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DEVELOPMENT AND PHYSICAL EVALUATION OF POLY HERBAL SYRUP FOR DEXAMETHASONE INDUCED OSTEOPOROSIS

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ABSTRACT

Osteoporosis, the silent disease is characterized by low bone mass and microarchitectural deterioration of bone tissue leading to increased bone fragility and consequent increase in fracture risk. Indians life style and skin complexion are responsible for such poor bone microarchitecture (low vitamin D levels, Calcium deficient diet etc). Drugs also acts as the second leading cause of secondary osteoporosis and steroids acts as a leader among them. Biphosphonates on chronic use lead to the development of many adverse effects and so an attempt is made to analyze the anti-osteoporotic potential of herbs based on the traditional literature. The aim of the present work is to formulate and investigate the anti-osteoporotic potential of Polyherbal syrup (Soya, Raagi, Moringa oleifera leaves extract and honey) in the management of dexamethasone induced osteoporosis. Four different formulations are prepared and subjected to stability studies. Female wistar rats are used in the study and were administered decdan at the dose of 7mg/kg for 4 weeks. After four weeks, body weight and biochemical parameters like ALT, AST & ALP were assessed. Polyherbal syrup A-2 have shown the osteoblastogenic potential similar to bisphosphonates and is proven to be safe on chronic use.

Key words: Osteoporosis, Biphosphonates, Polyherbal syrup, ALT, AST, ALP.

INTRODUCTION

Osteoporosis, the most common bone disorder affecting humans is a skeletal disorder characterized by compromised bone strength, predisposing a person to an increased risk of fracture (Meeta & Jaypee Brothers, 2013). Bone strength (and, hence, fracture risk) is dependent on many qualities of bone, of which bone mineral density (BMD) is the most commonly measured. Expressed as grams of mineral per area or volume, BMD at any given age is a function of both peak bone mass (reached by age 30) and how much bone is subsequently lost (NAMS continuing medical educational society, 2012). Qualities of bone other than BMD (including degree of mineralization, hydroxyapatite crystal size, collagen structure, heterogeneity of bone microstructure, connectivity of trabeculae, and microdamage) are difficult or impossible to measure in clinical practice (Management of osteoporosis in postmenopausal women, 2006). The primary clinical goal of osteoporosis management is to reduce fracture risk. This may be accomplished by slowing or stopping bone loss, increasing bone mass or improving bone architecture, maintaining or increasing bone strength, and minimizing factors that contribute to falls (Neil C. Boland and Linda LaVelle, 2006).

The risk of osteoporosis fractures can be reduced with lifestyle changes and in those with previous

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osteoporosis related fractures, with medications. Lifestyle change includes diet, exercise, and preventing falls. Bisphosphonates are useful in those with previous fractures from osteoporosis but are of minimal benefit in those who have osteoporosis but no previous fractures (Wells GA et al., 2008). Osteoporosis itself doesn't project any symptoms. Its main consequence is the increased risk of bone fractures. Osteoporotic fractures are generally considered as fragility fractures and they mostly occur in vertebral column, rib, hip and wrist (Old JL and Calvert M, 2004). Primary or post menopausal osteoporosis becomes a serious health threat for aging post menopausal women predisposing them to an increased risk of fractures. Secondary or Senile osteoporosis and special type that include corticosteroid induced osteoporosis. Steroid induced osteoporosis is more prevalent in post menopausal female. It appears on the top of already existing primary osteoporosis (Moyer VA, 2013).

Glucocorticoid induced osteoporosis

Glucocorticoids are important therapeutic agents that have been used for their potent anti-inflammatory and immunosuppressive properties for over 50 years (Van Staa TP et al., 2002). Glucocorticoid-induced osteoporosis (GIO) is the most prevalent form of secondary osteoporosis (Mazziotti G et al., 2006). Of the multiple side effects that can occur with glucocorticoid therapy, osteoporotic fractures are one of the most devastating, affecting 30—50% of patients (Angeli A et al., 2006). The risk of fracture is increased by up to 75% during the first 3 months of treatment. It is recommended that clinicians assess BMD before initiating corticosteroid treatment which is likely to last longer than 3 months. While all patients should receive adequate calcium and vitamin D, these alone are not considered sufficient to counteract the adverse effects of long term corticosteroids on BMD (Tang BM et al., 2007).

Diagnostic tests for osteoporosis

Majority of the diagnosis aims at measuring the bone mineral density by the following tests:

1. Dual-energy X-ray absorptiometry (DXA or DEXA)
2. Quantitative computed tomography (QCT)
3. Qualitative ultrasound (QUS)
4. Single photon absorptiometry (SPA)
5. Dual photon absorptiometry (DPA)
6. Single energy X-ray absorptiometry (SEXA)

Among all the above DEXA is considered as the most cost effective approach & it works by measuring a specific bone or bones, usually the spine, hip, and wrist. The density of these bones is then compared with an average index based on age, sex, and size. The resulting comparison is used to determine risk for fractures and the stage of osteoporosis in an individual (Guglielmi G and Scalzo G, 2010).

Average bone mineral density = BMC / W [g/cm^2]

- BMC = bone mineral content = g/cm
- W = width at the scanned line

Pathophysiology of Osteoporosis

Bone contains living cells, including some that nourish the tissue and others that control the process known as bone remodelling. Throughout life, our bones are constantly being renewed by means of this remodelling process, in which old bone is removed (bone resorption) and replaced by new bone (bone formation) Bone remodelling is carried out through the coordinated actions of bone -removing cells called osteoclasts and bone-forming cells called Osteoblasts . During childhood and teenage years, new bone tissue is added to the skeleton faster than old bone tissue is removed. As a result the bones grow in both size and strength increasing its mass. The peak bone mass is achieved between 18-25 years of age. After this period, the bone mass remains stable or decrease very gradually for a period of years depending on a variety of life style factors such as diet & Physical activity. The three main mechanisms by which osteoporosis develop are an inadequate peak bone mass, excessive bone resorption and inadequate formation of new bone during remodelling. Estrogen deficiency is the most common factor contributing to bone loss after menopause. It leads to decreased apoptosis of osteoclasts thereby enhancing the osteoclasts number and bone resorption (Anonymous 1).

Pharmacological treatment

Choice of pharmacological treatment is influenced by patient gender, menopausal status, medical history, whether it is for primary or secondary fracture prevention, patient preference and eligibility for government subsidy. Calcium and vitamin D are usually provided to all participants in randomized controlled trials (RCTs) evaluating the efficacy of pharmacological treatments for osteoporosis. The combined use of calcium and vitamin D is recommended with all anti-osteoporosis treatments if intake is inadequate. Epidemiological and small scale clinical studies suggest vitamin K plays an important role in bone health. However, on the basis of the current evidence, vitamin K is not routinely recommended for osteoporosis treatment or prevention. While hormone therapy (HT) may have a role in the management of postmenopausal osteoporosis in women under the age of 60 years, it is not routinely recommended as an osteoporotic treatment for older women, or for those whose last spontaneous menstrual period occurred more than 5 years ago, due to the possible adverse effects.

Anti-resorptive agents:

Bisphosphonates

Bisphosphonates slow bone loss, improve BMD and reduce fracture rates. Alendronate, risedronate and zoledronic acid are recommended as first line therapy in men and women for primary and secondary prevention of vertebral, nonvertebral and hip fractures Etidronate is recommended for the secondary prevention of osteoporosis. Zoledronic acid is an intravenous infusion that is administered once per year for osteoporosis treatment and prevention.

Adverse effects of Bisphosphonates

Gastrointestinal adverse effects are the most common and upper gastrointestinal disorders or esophageal abnormalities are considered a contraindication to oral bisphosphonate use. Atypical femoral stress fractures with long term bisphosphonate use have been reported, but the increased risk is approximately five fractures per 10 000 patient years.

Osteonecrosis of the jaw (ONJ) has also been reported with bisphosphonate treatment, although the risk is low (approximately one per 10 000 to one per 250 000 patients receiving oral bisphosphonate) and more than 95% of cases occur in people receiving treatment for cancer rather than for osteoporosis.

Adherence to treatment

Approximately half of the subjects discontinue taking OP treatment within the first 6 months, and two-thirds discontinue within the first year. Discontinuation of treatment has been linked to gastrointestinal adverse effects and several other kidney related complications to bisphosphonates. Hence, the current research aimed at utilizing herbs to prevent dexamethasone induced osteoporosis. The current pharmacological approaches are associated with severe adverse effects like neoplasm, Gastric distress, ulcers and renal failure. Hence the present research is aimed at utilizing the nature gifts (Herbs) in prevention and treatment of osteoporosis with minimal or no adverse effects.

HERBS IN THE TREATMENT OF DEXAMETHASONE INDUCED OSTEOPOROSIS

Medicinal plants are being used since times immemorial among indigenous communities of India. These plants are more likely to have pharmacologically active compounds and so are called as sleeping pharmaceutical giants. In the past decade herbs are neglected for study as there are very less methods for standardizing the active principles in them. In the position of plants allopathic medicine took the flight as they gave immediate relief from the ailments. Recently society has focused on the adverse effects shown by allopathic medicines and is accepting the use of herbs. Several researchers have suggested the importance of phytochemicals in the prevention and treatment of corticosteroid induced osteoporosis. Among the herbs Soy is considered as an herb which supplies high amount of Phytoestrogens and these phytoestrogens have the capability to prevent osteoporosis and hence it is employed in the present study (Luigi Mario Chiechi and Loredana Micheli, 2005). Some research methods also proved to increase the trabeculae bone mass in ovarectomized rats (OVX) with honey and hence honey is employed (Siti Sarah Mohamad Zaid et al., 2012). As finger millet (raagi) is found to be a rich source of calcium (Rama bhide et al., 2013), it has been employed and *Moringa oleifera* leaves aqueous extract has been a rich source of vitamin D it is employed in this study (Chirag Patel et al., 2013).

MATERIALS & METHODS

Collection and Extraction of Plant material

The leaves of *Moringa oleifera* was collected from medicinal garden, M.A.M College of Pharmacy, Kesanupalli, Narasaraopet, Andhra Pradesh. Soy bean seeds, Raagi seeds powder & Honey were procured from the local market.

Preparation of aqueous extract of *Moringa oleifera* by Maceration

The leaves of *Moringa oleifera* were extracted using water as a solvent by hot maceration method.

Preparation of Soy bean extract

The seeds of soybean were soaked in water overnight and made into a slurry. The slurry was diluted with water and passed through a muslin cloth and the extract is boiled in order to remove any traces of impurities (Anonymous 2).

Preparation of Raagi extract

The procured raagi powder was boiled with water and filtered and the extract was used in the preparation of formulation (Anonymous 3).

Method of Preparation of Polyherbal syrup

All the extracts were combined randomly in different ratios and formulated as four different formulations. Required quantity of sodium benzoate was added to the above mixture as a preservative. Solubility was checked by observing the clarity of the solution visually. The final herbal syrup was then subjected to evaluation of quality as per official standards.

Evaluation of Polyherbal syrup

Organoleptic evaluation

Color Examination

Few ml of the syrup was taken into a watch glass and placed against white background in white tube light. It was observed for its color by naked eye.

Odour Examination

Few ml of the syrup was smelled individually and the time interval between two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste Examination

A pinch of final syrup was taken and examined for its taste on taste buds of the tongue.

Determination of p^H & Density

The p^H of Polyherbal syrups was determined using p^H meter. The p^H meter was calibrated using distilled water till constant values are obtained. The syrup was evaluated for its density.

Determination of Viscosity

Ostwald viscometer was used to determine the viscosity of Polyherbal syrup and the method adopted was according to the standard procedure.

Determination of Specific Gravity

Pycnometer was used to determine the specific gravity at 25ºC. It was determined by dividing the weight of sample by the weight of water.

Stability testing of the formulated syrups

The stability testing of the formulated syrups were carried out for 72 hours. The syrups were kept at 40 ºC ± 2 ºC, 2 to 8 ºC and stored in an amber colored bottle.

Animal study

A total of 24 female rats weighing between100-150g were procured from albino labs and housed in the animal house. The rats were housed in sanitized polypropylene cages containing paddy husk as bedding. The animals were maintained under controlled conditions of temperature and humidity and a 12h light –dark cycle. All animals were allowed free access to water and fed on a commercial diet. Decdan sodium vials were purchased from the local stores.

Induction of Osteoporosis

After 5 days of acclimatization, experimental animals were divided into four groups (each group having 6 animals each) and osteoporosis was induced by administering decdan sodium (dexamethasone sodium phosphate) at 7mg/kg body weight intramuscularly once in a week up to four weeks. Weights of rats were observed during induction of osteoporosis and their treatment.

Anti-Osteoporotic study

Anti-osteoporotic study was performed using dexamethasone induced osteoporosis in rats. Animals were divided into six groups. Group-I was kept as normal control, Group-II served as osteoporotic control, Group-III was given dexamethasone once a week and sodium Alendronate was given daily till four weeks. Group-IV was given dexamethasone and A-2 syrup, In groups- III & IV dexamethasone was administered once in a week up to four weeks and standard, formulated syrup was given daily.

Evaluation of Bio-chemical Parameters

Blood of each rat is collected through intravenous route and centrifuged at 3000rpm for 20 minutes to obtain the serum and the obtained serum was refrigerated for future use. Serum levels of ALT, AST & ALP were measured by spectrophotometric method (Antai AB et al., 2009; Neil B.Madsen & Jules T, 1952).

RESULTS & DISCUSSION

Table 1.Composition of Polyherbal syrups

S.No	Formulation code	Quantities of the extracts used in formulation			
		Moringa oleifera	Soya extract	Raagi extract	Honey
1.	A-1	10ml	35ml	35ml	20ml
2.	A-2	15ml	40ml	25ml	20ml
3.	A-3	15ml	35ml	35ml	15ml
4.	A-4	15ml	30ml	30ml	25ml

Different formulations were prepared by varying the proportions of all the extracts. As all the extracts used here are considered to be safe even at high doses and extensive literature has been reported on their use, toxicity studies have not been performed. Benzoic acid is added as a preservative in all the above formulations.

Results of Physicochemical parameters of the prepared Polyherbal syrups

Table 2. Physicochemical parameters of Syrup A-1

S.No	Physicochemical parameters	Observed values
1.	Colour	Light brown
2.	Odour	Aromatic & Pleasant
3.	Taste	Sweet
4.	Density wt/ml at 25ºC	1.09g

5.	Specific gravity	0.732 g/ml
6.	Viscosity	1.75 poises
7.	p^H	4.5

Table 3. Physicochemical parameters of Syrup A-2

S.No	Physicochemical parameters	Observed values
1.	Colour	Light brown
2.	Odour	Aromatic & Pleasant
3.	Taste	Sweet
4.	Density wt/ml at 25ºC	1.067g
5.	Specific gravity	1.313 g/ml
6.	Viscosity	1.282poises
7.	p^H	4.41

Table 4. Physicochemical parameters of Syrup A-3

S.No	Physicochemical parameters	Observed values
1.	Colour	Light brown
2.	Odour	Aromatic & Pleasant
3.	Taste	Sweet
4.	Density wt/ml at 25ºC	0.906g
5.	Specific gravity	1.159 g/ml
6.	Viscosity	1.132poises
7.	p^H	4.6

Table 5. Physicochemical parameters of Syrup A-4

S.No	Physicochemical parameters	Observed values
1.	Colour	Light brown
2.	Odour	Aromatic & Pleasant
3.	Taste	Sweet
4.	Density wt/ml at 25ºC	1.456g
5.	Specific gravity	0.250g/ml
6.	Viscosity	0.94poises
7.	p^H	4.7

Table 6. Stability studies through physicochemical parameters of developed syrups

Sample	Time duration (hrs)	Temperature ºC	Physicochemical parameters				
			Colour	Odour	Taste	P^H	Turbidity/Homogenity
A-1	24 hrs	2-8 ºC	No change	No change	No change	4.5	No turbidity
A-2		Room temperature	No change	No change	No change	4.41	No change
A-3		47 ºC	No change	No change	No change	4.6	Homogenity
A-4							
A-1	48 hrs	2-8 ºC	No change	No change	No change	4.54	No turbidity
A-2		Room temperature	No change	No change	No change	4.41	No change
A-3		47 ºC	No change	No change	No change	4.68	Homogenity
A-4							
A-1	72hrs	2-8 ºC	No change	No change	No change	4.57	No turbidity
A-2		Room temperature	No change	No change	No change	4.41	No change
A-3							
A-4		47 ºC	No change	No change	No change	4.75	Homogenity

When the study has been extended to three months only A-2 Syrup was found to be stable and so A-2 Syrup is employed in further study.

Table 7. Body weight of rats after 4 weeks of decdan administration

Rat No	Group-I (Normal Control)	Group-II (Osteoporotic control)	Group-III (Standard)	Group-IV (A-2 Syrup treated group)
Body weights (gm)				
1	115	112	113	114.3
2	123	120	121	122.8
3	148	145	146	147
4	125	122	123	124
5	120	115	118	119
6	135	130	134	134.5
Mean	127.66	124	125.83	126.9
S.D	11.96	12.01	12.08	11.90
S.E.M	4.88	4.905	4.93	4.858
Body weight (Mean ±SEM)	127.66±4.88	124±4.905	125.83 ± 4.93	126.9 ± 4.85

Table 8. Measurement of ALT, AST &ALP

Bio-chemical Parameters	Group-I (Normal Control)	Group-II (Osteoporotic control)	Group-III (Standard)	Group-IV (A-2 Syrup treated group)
ALT(U/L)	22.1±1.6	24.25 ±1.23	22.23 ±1.78	21.9 ±1.59
AST(U/L)	52.05 ± 2.87	54.24 ±3.01	53.31 ± 3.05	52.25 ±2.86
ALP(U/L)	115.708 ±2.99	118.85 ±2.66	117.17 ±2.96	116.6 ±2.84

U/L- Units per Litre.
All the values are expressed as Mean ± SEM.

Among all the syrups formulated A-2 syrup was found to be most stable even after three months and so the same is utilised for Anti-osteoporotic study. Body weights of osteoporotic rats reduced significantly after 4 weeks of decdan and treatment with Alendronate raised the body weights. Treatment with the syrup raised the body weights of osteoporotic rats to almost normal level which indicates the osteoblastogenic potential of the syrup.

CONCLUSION

From our present study it is clear that the synergistic action of Soya, Raagi, *Moringa oleifera* and honey have decreased the function of osteoclasts and also enhanced the osteoblastogenic potential. However no clear conclusion can be drawn at this stage as it is a preliminary analysis. It needs to be formulated and subjected to extensive pre-clinical and clinical investigations.

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CONFLICTS OF INTEREST: NIL

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