SCREENING OF POST-MENOPAUSAL OSTEOPOROSIS BY ANTIOSTEOPOROTIC ACTIVITY

Seema Singh¹, Santosh Kumar², Sunita Singh³, Chetna Mishra⁴, Dinesh Tripathi⁵, Mrinal Gupta⁶

^{1,2}Department of Respiratory Medicine, King Georges Medical University, Lucknow, Uttar Pradesh-226003, India.

³Department of Microbiology, King Georges Medical University, Lucknow, Uttar Pradesh-226003, India.

^{4,5}Department of physiology, King Georges Medical University, Lucknow, Uttar Pradesh-226003, India.

⁶Department of Biochemistry, King Georges Medical University, Lucknow, Uttar Pradesh-226003, India

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Corresponding author: Dr. Seema Singh

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Abstract

Postmenopausal osteoporosis, a quiet plague, has become a significant wellbeing danger, harassing about half of postmenopausal ladies around the world, and is accepted to be a malady that is one of the most well-known face to face who is encountering dementia achieved by mature age. It is a constant, dynamic condition, related with infinitesimal weakening of bone tissue, bringing about diminished bone mass, diminished bone quality which builds the danger of break. Ladies are bound to create osteoporosis than men because of decrease in estrogen during menopause which prompts decrease in bone-development and increment in bone-resorption action. Estrogen can stifle creation of proinflammatory cytokines like IL-1, IL-6, IL-7 and TNF- α . This is the reason these cytokines are raised in postmenopausal ladies. This paper manages the different techniques and parameters most every now and again utilized for screening of antiosteoporotic movement in post-menopausal osteoporosis. The ovariectomized creature model is the most proper model for considering the adequacy of various medications to forestall bone misfortune in postmenopausal osteoporosis. Different parameters dissected are: Biomechanical parameters as Three point bowing of tibia, Compression IV lumbar vertebra, Loading trial of femoral neck, Bone mineral thickness estimation; Biochemical parameters viz. serum calcium and inorganic phosphorus, serum basic phosphatase (ALP), Tartrate safe corrosive phosphatase (TRAP), protein profile, serum ACTH, corticosterone, IL-6, TNF- α , osteoprotegerin (OPG) and deoxypyridinoline crosslinks to creatinine proportion (DPD/Cr); Physical parameters like thickness and the length of the femur, weight of femur, Femur bone volume, bone thickness and so forth; Histopathology of femur to watch histopathological changes like size, shape and bone design; problematic and lytic changes, and fibrocartilagenous lattice with osteodystrophy; therapeutic advancement with mineralization alongside genuinely very much dispersed osteocytes; trabeculae and grid, and shaft size and so on; Histopathology of tibia to watch bone zone, bone volume per tissue volume, bone edge, outright number of dynamic osteoblasts, the proportion of indisputably the quantity of dynamic cuboid osteoblasts per bone border, the proportion of without a doubt the quantity of osteoclasts per osteoclast edge which speak to part of the trabeculae that are secured with osteoclasts, trabecular thickness, trabecular partition, trabecular number, mineralized bone volume per tissue volume, the osteoid volume per bone volume, the osteoid surface per bone surface, the osteoblast surface per bone surface, the disintegrated surface per bone surface. Different parameters as histopathology of uterus and mammary organ tissue, immunohistochemical recoloring to quantify ER level, pee examinations, body weight, organ weight, nourishment utilization, estrogen receptor ligand restricting test (ER-LBA), assurance of oxidative pressure, social test by constrained swimming test, neonatal mouse parietal bone resorption measure and so forth can likewise be completed.

1. Introduction

Menopause is the hour of life when menstrual cycles stop, and is brought about by diminished emission of the ovarian hormones estrogen and progesterone. Despite the fact that menopause is an ordinary occasion for ladies, singular encounters fluctuate, and a few ladies look for clinical guidance for the administration of indications (1). Numerous side effects have been ascribed to menopause, yet just vasomotor brokenness and vaginal dryness are reliably connected with this season of life in epidemiological investigations (2). At menopause estrogen

insufficiency adjusts the ordinary procedure of bone rebuilding by expanding osteoclastic resorption action without a comparing increment in osteoblastic action. There is an expanded creation of tumor putrefaction factor (TNF α) and cells of the stromal/osteoblastic heredity become increasingly touchy to IL-1. IL-1 and TNF animates stromal cells/preosteoblasts to discharge a few cytokines like IL-6, macrophage province invigorating variable (M-CSF), IL-11, granulocyte macrophage state invigorating element (GM-CSF), changing development factor (TGF) (3). The last cytokine in the osteoclastogenesis course is RANK ligand (receptor activator of atomic factor B ligand) which

is created from osteoblasts and tie to its receptor RANK on osteoclasts. RANKL has a characteristic enemy osteoprotegerin (OPG) that is a solvent receptor that is discharged by the stromal osteoblast ancestry cells (4). Estrogen invigorates OPG. These components increment bone resorption by expanding the pool size of pre-osteoclasts in bone marrow and down directed by estrogen. Estrogen expands OPG emission and diminishes M-CSF and RANK (5).

Postmenopausal osteoporosis, a quiet pandemic, has become a significant wellbeing risk, tormenting about half of postmenopausal ladies around the world, and is believed to be an illness with probably the most noteworthy occurrence in feeble individuals (6). As per World Health Organization (WHO), osteoporosis happens when bone mineral thickness tumbles to more than 2.5 standard deviations (SD) beneath the standard reference for most extreme bone mineral thickness of the youthful grown-up female (7). It is a constant, dynamic condition related with small scale engineering decay of bone tissue that outcomes in low bone mass, diminished bone quality that inclines to an expanded danger of crack; it is portrayed by a quick loss of mineralized bone tissue, interruption of trabecular design of the bone and changes in the crystalline properties of mineral stores, which bring about the basic disappointment (break) of destinations wealthy in cancellous bone, for example, the vertebrae, hip and distal lower arm (8). Ladies are bound to create osteoporosis than men because of decrease in estrogen during menopause which prompts decrease in bone-arrangement and increment in bone-resorption movement. Estrogen can stifle creation of proinflammatory cytokines like IL-1, IL-6, IL-7 and TNF- α . This is the reason these cytokines are raised in postmenopausal ladies (9). Treatment centers around easing back down or halting the mineral misfortune in this manner forestalling bone cracks and controlling the torment related with the malady. Medications accessible increment bone thickness either by repressing resorption (for example bisphosphonates, raloxifene, calcitonin, hormone substitution treatment and nutrient D), by expanding development (for example Terparatide) or by acting the two different ways (for example strontium ranelate), however every single one of them is related with symptoms, for example, hypercalcemia, hypercalciurea, expanded danger of endometrial and bosom malignant growth, bosom delicacy, period, thromboembolic occasions, vaginal draining and hot flushes (10). Thusly, ladies are progressively utilizing home grown cures as an elective treatment. The current survey centers around various parameters utilized for screening of postmenopausal osteoporosis.

2. Methods

Web perusing from Google Scholar database was utilized for downloading research papers and digests regarding screening of antiosteoporotic action by utilizing watchwords (antiosteoporotic action + plant + menopause) in the long stretch of July 2019. At that point articles were chosen based on screening of antiosteoporotic movement, techniques for the test and assessment parameters for screening.

2.1 Screening of antiosteoporotic activity

Despite the fact that osteoporosis influences both more established people, postmenopausal ladies have been the essential focal point of osteoporosis. An emotional insufficiency of female hormone is the most significant factor to postmenopausal ladies osteoporosis. The ovariectomized (OVX) rodent model is most ordinarily utilized in look into on postmenopausal osteoporosis, in which ovariectomy brings about an abundance of bone resorption over bone development at first and causes bone misfortune.

The OVX rodent is the most proper model for contemplating the adequacy of various medications to forestall bone misfortune in postmenopausal osteoporosis in view of a few similitudes between the grown-up rodent model of ovarian hormone insufficiency bone misfortune and postmenopausal osteoporosis (11). Bone redesigning including a period of actuation, osteoclastic resorption, and a period of osteoblastic bone arrangement happens in trabecular bone in rodents just as in people. In rodents, bone shortfall from estrogen inadequacy results from in any event two imperfections: a diminishing in intestinal assimilation of calcium and a net increment in bone resorption (12). Ovariectomy in rodents is in this manner followed by an expansion in bone turnover (with resorption surpassing development) related with bone mass misfortune at a few locales wealthy in trabecular bone and in an abatement in bone thickness, mineralization and quality (13). So also, in ladies the bone misfortune following menopause is related with elevated levels of biochemical markers of bone turnover, for example, basic phosphatase. Fast postmenopausal bone misfortune, which happens in female rodents following ovariectomy, is portrayed by a diminishing in trabecular thickness and a weakening of bone design, particularly an abatement of the all out number of trabeculae and an expansion of the quantity of their holes (14). The quantitative misfortunes of bone, just as the progressions of its interior structure, are viewed as answerable for the expanded break hazard in postmenopausal osteopenia.

2.2 Ovariectomy

Ovariectomy should be possible by the technique depicted by (15). The creatures are anesthetized with an intraperitoneal infusion of ketamine hydrochloride (80 mg/kg body weight). A solitary transversion is made on the

dorsolateral side of the back. The entry point moved promptly from one side to the opposite side in order to lie over every ovary thusly. A little scratch is made over the site of the ovary. The ovary is seen through the stomach divider, implanted in cushion fat. The top pair of a forcep is presented and fat around the ovary is gotten a handle on. Care to be taken with the goal that container around ovary isn't cracked. The tip of uterine horn is ligated and ovary, together with the fallopian tube is evacuated with a solitary cut by some scissors. The ovary of the opposite side is likewise evacuated similarly. Skin wound is shut with cotton sutures. The creatures ought to be housed exclusively in polypropylene confines during recuperation time of 21 days. Achievement of ovariectomy is affirmed at necropsy by neglecting to recognize the ovarian tissue and by perception of checked decay of the uterine horns. In the investigation regularly the creatures are haphazardly partitioned into various gatherings: a) hoax worked and filling in as basal control, b) got vehicle and fills in as ovariectomized control, c) bunch getting standard medication d) bunches controlled with test drug(s). Toward the finish of the treatment time frame blood tests from all the gatherings were pulled back by standard technique to survey biochemical parameters. The creatures were then relinquished and bones were separated for biomechanical testing and to contemplate histopathological changes.

Evaluation parameters

Biomechanical parameters

Ovariectomy causes bone fragility and decrease of bone strength. So, the freshly isolated bones (right femur, tibia and fourth lumbar vertebra) may be assessed for their biomechanical strength by using the tensile strength testing machine (16).

Three point bending of tibia

A supporter with two stacking focuses, 13 mm separated from one another is utilized on the phase of the testing machine. Horizontal surface of the tibia at tibio-fibular intersection is set upon the principal point and proximal tibia upon the other. An adjusted press head compacted the center of the tibial shaft until break happens (17). So as to gauge the mechanical quality of the tibia, trial of straightforward footing are completed at a consistent speed utilizing footing test device. The tibias are pulled at the two limits until they broke and the evaluated breaking quality is hence determined in closeness of the breakage (18).

Right femur can be surveyed for biomechanical parameters by three twisting test utilizing a MTS instrument.

The femur is set in the material testing machine with two backings isolated by a separation of 24 mm, at that point pack the center of the femur shaft by an adjusted press at 5 mm/min until break happens. The biomechanical properties decided from the heap disfigurement bend incorporate the accompanying: most extreme burden, greatest pressure, most extreme stroke, greatest strain, youthful's modulus, versatile and vitality (19). Burden distortion bends could be recorded during the bowing procedure utilizing Hounsfield material testing machine. The firmness and vitality mirroring the auxiliary properties of the bones are determined via robotized calculation (20).

Compression of IV lumbar vertebra

The fourth lumbar vertebra is located and then isolated. The fresh vertebra is placed on the flat metallic stage and compressed until it fractured. The reading is recorded in Newtons (21). The fresh vertebra was placed in digital hardness tester and compress until it got fractured and the reading was recorded in Newtons (N) (16).

Loading test of femoral neck

The head of the femur is loaded with the force parallel to the shaft of the femur until fracture. A metallic clamp with flat surface is used to fix the proximal part of the femur perpendicularly and tightly. A concave compressing head (2.5 mm) is used for loading the head. Force required to break the head is noted (22).

Bone Physical parameters

The thickness and the length of the femur are measured with calipers, then dried in an evacuated oven at 110°C for 48 h and weighed. Femur bone volume is measured using the plethysmometric method (Archimedes' principle) and bone density (g/ml bone volume) is calculated (23). Femur external diameter can also be measured at the midshaft. Fresh isolated femurs were weighed using an electronic scale. Length of the femurs was measured using a digital slide calipers. The length was measured from the proximal tip of the femur head to the distal tip of the medial condyle (24).

Bone mineral density measurement

Ovariectomy is related with an expansion in bone turnover, negative bone parity and bone mineral thickness (BMD) misfortune in trabecular area of long bones and decrease in BMD at metaphysic of tibia. Estimation of BMD utilizing double X-beam absorptiometry (DXA) is considered as a brilliant standard evaluation for deciding those with low BMD (25). BMD and BMD rate changes from gauge estimation of entire and proximal tibia should be possible by double vitality X-beam absorptiometry (DXA) utilizing a Lunar DPX-L (26). Fringe quantitative PC tomography (QCT) could likewise be performed at the proximal metaphysic of tibia utilizing STRATEC XCT 4.50

gear. The scanner is set at the left metaphysis of tibia and a coronal figured radiograph distal way is completed. The scout see is utilized to situate the scanner at the site of estimation. Record three tomographic slides in 3.75, 4.25 and 15 mm distal reference line. Picture obtaining, handling and estimation of results is finished by programming (XCT 5.40). Cancellous thickness is determined from the third slide by procuring information inside the default edge of 280-710 mg/cm³. Tissue with a thickness over 710 mg/cm³ is considered as the cortical bone. BMD can likewise be determined utilizing the bone mineral substance (BMC) of a deliberate territory. BMD of the correct femur and the lumbar vertebra (LV1–LV6) can likewise be estimated utilizing double vitality X-beam absorptiometry (PIXImus, Lunar Corp./GE, USA). Lumbar vertebra 1 through 6 (LV1–LV6) is estimated unblemished. Spot each correct femur and vertebra (LV1–LV6) in the focal point of a plastic compartment, which contains 40mL of refined water to imitate delicate tissue (27). BMD could be consequently determined (28).

Bone calcium content measurement

Ovariectomy was found to cause noteworthy misfortune in bone calcium which was predictable with osteoporosis because of estrogen inadequacy. The bones were dried in a stove at 1000C for 24 h, at that point ashed in a heater at 700-8000C for 8-12 h until the debris arrived at steady weight to gauge femur debris weight, debris rate and calcium content. The debris was gauged and disintegrated in 3ml nitric corrosive and afterward weakened in lanthanum chloride. Calcium chloride was estimated with an Atomic Absorption Spectrophotometer (AAS) at 422.7 nm (29). The debris test is can likewise be broken down with water fortis and weakened with deionized water to assess calcium content utilizing AAS (30).

Biochemical parameters

Biochemical markers of bone turnover also respond sensitively towards change in bone remodeling. Serum alkaline phosphatase, osteocalcin and cross-laps, the established markers for bone formation and resorption are often increased in osteoporosis. Along with the above few more biochemical parameters as discussed below are also determined in screening of menopausal osteoporosis.

Serum calcium and inorganic phosphorus

Serum calcium and inorganic phosphorus can be resolved in vitro by utilizing demonstrative reagent unit. The calcium present in the serum is hastened with naphthyl hydroxamic corrosive (calcium reagent). The encourage is then broken down in EDTA reagent and calcium from this arrangement can be complexed with shading reagent to give a hued complex which is estimated colorimetrically (31). Pee calcium and phosphorus taxi likewise be

controlled by standard colorimetric strategies utilizing programmed analyser.

Serum alkaline phosphatase (ALP)

In vitro determination of ALP by Kind and King's method can be carried out by using diagnostic reagent kit. ALP from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of oxidizing agent potassium-ferricyanide and forms an orange red colored complex, which can be measured colorimetrically. The color intensity is proportional to enzyme activity (32).

Tartrate resistant acid phosphatase (TRAP)

In vitro assurance of TRAP by King's strategy might be completed by utilizing indicative reagent packs. Corrosive phosphatase from serum changes over phenyl phosphate to inorganic phosphate and phenol at pH 4.8. Phenol so shaped responds in soluble medium with aminoantipyrine in nearness of oxidizing operator potassium-ferricyanide and structures an orange red shaded complex, which can be estimated colorimetrically. The shading force is relative to chemical action. Since tartrate represses the prostratic division of the catalyst, the distinction in corrosive phosphatase movement without and with tartrate speaks to the action of prostratic part (33).

Determination of protein profile and serum estradiol

Estrogen misfortune in ovariectomy may prompted aggregation of vitality stores prompting amassing of fat (reflected in the ascent of cholesterol, HDL and LDL) and thus weight gain. In this manner, the protein profile in the serum of different treatment gatherings can be estimated by utilizing biochemical units (34). Serum hormone level was controlled by radioimmunoassay (RIA). The estradiol RIA was performed by guidelines going with a Coat-a-Count pack (He et al., 2010). It is notable that serum estradiol (E2) level is generally acknowledged phenotype marker for bone digestion. Henceforth, Serum E2 level can likewise be resolved utilizing ELISA examine unit adhering to producer's guidance (35).

Determination of serum ACTH, corticosterone, IL-6, TNF- α , osteoprotegerin (OPG) and deoxypyridinoline crosslinks to creatinine ratio (DPD/Cr)

Serum DPD/Cr is a biochemical marker for bone resorption. Ovariectomy actuates high bone turnover in creatures and expands serum DPD/Cr. OPG is an endogenous protein of the tumor rot factor receptor superfamily created by osteoblastic cells, which hinders arrangement, actuation, and endurance of osteoclasts by interfering with the association between receptor activator of NF-kappa B ligand (RANKL) and its receptor (36). IL-1 β and TNF- α have been accounted for to upregulate the

outflow of OPG just as RANKL in human osteoblast genealogy cells, while IL-6 has no impact on guideline. In this way, the impact of these cytokines in invigorating osteoclastogenesis may rely upon the OPG:RANKL produced in the bone microenvironment (37). Ovariectomy diminishes serum OPG levels, and expands serum IL-6 and TNF- α levels. Ovariectomy additionally influences the serum ACTH and corticosterone levels in rodents. It is discovered that serum corticosterone levels and the quantity of positive ACTH cells are essentially expanded in ovariectomized rodents following 12 weeks of medical procedure (38). Decreased estrogen level after ovariectomy enacts pituitary ACTH cell work, continuously invigorates adrenocortical cell expansion, and expands the creation of glucocorticoids. Glucocorticoids alienate gonadal capacity, repress the osteoanabolic activity of sex steroids, and diminish intestinal assimilation of calcium, prompting a negative calcium balance. Subsequently these can be evaluated by utilizing ELISA pack as per the producer's guidance (39).

Serum osteocalcin

Osteocalcin is a non-collagenous protein combined by develop osteoblast and is for the most part viewed as a particular marker for osteoblastic movement and for bone arrangement (40). Serum osteocalcin (OCN) and antacid phosphate (ALP), bone-explicit soluble phosphatase (BALP) levels are as often as possible utilized as the bone arrangement markers to screen medicate activities (41); while, urinary free deoxypyridinoline (DPD) serves a comparable job in observing bone resorption (42). OVX increments in serum ALP, OCN and pee DPD. Thus serum OCN level can be resolved utilizing ELISA unit and pee DPD by serious catalyst immunoassay (20). The osteocalcin level was seen as brought down in ovariectomised rodents, relating to decreased bone development. Osteocalcin level can be resolved in serum test utilizing Elecsys framework and cross-laps by a particular radioimmunoassay (34).

Determination of C-terminal telopeptide of type 1 collagen (CTX)

CTX are peptide fragments from collagen degradation by cathepsin enzyme that is released by osteoclasts during bone resorption. These collagen crosslinks enter the blood circulation which can be measured as biochemical markers of bone resorption (40). It is sensitive and specific in detection of osteoporosis as it has a low coefficient of variation (43). The CTX level raises in ovariectomized rat model, consistent with other studies which have shown that the CTX level was raised after menopause. The CTX level was found to be reduced when antiosteoporotic treatment is started (43). CTX can be measured by ELISA reader using kits.

Serum and Urine Hydroxyproline (HOP)

HOP is known to be important biochemical markers associated with bone metabolism and their levels are the most common indicators of osteogenic properties. The levels of those markers are increased in osteoporosis and other bone metabolic disorders due to increased bone turn over (44). The HOP level can be measured using commercial assay kits on a microplate reader.

2.3 Histopathology

Around the completion of the treatment plan, the animals are surrendered to separate femur, tibia, uterus and mammary organ which are presented to histological examinations. Territories of decalcified femurs fixed in 10% impartial padded formalin and introduced in paraffin wax can be prepared using a microtome and recolored with haematoxyline and eosin. The portions are mounted, cut into longitudinal territories of 5 μ m thickness and looked for histopathological changes like size, shape and bone plan; tricky and lytic changes and fibrocartilageneous connect with osteodystrophy; helpful progression with mineralization close by truly all around appropriated osteocytes; trabeculae and cross section, and shaft size, etc (16). The sections can be recolored with hematoxylin-eosin and tartrate-safe destructive phosphatase, a cytochemical marker for osteoclasts in conclusion counterstained with hematoxylin. Osteoclasts are recognized as multinucleated cells. The amount of emphatically recolored osteoclasts in the zones of the center section of the whole femora may be recorded (29). The femur zone was broke down for any histological changes like designing, bone conservativeness, delicacy with tricky, lytic changes, reducing of the trabecule coming to fruition in intertrabecular spaces broadening; extended solidifying, mineralization and extended osteoclastic activity and diminished bone resorption, which shows the recovery with principal features of common bone (45).

Fast postmenopausal bone mishap, which occurs in female rodents following ovariectomy is portrayed by a decrease in trabecular thickness and debilitating of bone designing, especially a reducing of outright number of trabeculae and a development of number of their gaps. The quantitative loss of bone similarly as the movements of its inside structure, are considered for the assemblies break danger in postmenopausal osteopenia (46).

Around the completion of the preliminary, in the wake of surrendering the animals the right tibia is emptied, by then fixed in 10% supported formalin, models are cut longitudinally, got dried out and embedded in methylmethacrylate. Fragments of 5 μ m are gotten using microtome and recolored with Goldnerd by Villanueva Goldner's trichome methodology for partition among mineralized and unmineralized bones and besides for ID of cell portions. Territories should be seen by light amplifying focal point and assessed with a semi-automatised bone

histomorphometry system. The parameters which may be picked for study are: bone area, bone volume per tissue volume, bone fringe, inside and out number of dynamic osteoblasts, the extent of no ifs, ands or buts the amount of dynamic cuboid osteoblasts per bone edge, the extent of obviously the amount of osteoclasts per osteoclast edge which address some portion of the trabeculae that are made sure about with osteoclasts, trabecular thickness, trabecular segment, trabecular number, mineralized bone volume per tissue volume, the osteoid volume per bone volume, the osteoid surface per bone surface, the osteoblast surface per bone surface, the broke down surface per bone surface (47) .

Uterus is the most delicate organ towards estrogen. Hormone decline after ovariectomy rapidly backslide the organ and in this manner weight decrease. Affection of IGF-1 and limitation of ER β quality enunciation are the nitty gritty instruments by which estradiol mediate this effect (48). IGF-1 is an improvement factor which causes increase in uterus by growing the thickness of endometrium and myometrium layers, and the greatness of uterus moreover augments. Mammary organs are moreover center for estrogens and it has been demonstrated that estradiol strengthens augmentation by acting through ER α (49). Augmentation is with the ultimate objective that release is in like manner clear in courses. Number of channels and lobuli also augments confirming the prompting. Along these lines, Formalin fixed uteri and mammary organ tissue can in like manner be thought under histological assessments. Three micrometer portions of the squares are recolored with haematoxylin and eosin. Thickness of epithelium, endometrium and myometrium layers are evaluated in the uteri. In mammary organ number of channels and lobuli are estimated per μm^2 region (50).

Micro-CT analysis

The impacts of medication treatment on the trabecular structures of the six agent femora metaphysis are distinguished utilizing eXplore Locus SP Pre-Clinical Specimen microcomputed tomography (GE Healthcare, USA). The femora are checked from the proximal development plate the distal way (16 $\mu\text{m}/\text{cut}$). This area incorporates 350 pictures acquired from every femur utilizing a 1024 $\times$ 1024 lattice, bringing about an isotropic voxel goals of 22 μm^3 . The volume of intrigue (VOI) was chosen as the district 25 cuts from the development plate at the proximal finish of the femur, going to 125 cuts. The 3D pictures are acquired for perception and show. The accompanying morphometric parameters are estimated by breaking down the VOI: the relative bone volume (BV/TV), trabecular number (Tb.N), trabecular partition (Tb.Sp), trabecular thickness (Tb.Th), availability thickness (Conn.D) and structure model list (SMI). The administrator directing

the sweep investigation ought to be blinded to the medicines related with the examples (20, 41).

Immunohistochemical staining

At the molecular level, the effect of estrogen is achieved by acting on the genomic pathway involving estrogen/estrogen receptor interaction. The molecular mechanism of the protective effect of drugs on bone loss may be estrogen response element (ERE)-dependent and mediated through the activation of estrogen receptors (ERs). Therefore, measurement of ER level in the endometrium after drug treatment could be evaluated. Selected uteri are fixed in 10% formalin for 2 days at room temperature, then tissues are washed, dehydrated in gradient alcohol, embedded in paraffin wax, and cut into serial sagittal sections (4 μm thick) with a microtome. Immunohistochemical localization of ER is done with ER polyclonal antibody according to manufacturer's instructions (35).

Others parameters

Weight gain in ovariectomized rodents has been settled. Estrogen deficiency is believed to be to a great extent liable for an expansion in adiposity during menopause on the grounds that postmenopausal ladies under estrogen substitution treatment don't show the trademark example of stomach weight gain ordinarily connected with menopause (45). This is considered as a reaction of the body to secure long bones against osteopenia. Estrogen like mixes act by applying antilipogenic impact on fat tissue to keep up the body weight (46). Also, estrogen misfortune in ovariectomy may prompted gathering of vitality stores prompting collection of fat (reflected in the ascent of cholesterol, HDL and LDL) and thus weight gain. Uterus is the most delicate organ towards estrogen. Hormone decrease after ovariectomy quickly relapse the organ and subsequently weight reduction. Nourishment utilization, body weight, organ weight (uterus, heart, liver, spleen, lung, kidney, thymus, mind) and organ list spoke to as organ weight isolated by body weight are likewise assessed (41)

Urine analyses

The ovariectomized rodent showed a large portion of the attributes of human postmenopausal osteoporosis (51). The diminished bone mineral substance was seen with ovariectomized rodents and further it was prove by the decrease of absolute debris weight, debris percent and debris calcium content, attesting its job in the counteraction of bone misfortune. What's more, increment in loss of calcium, phosphorous and creatinine through discharge in pee are supporting element for bone misfortune in the ovariectomized rodents (52). Henceforth, pee tests are gathered in metabolic pens without giving nourishment 1 day before euthanizing the creatures and fermented with 2 ml 1 mol/l HCL, at that point the degree

of pee calcium, phosphorus and creatinine could be assessed utilizing programmed or self-loader analyser and utilizing economically accessible test units (45). Urinary discharge of Ca, P and DPD are additionally communicated as the proportion to Cr fixation (Ca/Cr; P/Cr; DPD/Cr) (53).

Estrogen receptor ligand binding assay (ER-LBA)

Antiestoporotic activity of plant extracts are thought to be due to compounds called phytoestrogens which acts as estrogen receptor agonists. Hence Estrogen receptor ligand binding assay can be performed with cytosolic fraction from porcine uteri. This preparation contains ER α , ER β and possibly other ER binding proteins. Based on studies with ER knockout mice and with subtype specific agonists it appears that the osteoprotective effects of estrogens are mediated via the ER α (54), but as cytosol preparation from uteri contain both type of ER, the positive effect produced in this assay may be binding with ER α or both receptor subtypes.

Oxidative Stress

Osteoporosis has been demonstrated to be related with oxidative pressure. Low estrogen levels in postmenopausal ladies have been related with oxidative pressure while physiological degrees of estrogen have been appeared to ensure low thickness lipoprotein against oxidation (55). Moreover, estrogen can likewise be considered as a cell reinforcement as it was found to display cancer prevention agent insurance of lipoproteins in the watery framework and was additionally appeared to build the outflow of glutathione peroxidase in osteoclasts. Nutrient E, a strong enemy of oxidant had the option to secure ovariectomised rodents against osteoporosis. Some in vitro examinations or creature models indicated that oxidative pressure importantly affects the separation and working of osteoclasts. Ovariectomy actuates an expansion in lipid peroxidation and H₂O₂ and an abatement in enzymatic cell reinforcements like SOD and GSH-Px in rodents (56). Thusly, these catalysts could be estimated in the serum by standard strategies.

Behavioral Test

Melancholy and nervousness of menopausal disorder in ladies are clinically improved by estrogen substitution treatment (57). With respect to upper impact of estrogens, it has been proposed that estrogens may improve the capacity of focal adrenergic frameworks through the expansion of noradrenaline content and the decline of monoamine oxidase movement (58). Notwithstanding this upper impact, it has likewise been affirmed that estrogens can improve memory in both sound ladies and female patients with Alzheimer's infection potentially through the incitement of the growing of axons and dendrites of neurons (59). In this way, estrogens are probably going to

emphatically initiate the sensory systems taking an interest in the procedures of learning and memory.

Ovariectomy in mice shows not just a drawn out fixed status time in constrained swimming test yet in addition local skin temperature changes the same a hot-flush side effect in ladies (60). Subsequently, constrained swimming test can be acted in mice after medication treatment by revealed techniques (61). Quickly, mice are put separately into a vertical polycarbonate chamber (40 cm in stature and 15 cm in breadth) containing water at 25°C at a profundity of 15 cm. After 15 min free swimming, the creature is expelled from the water and permitted to dry the body surface for around 15 min in a warmed nook (32°C) before being come back to its home pen. During this 15 min constrained swimming, the mouse shows a condition of stability (gliding latently) a few times. The mouse is somewhat hypothermic (- 30°C) on expulsion from the water, and afterward keeps on being hypoactive for periods up to 30 min. After 24 h, the mice are supplanted in the chamber and the all out span of idleness is estimated during a swimming time of 6 min. The mouse is decided to be stationary at whatever point it stays gliding inactively in water in a somewhat slouched upstanding situation with its head being simply over the water surface.

Neonatal mouse parietal bone resorption assay

Parathyroid hormone (PTH), a polypeptide discharged by the parathyroid organs is answerable for generally little changes in serum Ca²⁺. Together with the nutrient D endocrine framework, calcitonin and a few putative phosphatonic peptides, PTH assumes a significant job in calcium homeostasis (62). Persistent introduction of PTH causes a calcium discharge from bone in vitro transcendently because of incitement of osteoclast separation and movement by means of consequences for osteoblast cytokine creation (63). Subsequently, Evaluation of hostile to bone resorption action can be controlled by the revealed technique (64). Quickly, 2-day-old mice were infused subcutaneously with 45CaCl₂ (2 μ Ci). After two days, the parietal bones were taken out. The bones were haphazardly doled out to ordinary and treated gatherings and each gathering contains seven bones. The bones were refined in 24-well plates on the treated steel frameworks on the highest point of glass rings to help the bones close to the gas–fluid interface in a Ham's F-12 medium containing 5% (v/v) heat-inactivated horse serum. Subsequent to preculturing for 24 h, the medium was evacuated and new medium containing PTH (2 nM) and medication was included, at that point the bones were hatched for 144 h. After 72 h, the medium was changed with new medium. In the wake of completing the way of life, bones were placed into 0.01M EDTA support arrangement (pH 5.5) to tranquilize 45Ca contained in bones. 45Ca discharged into the medium at 72 and 144 h

and the EDTA arrangement were countered independently. Bone resorption was evaluated as the level of ^{45}Ca that discharged into the medium to the absolute ^{45}Ca . All societies were kept in a hatchery under sodden state of 5% CO_2 in air at 37°C

Evaluation of estrogenic activity

Estrogenic activity was evaluated using vaginal cytology. Weekly vaginal smears were taken using an eye dropper containing saline, placed on slides and observed under a light microscope after staining with hematoxylin–eosin in order to monitor cellular differentiation. Cells were identified as either leukocytes (indicating a diestrous stage), or nucleated or cornified epithelial cells (indicating an estrous stage) (29).

3. Discussion and conclusion

Ovariectomized creature model, most much of the time utilized technique for antiosteoporotic movement screening, causes sensational reduction in body weight, organ weight (fundamentally uterus), BMD, biochemical quality, and bone quality, and these progressions are to some extent because of estrogen lack. Biochemical parameters related straightforwardly or in a roundabout way with bone digestion, pee science and bone histopathology are additionally talked about. Some in vitro parameters as Osteoblastic multiplication, Osteoblastic antacid phosphatase action and Osteoclastic tartrate-safe basic phosphatase action are additionally utilized for assessment of antiosteoporotic capability of medications (65). A portion of the ongoing distributions of restorative plants and the different parameters decided for assessment of antiosteoporetic action in ovariectomized creature model are portrayed beneath. This audit is prepared reference on the parameters utilized for antiosteoporotic screening in menopausal osteoporosis

Shirwaikar et al., in 2003 considered three point bowing of tibia, pressure of IV lumbar vertebra, stacking trial of femoral neck in ethanolic concentrate of *Cissus quadrangularis* (Vitaceae). For this examination entire plant was utilized. Term of study was 03 months. It was acted in femur area in ovariectomized creature model Biomechanical parameters examined were serum calcium, serum ALP, serum TRAP (16).

Dontas et al., in 2006 studied Tibia- bone area, bone volume per tissue volume, bone perimeter, absolute number of osteoblast, ratio of the absolute number of active cuboid osteoblasts per bone perimeter, absolute number of osteoblast, ratio of the absolute number of osteoclasts per osteoclast perimeter, trabecular thickness, trabecular separation and trabecular number in methanolic extract of *Onobrychis ebenoids* (Leguminosae).

For this study whole plant was used. Duration of study was 06 months. This study was performed in ovariectomized animal model and parameters evaluated were Bone mineral density: proximal tibia and total tibia. Others: body weight, uterine weight and tibia length (26).

Kapur et al., in 2008 studied in ethanolic extract of stem of *Tinospora cordifolia* (Menispermaceae) for 04 weeks. Parameters were evaluated Estrogen receptor ligand binding assay Bone mineral density: tibia Biochemical parameters: serum osteocalcin, serum cross-laps, ALP, lipid profile (total cholesterol, HDL, LDL). Histopathology: uteri and mammary gland Others: body weight, uterine weight in ovariectomized animal model (34).

Cao et al., in 2008 studied ethanolic extracts of rhizomes of *Curculigo orchoides* (Amaryllidaceae) for 12 weeks. Bone mineral density: tibia Bone mineral content: tibia Biochemical parameters: serum calcium, phosphorus, ALP, TRAP, creatinine, ACTH, corticosterone, IL-6, TNF α , osteoprotegerin, deoxypyridinoline Others: body weight, uterine weight parameters were evaluated in animal model (66)

Mori-Okamoto et al., in 2004 studied Seed and juice extract of *Punica granatum* (Punicaceae) for 2 weeks in animal model. Behavioral test, Bone mineral density: tibia, Histopathology: tibia- total bone volume per tissue volume, mineralized bone volume per tissue volume, trabecular thickness, trabecular number, trabecular separation, osteoid volume per bone volume, osteoid surface per bone surface, osteoblast surface per bone surface, eroded surface per bone surface, number of osteoclast per bone perimeter, osteoclast surface per bone surface, Others: body weight, uterus weight were evaluated (47)

Zhang et al., in 2006 studied ethanolic extracts of roots of *Lepidium meyenii* (Brassicaceae) for 28 weeks in animal model. Femur physical parameter: weight, length, external diameter, bone volume Bone mineral density: femur and lumbar vertebra (LV1-LV6), Biomechanical properties of femur: three point bending test for properties as max-load, max-stress, elasticity, energy, max-stroke, max-strain. Biochemical parameters: serum calcium, phosphorus, ALP, osteocalcin was performed in animal model. Urine analysis: calcium, phosphorus. Histopathology: lumbar vertebra (LV2). Others: femur ash weight and calcium content, organs weight (liver, spleen, suprarenal gland, uterus) (69).

Zhang et al., in 2007 studied ethanolic extract of Stem bark of *Erythrina variegata* (Leguminosae) for 14 weeks in animal model. Serum chemistry: calcium, phosphorus, osteocalcin, alkaline phosphatase Urine chemistry: calcium, phosphorus, deoxypyridinoline Biomechanical parameters: femur- three point bending test (stiffness and

energy). Histopathology: tibia- cancellous bone in the proximal tibial metaphysis, trabecular thickness, percentage of trabecular area. Others: body and uterine weight were performed (20).

He et al., in 2010 studied in Hexane, ethyl acetate, n-butanol and water fractions of methanolic extract of roots of *Achyranthes bidentata* (Amaranthaceae) for 6 weeks in animal model. Neonatal mouse parietal bone resorption assay Serum parameters: estradiol, total cholesterol, triglyceride Bone mineral density: lumbar spine (L 2-4) Others: body weight, uterine weight (67).

Shuid et al., in 2011 studied in roots of *Labisia pumila* (Myrsinaceae) for 8 weeks. The biochemical parameters which were studied is as follows: serum osteocalcin, C-terminal telopeptide of type 1 collagen. Bone weight and calcium content: fifth lumbar vertebra. Others: body weight (68)

Yogesh et al., in 2011 studied Aqueous-methanol extract of stem bark of *Berberis aristata* (Berberidaceae) for 42 days in animal model and parameters which were measured were Femur physical parameter: weight, length, volume and density. Lumbar compression test: fourth lumbar vertebra. Biochemical parameters: serum calcium, inorganic phosphorus, alkaline phosphatase. Urine analysis: calcium, inorganic phosphorus, creatinine. Histopathology: femur-bone architecture and compactness Others: body weight, uterine weight; femoral ash weight, ash percentage and ash calcium (45).

Li et al., in 2013 studied Echinacoside of *Cistanche tubulosa* (Orobanchaceae) for 12 weeks in animal models. Parameter were analysed are as follows: Urine analysis: calcium, phosphorus, hydroxyproline. Serum analysis: calcium, phosphorus, hydroxyproline, estradiol. Bone mineral density: femur Micro-CT analysis: trabecular structure of femur metaphysis- relative bone volume, trabecular number, trabecular separation, trabecular thickness, connectivity density, structure model index Biomechanical evaluation: three point bending test of tibia- maximum load, extrinsic stiffness, energy to ultimate load. Histopathology: femur- cortical bone thickness, trabecular bone thickness, number of trabecular, number of osteoblast and number of osteoclast Immunohistochemical staining: evaluation of estrogen receptor expression. Others: body weight and uterus weight (35).

Li et al., in 2009 studied ethanolic extracts of roots of *Morinda officinalis* (Rubiaceae) for 12 weeks in animal models. Bone mineral content parameters were evaluated like total bone content, trabecular bone content, cortical bone content. Bone mineral density parameters were evaluated like total BMD, trabecular BMD, cortical BMD. At last, biochemical parameters were evaluated like serum

calcium, phosphorus, alkaline phosphatase, tartrate-resistant alkaline phosphatase, creatinine, ACTH, corticosterone, IL-6, TNF α , DPD, osteoprotegerin (OPG).

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