Original Research Paper

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TO EVALUATE OSTEOBLASTIC ACTIVITY OF JAPAKUSUMA IN WISTER ALBINO RATS

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ABSTRACT Bone homoeostasis is a dynamic equilibrium, regulates by multiples but coordinated cellular molecular and hormonal events .Osteoblast and osteoclast are the two types of cells that are majorly responsible for bone metabolism. The calcium plays an important role ln close cooperation between osteoblast and osteoclast cells and its metabolism[1,2].Bone metabolism and control growth rest on multifaceted signalling trails to achieve proper rates of growth, differentiation and these factors include the action of steroids mainly estrogen. The major physiological effect of estrogen is to inhibit bone resorption and helps in not just remodelling but plays an important role in growth and maturation which is an active continual process3. Hence the present study has been planned to assure to evaluate the osteoblastic activity of japakusuama an oral formulation in Wister Albino rats . The test formulation was administered to animals for 21 consecutive days. Histology parameter of bone carried out. The osteoblastic activity of japakusuma was found to be responsible for plays significant role bone formation or metabolism.

KEYWORDS : Japakusuma, Osteoblastic activity.

INTRODUCTION

Osteoblasts and osteocytes are involved in the formation and mineralization of bone; osteoclasts are involved in the resorption of bone tissue. . The osteoblast creates and repairs new bone by actually building around itself. Osteoblasts and osteocytes are derived from osteoprogenitor cells.[4,5]

The major physiological effect of estrogen is to inhibit bone resorption. Osteoclast apoptosis is regulated by estrogens. Therefore, there is considerable interest in unraveling the pathways by which estrogen exerts its protective effects on bone. While the major consequence of the loss of estrogen is an increase in bone resorption, estrogen deficiency is associated with a gap between bone resorption and formation, indicating that estrogen is also important for maintaining bone formation at the cellular level[6].

MATERIALS AND METHODS

DRUG SAMPLE

Kanjibhavit japakuausma consist of japakusuma flower , purana guda and kanji. 70g of japakusuma flower was added with equal amount of purana guda and made into kalka form. 20 ml kanji was taken to which 400 mg of above kalka dravya was added and bhavana was done. Dose was fixed according to the body weight and was administered orally. Kanji bhavit japakusuma kalka taken as test drug to be given internally

ANIMALS

Wistar strain albino female rats weighing between 150-250g were used for experimental study with following conditions.

The animals were obtained from the animal house attached to the pharmacology laboratory, SDM centre for research in ayurveda and allied science. Udyavara.

They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature, humidity. They were fed with saidurga feeds, bengalooru rat pellets and tap water ad libitum. The experiments would be carried out in conformity with guidelines of the institutional animal ethical committee (IAEC) after obtaining its permission. Ethical CLEARENCE NO. SDMCRA/IAEC/UD-PT-16 ON 29/5/2017.

Animal grouping: the selected animal grouped into five groups with 6 animals each. Group A, only water and normal diet administered . Whereas group B ovariectomized control group only water and normal diet administered and served as positive control group.

In group C ovariectomized animals treated with kanji bhavita japakusuma at therapeutic dose. In group E normal rats treated with kanji bhavita japakusuma at therapeutic dose. The group D ovariectomized animals treated with standard drug.

Animal grouping

Group	No . Of rats	Drugs
A	6	Normal control
B	6	Positive control (oophorectomized)
C	6	Test drug + (oophorectomized)
D	6	Standard drug (Norethisterone)
E	6	Test drug

Dose calculation: the dose determination of test drug will be carried out based on acute oral toxicity report AOT will be carried out following OECD GUIDELINES 425

TEST DRUG: kanji bhavit japakusuma kalka taken as test drug to be given internally. Acute oral toxicity study

The test drug kanjibhavit japakusuma was screened for acute toxicity to OECD425 GUIDELINES.

Drug administration

Direct administration of the drug was done without any vehicle as the formulation was liquid . Drugs were administrated by oral route by the help of feeding tube with injection syringe and duration for 21 days .

OBSERVATION PARAMETERS

(I) Histopathology : bone

RESULT

Ovariectomised control group

Rat no. and section	Changes observed	Remarks
1	Necrosed in 1 area. Thin trabeculae. Trabaculae discontinuity in 1 area. More inter trabecular space.	Severe osteoporotic activity
2	Very less trabeculae. Hemorrhage seen. Discontinuity of trabeculae.	Moderate osteoporotic activity

3	Thick trabeculae.	No osteoporotic activity
4	Less trabeculae. Discontinuity of trabeculae seen.	Moderate osteoporotic activity
5	Slight necrosis seen	Moderate osteoporotic activity

Test drug ovariectomised group

Rat no and section	Changes observed	Remarks
1	Increased trabeculae. More thickened cortex. Reduction in trabecular space. More hematopoietic cells	Reduction in osteoporotic activity
2	Increase in cartilage. Increase in trabeculae. Hypercellularity. Decreased intertrabacular space. More osteoblast rimming seen. Mild discontinuity in trabeculae.	Reduction in osteoporotic activity with osteoblastic activity
3	Increase in trabeculae. More hematopoietic cells. Decreased intertrabecular space	Reduction in osteoporotic activity
4	Increase in trabeculae. More hematopoietic cells. Decreased intertrabacular space. Mild discontinuity .hemorrhage	Mild osteoporotic activity
5	Thinner trabeculae with increased intertrabecular space. Hemorrhage. More hematopoietic cells. Increased osteoblastic cells	Moderate osteoporotic activity with osteoblastic bone formation.
7	Thickened trabeculae with decreased intertrabecular space. Increased osteoblasts. More hematopoietic cells.	reduced osteoporotic activity with osteoblastic activity

Standard group

Rat no and section	Changes observed	Remarks
1	No discontinuity in trabeculae. Reduction in trabecular space. Abundance of osteoblasts	Reduction in osteoporotic activity with osteoblastic bone formation.
2	Discontinuity in trabeculae with increase in intertrabecular space. Osteoblastic rimming seen	Moderate osteoporotic activity with osteoblastic bone formation.
3	Slightly thinner trabeculae with more intertrabacular space. Hypercellular. No discontinuity between trabeculae. Osteoblasts seen	Mild osteoporotic activity with bone formation.
4	Thinner trabeculae in some areas. Decreased intertrabacular space.	Mild osteoporotic activity
5	Thickened compact bone. Discontinuity of trabeculae seen more hematopoietic cells. Increased osteoblastic cells.	Mild osteoporotic activity with osteoblastic bone formation.

Test drug

Rat no and section	Changes observed	Remarks
1	Thickened trabeculae. Reduction in intertrabecular space. Mild discontinuity with hemorrhage seen	Mild osteoporotic activity
2	Discontinuity in trabeculae with increase in intertrabecular space. Less no of trabeculae. Osteoblastic rimming seen	Moderate osteoporotic activity with osteoblastic bone
3	Less no of trabeculae. Mild discontinuity with decreased intertrabecular distance	Mild osteoporotic activity.

DISCUSSION –

The role of phytoestrogens which are present in japakuauma ,like flavonoids exert their effect primarily binding to the estrogen receptor and reported for their estrogenic activity [7].

In ovariectomised control group, necrosis seen in few sections and more adipocytes. Thin trabeculae, increased intertrabecular space and discontinuity in trabeculae seen. Moderate to severe osteoporotic activity seen. in test drug ovariectomised group, most of the sections showed thickened trabeculae, reduced intertrabecular space without discontinuity. More hematopoietic cells were seen. Overall there was reduced osteoporosis compared to ovariectomised control. Osteoblastic rimming was seen in many sections suggesting bone formation.

In test drug , few animals showed thin trabeculae, increased intertrabecular space and discontinuity between trabeculae. Mild to moderate osteoporosis was seen in most of the sections. The osteoporotic activity reduced compared to ovariectomised control.

In standard group, few animals showed thin trabeculae, increased intertrabecular space and discontinuity between trabeculae. Mild to moderate osteoporosis was seen in most of the sections. The osteoporotic activity is reduced compared to ovariectomised control but more compared to test drug ovariectomised control.

CONCLUSION

The japakusuma formulation may play a significant role in bone formation or metabolism by improving osteoblastic activity to exhibit multifaceted therapeutic effects. In present study osteoblastic rimming was seen in many sections suggesting bone formation with the help of estrogen , has an osteoporotic activity through stimulating osteoblastic activity.8,9 Japakusuma can be used as herbal drug for osteoblastic activity which is needed for further the improvement of several parameter .


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APPROVAL CERTIFICATE

This is to certify that the project title "An experimental evaluation of estrogenic activity of Jepakusuma/ Hibiscus Rosasinensis" submitted by Dr. Neetika Gautam Dept. Prasuti Tantra has been approved (Approval No: SDMCRA/IAEC/ UG-PT-14) by the IAEC in its meeting held on 25/05/17

(Chairman IAEC) (CPCSEA Nominee)

HISTO-PATHOLOGY -BONE

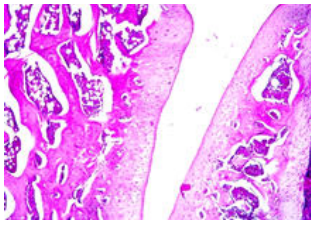


FIG.1 NORMAL CONTROL GROUP R1

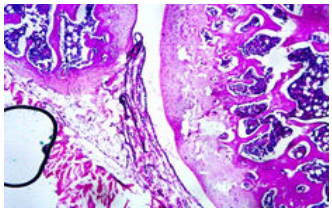


FIG. 2 NORMAL CONTROL GROUP R 2

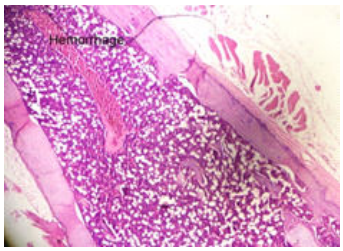


FIG.3 TEST DRUG OVARIECTOMIZED GROUP,R1

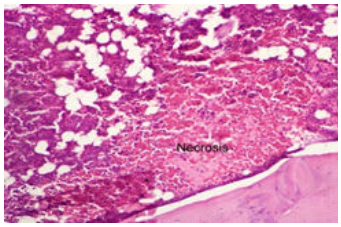


FIG.4 TEST DRUG OVARIECTOMIZED GROUP,R1

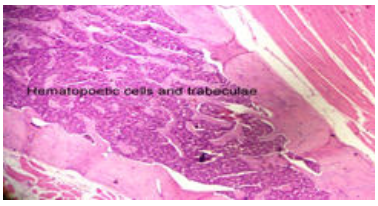


FIG.5 POSITIVE CONTROL GROUP,R1-

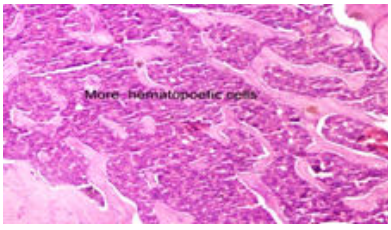


FIG.6 POSITIVE CONTROL GROUP, R2

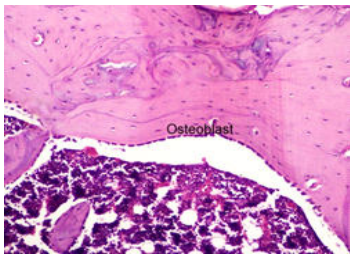


FIG.7 TEST DRUG OVARIECTOMIZED

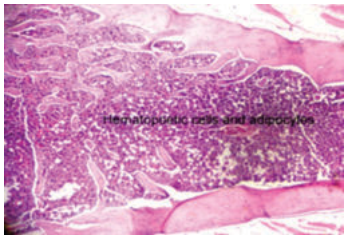


FIG.8 TEST DRUG OVARIECTOMIZED R2

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