Role of *Triticum aestivum* aqueous extract in glucocorticoid induced osteoporosis in rats

David Banji*, Otilia J F Banji, Vijaya Laxmi Chiluka & Saidulu Abbagoni
Department of Pharmacology, Nalanda College of Pharmacy, Charlapally, Nalgonda 508 001, India

Received 3 August 2012; revised 8 November 2013

Administration of aqueous extract of *T. aestivum* (200 and 400 mg/kg/day, po, for 30 days) and risedronate (20 µg/kg, sc, five times a week for 30 days) following methyl prednisolone sodium succinate (10 mg/kg, sc, thrice a week for 4 weeks) induced osteoporosis in Wistar rats showed an increase in the serum levels of bone mineral content markers, decrease in the serum and urinary levels of bone resorption markers. An incline in strength of femur and tibia was seen particularly with 400 mg/kg of *T. aestivum*. Maintenance of calcium homeostasis, formation of collagen and scavenging of free radicals can plausibly be the mode of action of aqueous extract of *T. aestivum* thereby combating osteoporosis induced by glucocorticoids.

**Keywords**: Bone resorption, Free radicals, Glucocorticoids, Neutraceutical, Wheat grass

Glucocorticoids are steroidal hormones possessing wide applicability and are extensively used for the treatment of autoimmune disorders, pulmonary disorders, malignancy, and organ transplantation. Glucocorticoids exert their action by binding to glucocorticoid receptors and regulate tissue differentiation, control intermediary metabolism and enable the body to cope with stress. Long-term treatment or high doses adversely affects bone health and is considered to be a leading cause of drug induced osteoporosis. Osteoporosis is a chronic bone disorder characterized by low bone mass and deterioration of the micro architecture of bone tissue.

Maintenance of bone health requires a healthy diet. Steroids deplete minerals causing an alteration in the mechanical and structural integrity of skeletal tissue. Neutraceuticals are widely employed to curb the spread of diseases owing to the presence of several antioxidant principles. *Triticum aestivum* (family: Poaceae) seedlings commonly called as wheat grass is consumed in the form of juice for healthy growth of human body. Wheat grass is used to reduce ulcerative colitis, and myelotoxicity. It is reported to be beneficial in mitigating thalassemia and possesses tremendous anti-oxidant potential.

*T. aestivum* seedlings are a rich source of minerals like magnesium, potassium, calcium, phosphorous, manganese, iron, zinc and vitamins like A, C, D, and E.

*T. aestivum* is rich in nutritional constituents and has not been explored for its potential in maintaining skeletal health. Therefore, the present study has been undertaken to investigate the effect of aqueous extract of *T. aestivum* on osteoporosis induced by methyl prednisolone sodium succinate, a glucocorticoid (GC) in rats by biochemical and biomechanical assessment.

**Materials and Methods**

**Drugs and chemicals**—Methyl prednisolone sodium succinate and risedronate sodium were procured from Sigma Aldrich, USA. The reagent kits for the measurement of calcium (Ca), inorganic phosphate (P), alkaline phosphatase (ALP) and creatinine (Cr) were procured from ERBA Diagnostics (Transasia Bio-medicals Ltd., Daman, India). All other reagents and chemicals were of analytical grade.

**Plant material**—Fifteen day old seedlings of *T. aestivum* were collected in the month of December in Nalgonda. They were authenticated by Prof. A Laxman Reddy, N.G. College, Nalgonda and a voucher specimen is maintained in the herbarium. Ariel parts of 15 day old seedlings were dried in shade and made into fine powder. Dried powder...
Chlorophyll b (mg/L) = 27.05 Abs
Chlorophyll a (mg/L) = 15.65 Abs

Proposed by Lichtenthaler and Wellburn, the concentration of chlorophyll was determined from the formula proposed by Lichtenthaler and Wellburn.

The determinations were carried out in triplicate and the presence of chlorophyll was done by recording the absorbance at 653 and 666 nm using a UV/VIS spectrophotometer. The determination of chlorophyll was done by recording the absorbance at 470 nm using a UV/VIS spectrophotometer. The total carotenoid content was measured after 4 min. Ascorbic acid as a standard (100-1000 µM) was processed in the same way.

Safety evaluation and dose selection—Safe dose of aqueous extract of *T. aestivum* was evaluated according to OECD guidelines number 407.

**Animals**—Young male rats (30) of Wistar strain weighing 150-250 g were procured from National Institute of Nutrition, Hyderabad. The experimental animals were housed in a temperature controlled room (24±1 °C) with 12:12 h L:D illumination cycle. All animals were allowed free access to distilled water and fed on a commercial diet. The experimental protocol has been approved by the Institutional Animal Ethical Committee (NCOP/IAEC/approved/18/2010).

**Induction of osteoporosis and experimental design**—Animals were divided into following five groups of 6 rats each. Gr. I were negative control animals treated with saline. Osteoporosis was induced in male rats by injecting methyl prednisolone sodium succinate, a glucocorticoid (GC) in a dose of 10 mg/kg thrice a week for four weeks by subcutaneous route. Gr. 2 animals received GC alone; Gr. 3 were GC treated animals which received risedronate sodium (20 µg/kg, sc) as a standard, five times a week for the next 30 days; Gr. 4 were GC treated animals which received the aqueous extract of *T. aestivum* (200 mg/kg) daily by the oral route for the next 30 days; Gr. 5 were GC treated animals which received aqueous extract of *T. aestivum* (400 mg/kg) daily by oral route for the next 30 days.

**Biochemical analysis**—After 60 days, rats were anaesthetized with enflurane and blood was withdrawn from the retro orbital plexus and collected into dry test tubes. It was centrifuged at 3000 rpm for 10 min for the separation of serum. The separated serum was used for biochemical analysis.

**Bone mineral content markers**—Calcium was estimated by OCPC method in which it complexes with o-cresolphthalein complexone and phosphorous was estimated by molybdate UV method.

**Bone resorption markers**—Creatinine was estimated by Jaffe’s kinetic method. Serum specific alkaline phosphatase was estimated by PNPP kinetic method in the serum using standard kits with a semi auto analyzer (Erba Chem 5 Plus V2).
Hydroxyproline was estimated in urine using modified Switzer and Summer\textsuperscript{19}. The levels of serum chemistries of bone mineral content and bone resorption markers were measured using a semiautomatic analyzer (ERBA Chem-5 Plus V2, Transasia Bio-Medicals Ltd, Mumbai).

**Physical and biomechanical parameters**—Animals were sacrificed on 60\textsuperscript{th} day after blood withdrawal. Femur and tibia were isolated, defleshed and immediately weighed. The length of bones was determined using dial calipers. Assessment of their biomechanical strength was done by using Universal testing machine (UTE 40 HGFL; Fuel Instruments and Engineers Ltd).

**Three point bending test of femur and tibia**—The bones were placed horizontally on two supporting bars separated at a distance of 15 mm and was loaded by a rounded press. Bones were subjected to test with loading at a speed of 1 mm/min each mm corresponding to 40 Newton (N) perpendicular to the bone longitudinal axis. Variables estimated were yield load which is the force causing the first detectable bone damage and ultimate load which is the force at which fracture occurs\textsuperscript{20}.

**Compression test of femur and tibia**—The bones were placed on the flat metallic stage and compressed until it fractured. The reading was recorded in Newtons\textsuperscript{21}.

**Statistical analysis**—*In vitro* data are expressed as mean±SD. *In vivo* data are expressed as mean±SE. Analysis of data was done by One-way ANOVA followed by Dunnett multiple comparison tests using Graph Pad InStat version 3.10 for Windows 2009 (Graph Pad Software). The statistical significance was set as 0.05 (P<0.05).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Gr 1 Control</th>
<th>Gr 2 GC</th>
<th>Gr 3 Res</th>
<th>Gr 4 AETA (200 mg/kg)</th>
<th>Gr 5 AETA (400 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.6±0.9</td>
<td>8.3±1.0\textsuperscript{*}</td>
<td>10.4±0.07\textsuperscript{*}</td>
<td>9.2±0.79\textsuperscript{*}</td>
<td>9.7±1.05\textsuperscript{*}</td>
</tr>
<tr>
<td>Phosphorous (mg/dL)</td>
<td>5.0±0.65</td>
<td>4.03±0.78\textsuperscript{*}</td>
<td>4.8±0.87\textsuperscript{*}</td>
<td>4.45±0.6\textsuperscript{*}</td>
<td>4.7±0.34\textsuperscript{*}</td>
</tr>
<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>129±2.0</td>
<td>262.5±2.4\textsuperscript{*}</td>
<td>223.0±2.1\textsuperscript{*}</td>
<td>255.6±2.06\textsuperscript{*}</td>
<td>243.6±1.8\textsuperscript{*}</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.42±0.01</td>
<td>0.64±0.04\textsuperscript{*}</td>
<td>0.50±0.02\textsuperscript{*}</td>
<td>0.58±0.006\textsuperscript{*}</td>
<td>0.47±0.007\textsuperscript{*}</td>
</tr>
<tr>
<td>Hydroxyproline (mmol/L)</td>
<td>15.8±1.2</td>
<td>17.0±0.68</td>
<td>16.3±0.57</td>
<td>16.4±0.54</td>
<td>14.03±1.06\textsuperscript{*}</td>
</tr>
</tbody>
</table>

*P*<0.05 as compared with the control, \# GC treated group.

Res-Residronate, AETA-Aqueous extract of *T. aestivum*, GC-Glucocorticoid

**Results**

**Phytochemical screening**—The presence of carbohydrates, amino acids, proteins, tannins, and flavonoids were detected in AETA. Three amino acids were clearly detected in the AETA with *R*\textsubscript{f} values of 0.5, 0.76 and 0.47 which coincided with the *R*\textsubscript{f} values of standard amino acids leucine, tyrosine and asparagine. The presence of ascorbic acid was detected in the aqueous extract of *T. aestivum* with an *R*\textsubscript{f} value 0.75 which was similar to standard ascorbic acid.

**In vitro evaluation of wheat grass powder**—The content of chlorophyll a in wheat grass was found to be 0.66±0.03 mg/g dry weight and the content of chlorophyll b was 0.15±0.02 mg/g dry weight. The total carotenoid content was 1.02±0.07 mg/g.

**FRAP assay**—Antioxidant activity of 1 mg/mL of AETA was equivalent to 300 µmol/L of ascorbic acid.

**Safety evaluation**—AETA was found to be safe up to a dose of 2000 mg/kg body weight.

**Effect of AETA on biochemical parameters**—GC induced biochemical changes by reducing serum calcium and phosphorus levels to 8.3±1.0 and 4.03±0.78 mg/dL respectively which was reversed by risedronate. AETA (400 mg/kg) caused a significant rise in serum calcium and phosphorus compared with GC treated group (P<0.05, Table 1). GC produced an elevation in levels of alkaline phosphatase and creatinine. Treatment with risedronate and AETA (400 mg/kg) evoked a reduction in the levels of alkaline phosphatase and creatinine compared with GC treated group (P<0.05).

**Table 1**—Effect of aqueous extract of *T. aestivum* on biochemical parameters in osteoporosis induced by GC in rats

[Values are mean ± SE from 6 rats each]
urinary hydroxyproline levels compared to negative control \( (P<0.05) \). Treatment with risedronate and AETA produced a decline in the levels of urinary hydroxyproline compared to GC treated group \( (P<0.05) \), Table 1.

**Effect of AETA on physical and biomechanical parameters**—A significant decrease in femur and tibia weight and length was observed in methyl prednisolone treated rats compared to negative control group \( (P<0.05) \). Bone weight and length significantly increased on treatment with residronate, and AETA respectively compared with GC treated group \( (P<0.05) \), Fig. 1a and b.

**Three point bending test of femur and tibia**—The ultimate load tolerated by femur and tibia in rats treated with GC declined to in the three point bending test and compression test compared with the control \( (P<0.05) \). Load tolerated by risedronate treated rats increased in both the three point and compression test. Maximum load required to fracture the femur and tibia increased significantly following treatment with AETA \( (400 \text{ mg/kg}) \) compared with GC treated group \( (P<0.05) \), Fig. 2a and b.

**Discussion**

The present study evaluated the effect of the aqueous extract of *T. aestivum* on osteoporosis induced by glucocorticoids as they alter skeletal integrity by affecting bone metabolism, reduce the life span of osteoblasts and inhibit osteoblastogenesis. Glucocorticoids create an imbalance in the rhythm between bone formation and bone resorption, thereby increasing the risk of fractures. Glucocorticoids hinder the synthesis of collagen and affect differentiation of osteoblasts causing rapid bone formation.

---

**Fig. 1**—Effect of the aqueous extract of *T. aestivum* on (a) bone weight and (b) bone length in GC induced osteoporosis in rats. [Values are mean±SE from 6 rats each. GC=glucocorticoid, Ris=risedronate, AETA= aqueous extract of *T. aestivum*. \( P \) values: *<0.05 compared with control, #<0.05 compared with GC treated group].

**Fig. 2**—Effect of the aqueous extract of *T. aestivum* on biomechanical strength using (a) three point test and (b) compression test in GC induced osteoporosis in rats [Values are mean±SE from 6 rats each. GC=glucocorticoid, Ris=risedronate, AETA= aqueous extract of *T. aestivum*. \( P \) values: * <0.05 compared with control, #<0.05 compared with GC treated group].
loss. Owing to this, glucocorticoids are considered ideal candidates for experimental induction of osteoporosis in rodents.

Calcium salts in bone are embedded in collagen fibrils of which 13% is hydroxyproline. During bone loss, breakdown of collagen fibrils occurs, resulting in the loss of hydroxyproline in urine and is considered to be an index of bone resorption. AETA was found to substantially reduce the excretion of hydroxyproline thereby maintaining bone structure. Treatment with AETA produced a significant decline in the levels of serum alkaline phosphatase in the osteoporosis induced animals. Serum alkaline phosphatase is a biochemical marker of bone turnover, good indicator of internal bone activity and is used to monitor metabolic bone disease.

Calcium is abundant in bone and is essential to maintain bone mineral density. A significant reduction in the level of calcium was observed on treatment with GC. This may be because GC enhances urinary excretion of calcium and reduces its intestinal absorption. The levels of phosphate in serum were also significantly altered in the GC treated group. Decline in the levels of phosphate could be due to enhanced renal excretion and alteration in their transport across the brush border membrane. Treatment with AETA produced an appreciable increase in the levels of calcium and phosphorous. AETA contains calcium which may contribute to the maintenance of calcium homeostasis and potassium which buffers metabolic acid loads as activation of osteoclasts occurs due to lowering of pH. Minor minerals like zinc and iron may be responsible for activation of osteoblast enzymes and collagen hydroxylation.

Bone mineral density is dependent on the supply of number of dietary factors like minerals and vitamins. Formation of collagen which is a prerequisite for normal bone development relies on several nutrients. Studies reveal that 15-day old seedlings of T. aestivum are power houses of minerals like calcium, potassium, phosphorous, magnesium, iron, zinc and copper. Abundant presence of ascorbic acid or vitamin C has been reported in the seedlings of T. aestivum; therefore the aqueous extract of 15-day old seedlings of T. aestivum was utilized in this study. Vitamin C is essential for the formation of type I collagen and healthy bones. Collagen needs to undergo hydroxylation so as to retain its structural integrity. Ascorbic acid serves as an essential coenzyme required to convert proline to hydroxyproline which plays a pivotal role in maintaining collagen structure. In addition, decreased bone density and increased bone loss has also been associated with low protein diet. Proteins present in AETA could promote bone health presumably by both these mechanisms.

Biomechanical tests have enabled us to discern the effect of AETA on the femur and tibia of osteoporotic animals. Physical parameters show increased weight and length of femur and tibia in AETA treated animals indicating improvement in the structure of the weight bearing bones. Biomechanical studies revealed improvement in the integrity of skeletal structures with AETA thereby reducing the risk of osteoporosis.

Free radicals enhance bone loss, by damaging the osteoblast cells. In vitro tests revealed the antioxidant potential of AETA which may help in the restoration of bone formation.

In conclusion, AETA has substantial capability of reducing the risk of glucocorticoid induced osteoporosis. Therefore supplementation of diet with T. aestivum as a nutrient will be economically justifiable as compared to the high cost of current therapies.

Acknowledgement
Support by Nalanda Education Society, Nalgonda-508001, India is acknowledged.

References


12 Benzie I F F & Strain J J, The Ferric Reducing Ability of solvents, carotenoids and chlorophylls A and B of leaf in different (W.B. Saunders in *tietz fundamentals of clinical chemistry*, 1127.


