteoporosis is a systematic skeletal disease characterized by a decrease in the amount of bones with microarchitectural deterioration to the point of spontaneous fracture or fracture following minimal trauma. This loss and deterioration of the structure of bone tissue are caused by long-term glucocorticoid therapy. Changes are more pronounced at the appendicular skeleton compared to the spine; the effects of glucocorticoids depend on the dose and duration of treatment (Ima Nirwana et al, 2010). These effects are partially reversible upon discontinuation of therapy (Silvia Pierotti et all,2008). Bone loss in glucocorticoid-induced osteoporosis is most pronounced in trabecular bone and cortical shell of vertebral bodies (Canalis et all, 2007) trabecular thickness was markedly reduced by the spatial distribution of the trabecular remained unchanged (Aaron, J.E et al, 1989; Chappard. D et al, 1996; Naohisa Miyakoshi et al, 1997).

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The pathogenesis of GIO involves multiple factors, of which decrease in the number and functions of osteoblast is main contributing factor (Ernesto Canalis, M.D, 2005; Robert S. Weinstein, 2010) Glucocorticoids inhibit osteoblastogenesis and reduce the life span of osteoblasts and osteocytes. They are also potent suppressor of osteoblast function and these cause reductions in bone formation (Weinstein, R.S et al, 1998).

MATERIALS AND METHODS

1. Plant Materials

The leaves of Ormocarpum sennoides DC collected from Irula Women Welfare Trust (IWWT), Kanchipuram dist., Tamil Nadu, India authenticated by the Botanical Survey of India/Ministry of Environment, and Forest (LtrNo. BSI/SRC/5/2013-14ech/550), shade dried leaves were ground to get a coarse powder that was stored in an airtight, high-density polyethylene container.

2. Preparation of Plant Extract

The coarse powder weighed accurately 600gms was soaked in n-hexane for defatting for 48 hours and then successively extracted in 80% ethanol at room temperature; the solvent was then removed by filtration and fresh solvent was added to the plant materials, the extraction process was twice repeated the combined filtrate were then evaporated under reduced pressure to give a dark-green viscous mass the extract was

stored at 0-4°C, 20% yield was acquired (Chappard et al, 1996).

3. Acute Toxicity Study

Acute toxicity of *Ormocarpum sennoides* DC was performed to ascertain safe dose by acute oral toxic class method as per clause 423 guidelines of Organization of Economic Co-operation and Development (Eva Schledeet al, 2005).

EXPERIMENTAL PROTOCOL

In the present study female Wister albino rats were used to create the rodent model of osteoporosis. Animals weighing 150-200gms were obtained from lab animal maintenance unit, King institute, Guindy, Chennai, Tamil Nadu, India. The animals were housed in sanitized polypropylene cages at room temperature the rats were exposed to a 12hrs light/dark cycle and were fed with commercially available pelleted food (manufactured by Provimi Animal Nutrition India pvt, ltd.,) and adlibitum water. All the studies conducted were approved by the Institutional animal ethics committee (ICL.No.409/19.10.10), SRM Medical College and Research Institute, Potheri, Kanchipuram, Tamil Nadu, India, followed by the guidelines of the control and supervision of experiments of animals (CPCSEA) committee, Government of India. The following groups were assigned (Table 1).

CT SCAN ANALYSIS OF BMD OF THE FEMUR

The CT scan analysis of bone in various groups (Control, Methylprednisolone induced; *EOS* and Alendronate treated) is given in the Figure1.The density of the bone was determined using software accompanied. The density of the femur was measured and expressed in HXU-Hounx Unit (Table2).

BLOOD COLLECTION

After 45days of the treatment period, all the rats were euthanized, and blood collected from carotid bleeding. The samples were collected in clean polypropylene tubes, left to clot at 37°c for 10 minutes, then centrifuged at 3000rpm for 20 minutes at 4°c, the resulting supernatant which is serum was transferred to sterile vial and frozen at -20°C until used for analysis of calcium, phosphorus and alkaline phosphatase (Shomali.T et al, 2010; Sanganna.C et al, 2010).

MEASUREMENT OF FEMUR LENGTH AND WEIGHT

The Femur length was measured as the distance between the greater trochanter and the medial condyle in right femurs using vernier calipers. The same femurs were then dried and weighed using a digital weighing device (Potu et al, 2009).

HISTOMORPHOMETRY ANALYSIS

Bone tissue: All the animals were euthanized by deepening the chloroform anesthesia and the femur, tibia were dissected for histomorphometry study. After the rats were sacrificed the femur, tibia was dissected free of soft tissue and fixed in 10% formalin, the bone tissues were then decalcified in formic acid for 10 days, tissues were dehydrated in graded alcohols and embedded in paraffin. 5µmm sections were cut and stained with hematoxylin and eosin for further histomorphometry analysis.

RESULTS

1. Effect of ethanol extract of Ormocarpum sennoides DC extract ((EOS) on bone mineral density (BMD)

CT scan analysis of femur showed a market decrease in bone mineral density in proximal end, mid shaft and

Table 1: Animal Grouping and Description

No	Group	Description
1	Control	Without any drug administration
2	MPA	Subcutaneous administration of Methyl Prednisolone Acetate 10mg/kg bw alternate days for six weeks
3	MPA + Alendronate	Methylprednisolone Acetate induced and treated with 2mg/kg bw oral administration of alendronate.
4	MPA + 100mg *EOS*	Methylprednisolone acetate induced and treated with 100mg/kg bw of ethanol extract of *Ormocarpum Sennoides* DC
5	MPA + 200mg *EOS*	Methylprednisolone acetate induced and treated with 200mg/kg bw of ethanol extract of *Ormocarpum Sennoides* DC
6	100mg *EOS* pre-treatment (132 days)	Stage1: 100mg/kg bw of *EOS* for 45days (before inducing osteoporosis) Stage2: 100mg/kg bw of *EOS*+MPA inducing for 42 days (Inducing period) Stage3: 100mg/kg bw of EOS for 45 days (Treatment period)
7	200mg *EOS* pre-treatment (132 days)	Stage1: 200mg/kg bw of *EOS* for 45days(before inducing osteoporosis) Stage2: 200mg/kg bw of *EOS*+MPA inducing for 42 days (Inducing period) Stage3: 200mg/kg bw of *EOS* for 45 days (Treatment period) period

*EOS – Ethanolic extract of *Ormocarpum sennoides* DC; MPA- Methyl Prednisolone Acetate; bw – Body weight

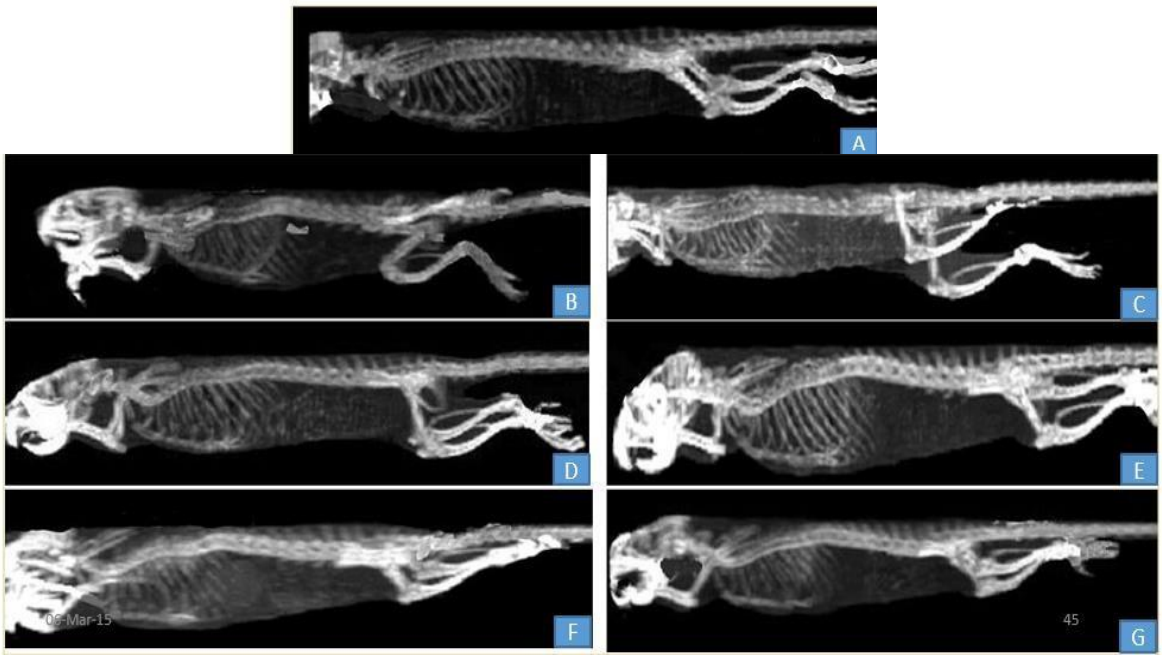

Figure 1: CT scan images of Shows CT scan sagittal images of various groups

A. Control; **B**. MPA; **C**. MPA + Alendronate; **D**. MPA + 100 mg EOS; **E**. MPA + 200 mg EOS **F**. MPA + 100mg pre - treatment EOS; **G**. MPA + 200mg pre - treatment EOS

distal end of femur in MPA treated group, whereas the *EOS* and alendronate treated groups showed increased ($p<0.001$) bone mineral density. This indicates the bone healing property of *EOS*.

2. Effect of ethanol extracts of Ormocarpum Sennoides on biochemical parameters

The results of serum parameters in animals of different groups are shown in Table3 the results indicate elevated levels of serum calcium and phosphorus in methyl prednisolone induced group compared to control and treated groups. Serum alkaline phosphate levels decreased in methyl prednisolone induced group in contrast to control and treated group, which showed elevated levels of alkaline phosphates.

3. Effect of ethanol extracts of Ormocarpum sennoides DC on femur weight and length

MPA treated group showed significant reduction in femur weight and length compared with control group ($p<0.001$). The treatment with *EOS* and alendronate significantly increased the femur weight and length in correlation with the control group ($p<0.001$) as shown in Table 4.

4. Effect of ethanol extracts Ormocarpum Sennoides on Histopathological evaluation

The femur and Tibial sections were examined for Histopathological changes. The control group showed normal architecture and bone compactness, MPA induced bone showed trabecular thinning, losses of trabecular connectivity, increased inter trabecular space.

Administration of ethanolic extracts of Ormocarpum Sennoides, and alendronate exhibit restorative progress with increase bone volume mineralization and decreased trabecular separation, which indicate the recovery with essential features of normal bone. Drastic decrease in the total number of osteoclast, and increase in the osteocyte number were observed in the Tibial metaphysics of *EOS* treated groups. In the present study, the number of adipocytes in bone marrow showed a marked increase with reduced vasculature in the methylprednisolone treated group than that in the control group and other treated groups (Figure2).

DISCUSSION

Excessive glucocorticoid administration induces a decrease in bone formation and an increase in bone resorption, which result in a decrease in BMD in various bones in human (Yujiang Wang et al, 2002) even in animal experiments it has been demonstrated that the administration of inordinate G.C induces a decrease in the total-body bone mineral content and results in a decrease in BMD (Chiodini.I et al, 1998). Our present study shows that *EOS* treatment dose dependently increased BMD compared to the alendronate treated group.

The decrease in serum alkaline phosphate activity detected in methylprednisolone induced group compared to the control was consistent with reduction in osteoblastic activity which correlated to histological observation in the present study (Yujiang Wang et al, 2002; Hayder.F.Saloom et al, 2012). An ethanol extracts of ormocarpum sennoides DC increased alkaline phosphatase activity indicates that the ormocarpum sennoides compounds exert a selective estrogen receptor modulator activity in the bone. As osteoclast causes bone resorption, serum, calcium and phosphorus levels are elevated in the MPA induced group compared to *EOS* and alendronate treated group.

Table 2: Shows the Bone Mineral Density of femur of the normal, induced and various treatment groups

BMD of Femur	Control	MPA	MPA+ Alendronate	MPA+100mg EOS	MPA+200mg EOS	Pre-100mg EOS	Pre-200mg EOS
Proximal End	695.17±71.8^{b}	515.33±6.2^{a}	752.17±69.2^{b}	695.83±72.7^{b}	695.33±20.5^{b}	693.83±53.8^{b}	681.1±43.8^{b}
Midshaft	569.55±43.1^{b}	465.83±22.4^{a}	568.17±28.2^{b}	592.67±79.2^{b}	605.17±25.8^{b}	562.33±70.8^{b}	560.50±7.2^{b}
Distil End	621.00±72.2^{a}	558.83±4.6^{ab}	663.67±39.0^{b}	614.00±44.8^{ab}	663.83±7.3^{ab}	616.00±19.1^{b}	748.83±30.5^{c}

*Values are expressed as Mean ± SEM. Different alphabet between groups denotes significant at (p<0.05) 5% level using Tukey HSD test, P values are <0.001.

Table 3: Biochemical parameters in serum

	Control	MPA	MPA+ Alendronate	MPA+100mg	MPA+200mg	Pre-100mg	Pre-200mg
Ca (mg/dl)	8.73±0.26^{a}	8.47±0.69^{a}	8.71±0.42^{a}	8.71±0.81^{a}	8.83±1.41^{a}	8.94±0.30^{a}	8.93±1.18^{a}
P (mg/dl)	4.28±0.28^{b}	4.48±0.48^{b}	3.22±0.53^{a}	2.95±0.23^{a}	2.88±0.35^{a}	3.4±0.38^{a}	3.15±0.53^{a}
ALP (IU/L)	73.42±2.73^{abc}	67.20±3.51^{a}	72.68±4.64^{abc}	78.33±7.49^{c}	89.93±7.28^{d}	70.80±7.22^{ab}	82.10±5.99^{cd}

*Values are expressed as Mean ± SEM. Different alphabet between groups denotes significant at (p<0.05) 5% level using Tukey HSD test, P values are <0.001.

Table 4: Shows the Femur length and weight of normal, induced and various treatment groups

Group	Femur Length (mm)	Femur Weight (g)
	Mean±SEM	Mean±SEM
CONTROL	30.78 ± 2.68^{b}	0.69 ± 0.06^{b}
MPA	22.99 ± 3.43^{a}	0.54 ± 0.09^{a}
MPA+Aendronate	29.23 ± 3.01^{ab}	0.60 ± 0.07^{ab}
MPA+100mg *EOS*	27.01 ± 3.08^{ab}	0.64 ± 0.11^{ab}
MPA+200mg *EOS*	30.30 ± 5.77^{b}	0.64 ± 0.06^{ab}
Pre-100mg *EOS*	25.85 ± 2.65^{ab}	0.62 ± 0.05^{ab}
Pre-200mg EOS	28.74 ± 3.79^{ab}	0.60 ± 0.06^{ab}

*Values are expressed as Mean ± SEM. Different alphabet between groups denotes significant at (p<0.05) 5% level using Tukey HSD test, P values are <0.001.

GCs have also been reported to promote the apoptosis of osteoblasts and osteocytes (Weinstein. R.S, 1998) The GCs at higher concentration's doses drastically reduce the proliferation of osteoblast precursors (Scutt.A et al, 1996) and inhibit the differentiation to mature osteoblasts (Scott, D. Boden et al, 1997). Stromal mesenchymal stem cells are known to be pluripotent and to have the capacity to differentiate into osteoblasts and adipocytes (Mark.E. Nuttal et al, 1998). These two lineages are thought to be reciprocally related (Nuttall. M.E et al, 2000; Sabatakos.G.Sims, 2000).

The present study increased the number of adipocytes in the bone marrow in MPA treated rats (Figure 3) were related to the decrease in the number of osteoblast on the bone surface. Bone marrow and the bone present a fight functional interdependence, the modification occurring at the bone marrow level, with the decrease of the cellularity paralleled by an increase of the adiposity at the medullar level are to be expected.

The increase of the adiposity is produced because of the diminution of the differentiation of the osteoblast within the stromal cell precursor in favor of adipocytary differentiation studies (Marcu.F.L et al, 2011; Compston.J.E, 2002). That also shows the increase of the adipose cells at the bone marrow level in osteoporosis.

Another cause that can be ascribed to the increase of the medullary Adiposity is linked to vascularization of the bone. In order to optimally perform its functions, the bone marrow, as well as the bone is dependent on an adequate blood supply. In osteoporosis, a reduction of the blood supply in the bone may take place, a decrease of the blood supply at medullary level being followed by the increase of the Adipose content of the marrow this can be explained by the fact that the marrow shown a fight functional dependency, the marrow cells being the precursor of the cells that will take part in the reshaping process then alteration having consequences on the reshaping process (James.F.Griffith et al, 2008). The disruption of marrow microvasculature would reduce the source of circulating progenitor cells supporting osteogenesis. The reduction in local and circulating progenitors would then lead to reduced

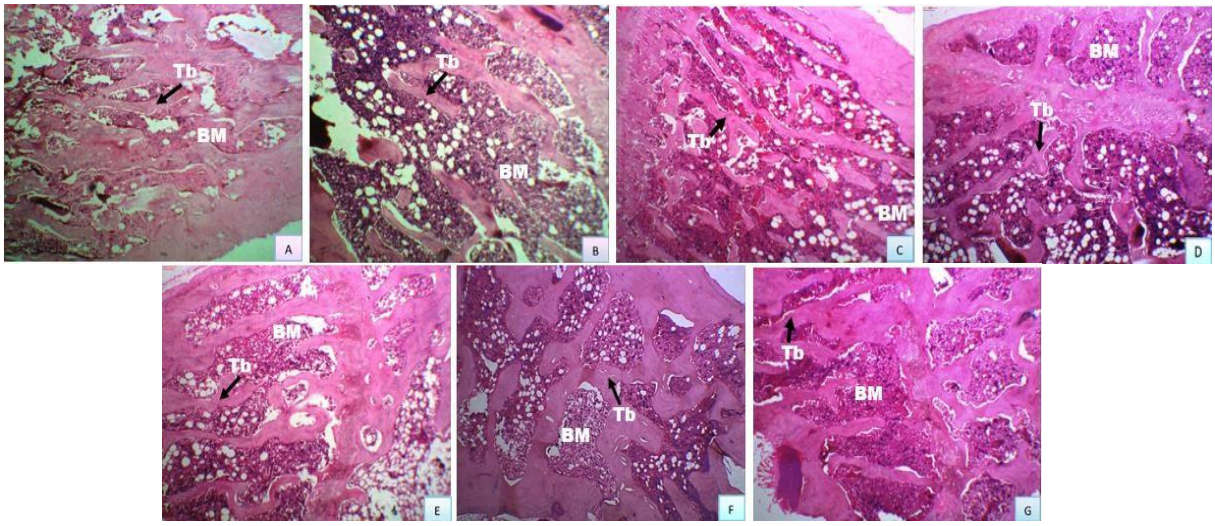

Figure 2: Trabecular pattern of epiphyseal end of femur in various treatment groups (H & E, 100x)

A. Epiphyseal region showing normal compact bone with inter trabecular space in control group.

B. Epiphyseal region showing sparse, thinning of trabeculae, loss of connectivity and widening of inter trabecular space in methylprednisolone treated.

C. Epiphyseal region showing normal trabecular thickening in Alendronate treated group.

D, E, F, G. Epiphyseal region showing moderately thick elongated trabeculae and narrow inter trabecular space in MPA+100mg, MPA+200mg, Pre-100mg, Pre-200mg of Ormocarpum sennoides treated groups.

Arrows: Trabecule (Tb), Bonemarrow (BM)

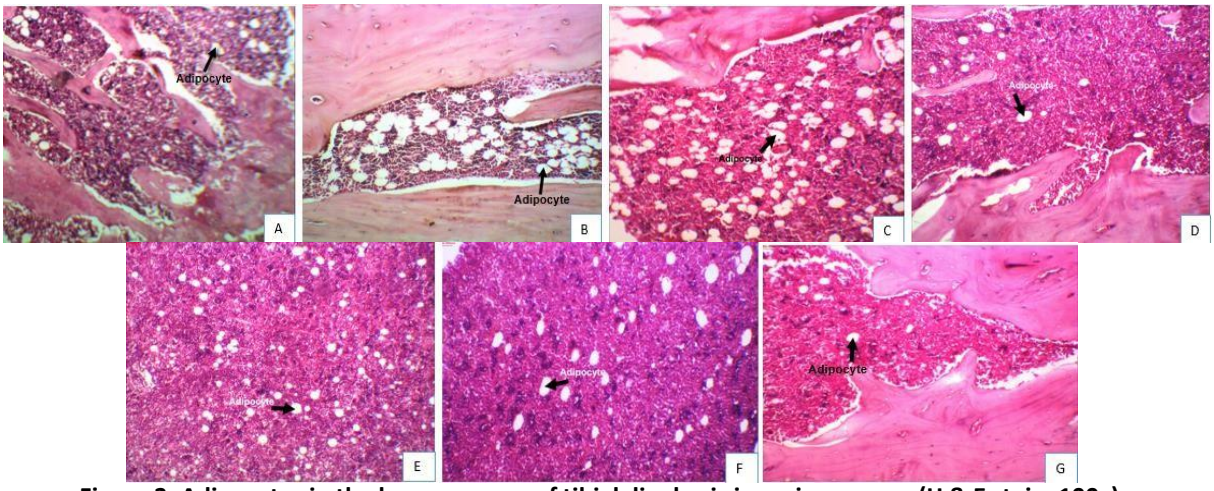

Figure 3: Adipocytes in the bone marrow of tibial diaphysis in various group (H & E stain, 100x)

A. Control group showing normal adipocytes and vasculature in bone marrow of tibial diphyses.

B. Methylprednisolone treated group showing increased adipocytes and reduced vasculature in bone marrow of tibial diphyses.

C. Alendronate treated shows reduced adipocytes and increased vasculature.

D, E, F, G. Showing decreased number of adipocytes and increased vasculature in MPA+100mg, MPA+200mg, Pre-100mg, Pre-200mg of Ormocarpum sennoides treated groups. **Arrows**: Adipocytes.

osteogenesis. Our finding suggests bone formation, bone marrow fat metabolism and microcirculation cue later two factors also contribute significantly to the development of GCinduced bone loss and the decline in bone strength (Robert.S.Weinstein, 2010; Amin Kerachian et al, 2009).

CONCLUSION

In correlation with biochemical analysis, Histopathological findings add a confirmatory note to the findings, that *Ormocarpum sennoides* prevents effects of methylprednisolone induced bone loss by the effectively lowering the number of mature osteoclasts by inducing osteoclast apoptosis and suppressing osteoclastogenesis thereby reducing bone resorption.Increased activity of osteoblasts, enhance bone mineralization, trabecular thickening and bone marrow vasculature with reduced adipocytes.

Our results clearly demonstrate that *Ormocarpum sennoides* could be considered as the natural alternative therapy to reverse the catabolic glucocorticoid effect on the bone by improving osteoblast function and the balance between osteoblast, osteocyte and osteoclast survival.

CONFLICT OF INTEREST

We have no conflict of interest.

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