Ameliorative Role of Lactoferrin on Osteoporosis Caused by Glucocorticoids

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ABSTRACT
Lactoferrin is derived from its past classification as a major iron-binding protein in milk, which was in bovine milk. Osteoporosis is a major disease affecting human bone, which is caused by endocrinological disorders and drugs. Glucocorticoids-induced osteoporosis is the most common form of secondary osteoporosis. The current study was aimed to study the curative role of natural substances (lactoferrin) on the osteoporosis caused by glucocorticoids (prednisone) and comparing its curative effect with sodium alendronate, the common therapy for osteoporosis. Our experiment included 8 groups, in each group, 10 adult’s albino rats. First group (normal females) and fifth group (normal males) were given saline for two months. Second group (control females) and sixth group (control males) were given prednisone orally (25 mg/kg b.w.) each day for two months. Third group (treated females) and seventh group (treated males) were given (25 mg/kg b.w.) each day for two months and then lactoferrin (0.85 mg/kg body weight) daily for two months. Fourth group (treated females) and eighth group (treated males) were given (25 mg/kg b.w.) each day for two months and then sodium alendronate (300 μg/kg b.w.) once weekly for two months. After treatment, blood samples were collected for estimating calcium, phosphorous, PTH and testosterone levels in serum. Right femur bones were removed for determining the density, calcium and phosphorous content. The results indicated that lactoferrin and prednisone were increased serum calcium and PTH levels (P < 0.05), in males and females animals and decreased serum phosphorous for the two sexes, but for testosterone there was a non-significant decrease for female animals and a significant decrease for male animals. Administration of lactoferrin was ameliorated the disturbances of bone. The result suggests that lactoferrin may improve prednisone-induced osteoporosis.

INTRODUCTION
Bone is a connective tissue that is undergoing continual remodeling. Bone remodeling involves osteoblast formation of the bone and osteoclasts resorption of the bone. With regular resorption and deposition of Ca in freshly deposited bone, bone undergoes continuous remodeling. Osteoporosis is a skeleton’s chronic, systemic, metabolic disease characterized by reduced bone mass, architectural defects and reduced mechanical resistance to injury, resulting in accumulated risk of fracture. (Kaczmarek et al., 2004). Milk is a rich biological fluid containing several...
growth sources and providing nutrition within the babe at a time of terribly rapid
growth and development of the skeleton, thus being thought of potential supply of
factors with anabolic effects on the bone. Lactoferrin was identified as a bone - active
factor by investigations of fractions of whey proteins extracted from milk. It is a
pleiotropic factor with powerful antimicrobial and immunomodulatory activities and
shows physiological anabolic effects in the bone (Dorit et al., 2012). Additional dietary
lactoferrin has improved bone mineral density, bone markers, and bone strength in a
number of recent studies in humans and experimental animals. The current study
discusses the bone health improvement effect of lactoferrin. Osteoporosis is
considered a major public health problem and is characterized by reduced bone density
resulting in fragility and fractures of the skeleton (McCarron and Heaney,
2004). Osteoporosis is three times more common in women than in men, partly because
of the hormonal changes that occur at the menopause. Osteoporosis has also adversely
affected elderly patients' quality of life. There are two types of osteoporosis: primary
osteoporosis resulting from aging and menopause, secondary osteoporosis resulting
from certain drugs as a side effect. Glucocorticoid administration resulted in the most
common secondary osteoporosis. Glucocorticoids are a class of steroid hormones
found in nearly every animal cell and play a key role in a wide range of physiological
responses. (Rhen and Cidlowski, 2005). Due to their powerful anti-inflammatory and
immunosuppressive effects, synthetic glucocorticoids called corticosteroids (e.g.
prednisone) have been widely used as a therapeutic option in pharmacology for a wide
range of autoimmune and inflammatory diseases (Barnes, 2006). However, the
benefits derived from the use of corticosteroids may be offset by the under occurrence
of corticosteroid-related osteoporosis American College of Rheumatology reported
that glucocorticoid medicines have both direct and indirect effects on bone tissue that
lead to bone loss. These drugs have a direct negative effect on bone cells, leading to a
lower rate of new bone formation. They can also interfere with calcium handling by
the body and affect sex hormone levels. Either of these issues may result in increased
loss of bone. Long administration of glucocorticoid causes osteoporosis (Mazziotti et
al., 2006). Glucocorticoids disturb calcium metabolism. They can also interfere with
calcium handling by the body and affect sex hormone levels. Either of these issues
may result in increased loss of bone. (Mazziotti et al., 2006). These actions may
theoretically lead to a secondary increase in parathyroid hormone concentrations
(Rubin and Bilezikian, 2002). These changes can have negative effects on bone
metabolism (Mazziotti et al., 2006).

Glucocorticoids reduce production of steroid by gonadal. Low serum testosterone
levels have been stated in glucocorticoid-treated men and may be due to both direct
effects on the testis and indirect effects on testosterone production mediated due to
destruction of gonadotropin hormone secretion (Mateo et al., 1995). Glucocorticoids
may have a direct effect on the renal handling of this mineral. They may also directly
inhibit gastrointestinal absorption of phosphate (Cosman et al., 1994). Long-term
treatment with glucocorticoids reduces bone mineral density leading to osteoporosis
(Reid, 1997).

We found that a variety of fractions of whey protein extracted from milk have growth
stimulatory effects in primary cultures of osteoblasts. High-performance liquid action
analysis of the main proteins within the active fractions known the presence of the
glycoprotein lactoferrin in most of those fractions (Naot et al., 2005).

Lactoferrin is a glycoprotein found in milk (Masson and Heremans, 1971
and Brock, 1980). It is structurally the same as transferrin it's terribly plentiful in
colostrum and little amounts may also be found in tears, saliva, within the secondary
granules of neutrophils, and most mucosal secretions like uterine fluid, vaginal secretion, semen, digestive fluid, bile and bowel secretions, (Kikuchi et al., 2003 and Baker and Baker, 2005). It made with the aid of mucosal epithelium and neutrophils and is released through these cells in reaction to inflammatory stimuli.

Bisphosphonates were evolved and used in the treatment of bone diseases, more often than not Paget’s disease, hypercalcemia of malignancy, and recently, osteoporosis. WHO changed into stated that bisphosphonate is the choice for osteoporosis. Alendronate is considered one of bisphosphonate organization and has an extra bone affinity. The endorsed weekly dose of alendronate at 70 mg is nearly double the potency of the encouraged dose of 35 mg rise Alendronate (Cheng et al., 2009).

### MATERIALS AND METHODS

**Material:**

**Animals:** 80 adult albino rats (male and female) at age (2-3 month) and weight about (180-200 g) were obtained from the Animal House, Faculty of Science, South Valley University, Qena, Egypt. They were kept under standard conditions of temperature (23±2°C), and 12h light/dark period, and fed with a standard pellet diet and water. The animals have divided into 8 groups each group contain 10 rats male or female (4 groups female and 4 groups male)

**Drugs and Chemicals:**

A-Prednisone (Hostacortine):

Hostacortine tablets 5 mg from Sanofi Aventis Egypt.

B-Lactoferrin:

Lactoferrin is an iron-binding protein. It extracted from human milk. It brought from the sigma Aldrich Company as a powder.

C-Fosamax (Sodium alendronate 70 mg tablets):

It was obtained from MSD. Which is described chemically as: (4-amino-1-hydroxybutylidene) bisphosphonic acid monosodium salt trihydrate.

**Experimental design:**

The experimental animals were classified into 8 groups, 10 rats of each group.

- **G1** (normal female group): this group was given only NaCl 0.9% and sacrificed after 2 months.
- **G2** (control female group): received prednisone (25 mg/kg body weight) (Jacobs et al., 1996) day after day orally for 2 months.
- **G3** (treated female group): received prednisone (25 mg/kg body weight) day after day orally for 2 months and then lactoferrin (0.85 mg/kg body weight) (Guo et al., 2009) daily for 2 months.
- **G4** (treated female group): received prednisone (25 mg/kg body weight) day after day orally for 2 months and then sodium alendronate (300 μg/kg body weight/week) (Lee et al., 2006).
- **G5** (normal male group): this group was given only NaCl 0.9%.
- **G6** (control male group): received prednisone (25 mg/kg body weight) day after day orally for 2 months.
- **G7** (treated male group): received prednisone (25 mg/kg body weight) day after day orally for 2 months and then lactoferrin (0.85 mg/kg body weight) daily for 2 months.
- **G8** (treated group): received prednisone (25 mg/kg body weight) day after day orally, for 2 months and then sodium alendronate 300 μg/kg body weight/week.
At the end of experiment, the animals were sacrificed by decapitation. Blood samples of all animals prepared from retro-orbital eye vein. Samples were collected in clean tubes at room temperature to clot then after an hour; serum was separated by centrifugation for 30 minutes at 3000 rpm. The serum was collected in labeled Eppendorf tubes and stored at -20 °C until used for biochemical analysis. Right femurs were removed, frozen at -20 °C until analysis.

**Methods:**

**biochemical Analysis:**

Calcium was estimated in serum by commercially available kit from Randox company using the colorimetric method according to (Cooper, 2013) by using 5010 spectrophotometers (semi-automatic). Phosphorous was estimated in serum by commercially available kit from GPL company using a colorimetric method according to (Farrell and Kaplan, 1984), by using 5010 spectrophotometers (semi-automatic). Parathyroid Hormone (PTH) was estimated in serum by a commercially available ELISA kit from Sigma Aldrich company by using (Bieglmayer et al., 2002) method. Testosterone Hormone was estimated in serum by a commercially available ELISA kit from Sigma Aldrich company (Nobert, 1995).

**Bone Density:**

The density of the femur was calculated from the mass in the air and in water by Archimedes principle (Kalu et al., 1984)

**Preparation of Bone Solution:**

1-Right femurs were manually cleaned from adhering soft tissue.
2-determine the volume.
3-dried 18 h at 100 °C and weighed.
4-digested overnight in 5ml of 60% HNO₃ by use of a modification of the method of (Brown et al. 1976).
5-the digits were heated to 80 °C, and after the addition of 0.3 ml 30% H₂O₂, filtered through What man filter paper.
6-Calcium and phosphorous concentrations were determined on the digested bone solution using spectrophotometry as the same methods of serum calcium and phosphorus.

**Statistical analysis:**

The varying degree of results was expressed as a Means S.D. The data were statistically analyzed by one-way ANOVA analysis of variance (prism computer program, year) and the least significant difference (L.S.D) was used to test the difference between treatments. The results were considered statistically significant when P < (0.05).

**RESULTS**

**1-Serum Calcium and Phosphorous:**

The effect of prednisone (25 mg/kg b.wt.) alone and the effect of lactoferrin (0.85 mg/kg b.w.) and sodium alendronate (300 μg/kg b.w./week) on serum calcium and phosphorous in female and male albino rats post-treated with prednisone:

In prednisone-treated female and male animals a significant increase in serum calcium level and a significant decrease in serum phosphorous when compared with normal animals at (p<0.05). Lactoferrin and sodium alendronate treated animals showed a significant decrease in calcium level and a significant increase in phosphorous when compared with the control group, calcium and phosphorous levels returned to the normal values in female and male animals when compared with normal (Tables 1 & 2) respectively.
**Table (1):** Effect of lactoferrin and sodium alendronate on serum calcium and phosphorous after prednisone treatment on female animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Serum calcium mg/dl M ± S.D</th>
<th>Serum phosphorous mg/dl M ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (normal females)</td>
<td></td>
<td>7.59 ± 0.96</td>
<td>5.81 ± 0.81</td>
</tr>
<tr>
<td>G2 (prednisone)</td>
<td></td>
<td>10.89 ± 0.99 ^a</td>
<td>3.83 ± 0.81 ^a</td>
</tr>
<tr>
<td>G3 (prednisone + lactoferrin)</td>
<td></td>
<td>6.99 ± 0.96 ^b</td>
<td>5.30 ± 0.81 ^b</td>
</tr>
<tr>
<td>G4 (prednisone + alendronate)</td>
<td></td>
<td>7.36 ± 0.70 ^b</td>
<td>5.76 ± 0.81 ^b</td>
</tr>
</tbody>
</table>

The result presented the mean ± S.D. Of 10 rats.
+ significant increase at (p<0.05).  a → significantly different from normal rats.
− significant decrease at (p<0.05).  b → significantly different from control rats.

**Table (2):** Effect of lactoferrin and sodium alendronate on serum calcium and phosphorous after prednisone treatment on male animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Serum calcium mg/dl M ± S.D</th>
<th>Serum phosphorous mg/dl M ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G5 (normal males)</td>
<td></td>
<td>9.44 ± 0.52</td>
<td>5.40 ± 0.88</td>
</tr>
<tr>
<td>G6 (prednisone)</td>
<td></td>
<td>11.54 ± 1.38 ^a</td>
<td>4.73 ± 0.64 ^a</td>
</tr>
<tr>
<td>G7 (prednisone + lactoferrin)</td>
<td></td>
<td>9.27 ± 0.68 ^b</td>
<td>5.19 ± 0.31 ^b</td>
</tr>
<tr>
<td>G8 (prednisone + alendronate)</td>
<td></td>
<td>8.86 ± 0.59 ^b</td>
<td>5.37 ± 0.41 ^b</td>
</tr>
</tbody>
</table>

The result presented the mean ± S.D. Of 10 rats.
+ significant increase at (p<0.05).  a → significantly different from normal rats.
− significant decrease at (p<0.05).  b → significantly different from control rats.

2-Serum PTH and Testosterone:
The effect of prednisone (25 mg/kg b.w.) alone and the effect of lactoferrin(0.85 mg/kg b.w.) and sodium alendronate (300 μg/kg b.w./week) on PTH and testosterone in female and male albino rats post-treated with prednisone:

In prednisone-treated female and male animals a significant increase in PTH when compared with normal animals at (p<0.05). Lactoferrin and sodium alendronate treated groups showed a significant decrease in PTH when compared with the control group, PTH returned to the normal values in female and male animals. While there is a non-significance decrease in testosterone level in females animals treated with prednisone also lactoferrin and sodium alendronate treated groups showed an insignificant decrease in testosterone level but in male rats treated with prednisone showed a significant decrease in testosterone level but lactoferrin and sodium alendronate treated groups showed a significant increase in testosterone level when compared with the control group but still lower than the normal group when compared with normal rats.(Tables 3 & 4).
Table (3): Effect of lactoferrin and sodium alendronate on PTH and testosterone after prednisone treatment of females animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>PTH (Pg/ml) M ± S.D.</th>
<th>Testosterone (ng/ml) M± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (normal females)</td>
<td></td>
<td>3.61 ± 0.25</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>G2 (prednisone)</td>
<td></td>
<td>4.06 ± 0.17 +a</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>G3 (prednisone + lactoferrin)</td>
<td></td>
<td>3.37 ± 0.21 -b</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>G4 (prednisone + alendronate)</td>
<td></td>
<td>3.39 ± 0.21 -b</td>
<td>0.12 ± 0.06</td>
</tr>
</tbody>
</table>

The result presented the mean ± S.D. Of 10 rats.
+ significant increase at (p<0.05). a → significantly different from normal rats.
- significant decrease at (p<0.05). b → significantly different from control rats.

Table (4): Effect of lactoferrin and sodium alendronate on PTH and testosterone after prednisone treatment on male animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>PTH (Pg/ml) M ± S.D.</th>
<th>Testosterone (ng/ml) M± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G5 (normal males)</td>
<td></td>
<td>3.46 ± 0.28</td>
<td>5.41 ± 0.32</td>
</tr>
<tr>
<td>G6 (prednisone treated males)</td>
<td></td>
<td>4.59 ± 0.23 +a</td>
<td>4.14 ± 0.21 -a</td>
</tr>
<tr>
<td>G7 (prednisone + lactoferrin)</td>
<td></td>
<td>3.41 ± 0.16 -b</td>
<td>5.06 ± 0.13 -z -b</td>
</tr>
<tr>
<td>G8 (prednisone + alendronate)</td>
<td></td>
<td>3.46 ± 0.22 -b</td>
<td>4.93 ± 0.21 -z -b</td>
</tr>
</tbody>
</table>

The result presented the mean ± S.D. Of 10 rats.
+ significant increase at (p<0.05). a → significantly different from normal rats.
- significant decrease at (p<0.05). b → significantly different from control rats.

3-Bone Calcium, Phosphorous and Density:
The effect of prednisone (25 mg /kg b.wt.) alone and the effect of lactoferrin (0.85 mg/kg b.w.) and sodium alendronate (300 μg/kg b.w./week) on bone calcium, phosphorous and density in female and male albino rats post-treated with prednisone:

In prednisone-treated female and male animals there is a significant decrease in bone density, calcium and phosphorous contents when compared with normal rats. Lactoferrin and alendronate ameliorated these disturbances where the results in table 5&6 indicated that the bone density, calcium and phosphorous contents increased significantly when compared with control group, which return to the normal values in female and male animals when compared with normal rats. (Tables 5 & 6).
Table (5): Effect of lactoferrin and sodium alendronate on femur calcium, phosphorous and density after prednisone treatment of females animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Femur density (g/cm³)</th>
<th>Femur calcium content (mg/dl)</th>
<th>Femur phosphorous content (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M ± S.D.</td>
<td>M ± S.D.</td>
<td>M ± S.D.</td>
</tr>
<tr>
<td>G1 (normal females)</td>
<td></td>
<td>0.524 ± 0.038</td>
<td>337.1 ± 3.436</td>
<td>123.1 ± 3.976</td>
</tr>
<tr>
<td>G2 (prednisone)</td>
<td></td>
<td>0.406 ± 0.094</td>
<td>326.3 ± 3.49</td>
<td>110.3 ± 4.99</td>
</tr>
<tr>
<td>G3 (prednisone + lactoferrin)</td>
<td></td>
<td>0.546 ± 0.036</td>
<td>339.4 ± 3.21</td>
<td>129.1 ± 4.45</td>
</tr>
<tr>
<td>G4 (prednisone + alendronate)</td>
<td></td>
<td>0.533 ± 0.0467</td>
<td>337.4 ± 4.12</td>
<td>127.4 ± 4.86</td>
</tr>
</tbody>
</table>

The result presented the mean ± S.D. Of 10 rats.
+ significant increase at (p<0.05).  a → significantly different from normal rats.
– significant decrease at (p<0.05).  b → significantly different from control rats.

Table (6): Effect of lactoferrin and sodium alendronate on femur calcium, phosphorous and density after prednisone treatment of males animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Femur density (g/cm³)</th>
<th>Femur calcium content (mg/dl)</th>
<th>Femur phosphorous content (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M ± S.D.</td>
<td>M ± S.D.</td>
<td>M ± S.D.</td>
</tr>
<tr>
<td>G5 (normal males)</td>
<td></td>
<td>0.541 ± 0.07</td>
<td>340.1 ± 3.29</td>
<td>126.6 ± 2.94</td>
</tr>
<tr>
<td>G6 (prednisone)</td>
<td></td>
<td>0.472 ± 0.027</td>
<td>326.6 ± 3.21</td>
<td>114.9 ± 1.95</td>
</tr>
<tr>
<td>G7 (prednisone + lactoferrin)</td>
<td></td>
<td>0.573 ± 0.02</td>
<td>338.6 ± 3.59</td>
<td>127.4 ± 3.21</td>
</tr>
<tr>
<td>G8 (prednisone + alendronate)</td>
<td></td>
<td>0.583 ± 0.037</td>
<td>338.1 ± 1.68</td>
<td>126.6 ± 1.90</td>
</tr>
</tbody>
</table>

The result presented the mean ± S.D. Of 10 rats.
+ significant increase at (p<0.05).  a → significantly different from normal rats.
– significant decrease at (p<0.05).  b → significantly different from control rats.

DISCUSSION

Plenty of attention has currently been targeted at the role and mechanism of motion of clearly happening compounds in the biological system as an example on bone osteoporosis.

Lactoferrin has powerful anabolic as well as potent inhibitory bone resorption properties and may increase bone formation in vivo (Cornish et al., 2004).In the current study, we have to compare the effect of lactoferrin on secondary osteoporosis caused by glucocorticoids (prednisone) with the effect of alendronate which belong to Bisphosphonates where The most common cause of secondary osteoporosis is glucocorticoid is prolonged high - dose glucocorticoid therapy (Ilona et al., 2012).

In the present work, we have to study the effect of Lactoferrin and alendronate on osteoporosis induced by glucocorticoid (prednisone) in females and males rats. From obtained results, we noticed the glucocorticoid caused the increase in serum calcium and the decrease in serum phosphorous levels, the similar result was obtained by (Elshal et al., 2013) showed that serum phosphorus concentration was significantly lower in glucocorticoid administration. On the other hand (Shomali and Fakhrzad, 2013) recorded in their study on rats an insignificant increase in calcium level in rats that treated with glucocorticoid. Also, Yonemura et al., (1999)Stated that the oral dose of prednisolone administration increases serum calcium and ionized calcium levels, possibly mediated by suppressed bone formation, increased calcium absorption in the intestine, and impaired calcium excretion in the urine. Also, an increase of serum...
calcium after glucocorticoid administration might occur as a result of increased release of Calcium from bone tissues (Elshal et al., 2013).

Hypercalcaemia differential is wide and can be categorized on the basis of levels of parathyroid hormone (PTH). PTH is secreted by the parathyroid glands. A unique calcium receptor (extracellular calcium - sensing receptor) on the parathyroid cell membrane quickly responds to changes in plasma calcium levels (Ayuk et al., 2010). Appropriate inhibition of PTH release should occur when the plasma calcium level is high. In the pathophysiology of hypercalcaemia, at least one of the following mechanisms is involved: (1) increased intestinal calcium absorption; (2) increased bone resorption; and (3) increased renal calcium reabsorption or reduced calcium excretion. Hypercalcaemia results mainly from the increased mobilization of calcium from the bone through the final common activation pathway of RANK (receptor activator of nuclear factor kappa B) receptors on the osteoclastic surface by RANK-ligand (RANKL) derived from osteoblasts, (Tanaka et al., 2005). Drugs used to treat dyspepsia or more commonly now osteoporosis can lead to hypercalcaemia mediated by high calcium intake plus metabolic alkalosis, which increases the reabsorption of calcium in the distal tubule. Hypercalcaemia is well known but rare in the context of vitamin D intoxication (Holick, 2007). The present study showed the glucocorticoid caused a significant elevate in parathyroid hormone level in both sexes but for androgen (testosterone) level the study was showed a significant decrease in the male rats, but there was an insignificant decrease in testosterone level of female rats. Several studies have found increased levels of PTH and excretion of urinary calcium. One of them suggests a vitamin D deficiency (Cosman et al., 1994) may contribute to reduced calcium absorption and increased PTH secretion. A direct effect of glucocorticoid on PTH's glandular secretion may also contribute to high PTH serum levels (Fucik et al., 1975). But as a result of these effects, glucocorticoid use could result in secondary hyperparathyroidism. However, a hyperparathyroidism state does not explain the bone disorder observed in glucocorticoid-induced osteoporosis.

Gonadal hormone reduction is an important mechanism through glucocorticoid inhibitory effects on pituitary gonadotropins (Lane and Lukert, 1998). In men, glucocorticoids can inhibit testosterone production through the suppression of gonadotropin hormone secretion due to direct effects on the testis and indirect effects (Luiz et al., 2006). Low levels of serum testosterone can help lower osteoblastic activation. Glucocorticoids blunt LH secretion in males and females in response to GnRH (Sakakura et al., 1975 and Luton et al., 1977). In the previous study, asthmatic men treated with prednisone at an average daily dose of 12 mg had significantly lower free and total testosterone levels than age-matched control subjects (Reid et al., 1985). Dolatabadi and Zarchii, (2015) stated that the testosterone level in male rats receiving glucocorticoid was shown to be lower compared to the control group. Gao et al., (2003) showed in their study that increase in the concentration of serum glucocorticoids due to stress caused controlling activity of testosterone making enzymes and reduction in leydig cells, then testosterone secreting would reduce. In a similar study corticosteroid, administration caused a suppression in androgen level in female rats (Brann and Mahesh, 1991).

While bone density, calcium and phosphorous femur contents showed a significant decrease in females and males rats treated by the glucocorticoid. A significant decrease in the level of phosphorus content of femur bone was observed in the lowest dose of prednisone treatment level (Lin et al., 2014), these results agree with our result. The data of the present study also agree with (Ima and Fakhrurazi, 2002) who founded that the administration of dexamethasone (120 µg/kg
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body wt. caused a decrease in femur calcium content. And (Thakur et al., 2013) who indicated that the administration of glucocorticoids caused a decrease in femur density. Hyperparathyroidism that can cause calcium changes associated with a compensatory increase in PTH resulting in the release of calcium from the skeleton resulting in the bone loss (Guillemant et al., 1999).

The present study showed that the lactoferrin improved the disturbances of serum (calcium, phosphorous, parathyroid hormone and testosterone) in both sexes that prednisone caused, also the lactoferrin improved the femur density, calcium and phosphorous values and became near to normal animals. The mechanisms of action of lactoferrin on bone cells also require further investigation as the pathways through which lactoferrin acts to inhibit osteoclastogenesis are largely unknown (Dorit et al., 2005). Cornish et al. (2004) showed the first evidence in their study that lactoferrin promotes osteoblast growth. It also shows that lactoferrin is osteoclastogenesis in vitro inhibitor and increases in vivo local bone formation. The physiological role of lactoferrin in the development of the skeleton or homeostasis (Dorit et al., 2005). Lactoferrin is expressed within the embryo and will play a role in the development and performance of chondrocytes and osteoblasts in the fetal skeleton (Dorit et al., 2005).

Sayed et al., (2013) suggested that the alendronate treatments were found to help in restoring the tibia phosphorous and calcium content in ovariectomized rats near to the normal rats, also alendronate decreased PTH level increase serum phosphorus level and increased bone mineral density in the same study and this agreement with our result. In an agreement with our study Sass et al., (1997) Alendronate treatment reported a reduction in osteocalcin by day 28 and a marginal decrease in total serum calcium. Bisphosphonates (alendronate) induce apoptosis of osteoclasts and inhibit bone resorption (Frith et al., 1997). Randomized clinical trials showed that treatment with bisphosphonates prevents corticosteroid-induced bone loss. Alendronate, a member of the bisphosphonate family, is positive in the prevention and therapy of glucocorticoid-induced osteoporosis and has been stated to inhibit bone loss and enhance the Bone mineral density of lumbar vertebrae by way of decreasing each bone formation and resorption and suppressing bone metabolism.

Conclusion

The present study indicated that lactoferrin as a natural product has a curative effect on osteoporosis caused by prednisone drug as well as the alendronate drug.

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