

## Study of Histological Changes in the Bones of Front and Hind Limbs of White Rat Treated with Ibuprofen and *Lepidium Sativum*

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### Abstract

*The current study was conducted to evaluate the morphological and histological changes in the front and hind limbs of Male albino rat belonging to the strain Sprague Dawley, treated with Ibuprofen and the possible protective effects of aqueous extract of *Lepidium sativum*. The study was performed in the animal house of Department of Biology Collage of Education for Girls University of Kufa. The study lasted seven months from September 2020 until February 2021 by following (80) male rats and were (16-20) weeks that weighted between (200-250)g which was divided in to eight groups each group consist of ten males. The first group was the orally given the normal saline only, and it was control group. The second group was treated orally with Ibuprofen at concentration of (400) mg/kg while the third group was administered Ibuprofen at concentration (400)mg/kg+ aqueous extract of *Lepidium sativum* seeds with concentration of (50) mg/kg. The fourth group was dosage Ibuprofen at (400)mg/kg+ extract of *Lepidium sativum* seeds with concentration (100)mg/kg orally too. The fifth group was treated with Ibuprofen at concentration (400)mg/kg +aqueous extract of *Lepidium sativum* at concentration (150) mg/kg orally. The sixth group was orally given the aqueous extract of *Lepidium sativum* seeds with concentration (50)mg/kg. The seventh group was treated with the aqueous extract of *Lepidium sativum* seeds with concentration (100)mg/kg orally. The last group was submitted to the aqueous extract of *Lepidium sativum* seeds with concentration (150)mg/kg by oral all groups were conducted once day from the first day until the sacrifice which was in two stages on (30 and 45)days. The current study included the process of recording the animal weights of body before and after the completion of experiment as well as weights of bones for front and hind limbs after the end of dose period The study included the histolgical sections of the limbs bones. It was absorbed through the macroscopic examination of males dosed with Ibuprofen drug at concentration (400)mg/kg lack of movement and ,anorexia and sluggishness during a period 45 days. The results of statistical analysis showed significant decrease ( $P<0.05$ ) in the body weight and the weight of limbs bones and the in treatment that were treated with Ibuprofen (30) and (45) days compared with control group. The results also showed decreased significant differences in body weight and weight of limbs in the groups were treated with Ibuprofen + water extract of lepidum sativum seeds (50)mg/kg also the result showed decrease ( $p<0.05$ ) in body weight in the group of Ibuprofen + water extract of lepidum sativum seeds at (100)mg/kg in,*

*the first dose 30 days, while there was no significant in the same group for the second dose(45) days. As for the group that was dose orally the aqueous extract of Lepidium Sativum seeds (50,100,150) mg/kg and for first and second dose showed significant increase ()in wight body and bones limb but no significant in length body and length of bones. Also pathological changes were observed in the histolglcal section of bone that dosed ibuprofen such as cellular changes in osteocytes, the lacuna looked empty without osteocyt and effected of cell components and cell life. The ather histolglcal change was show in the bone marrow that decrease in blood cells components with hypoplasia and replacement of heamatopia with adipose content in the marrow cavity. There was no histopatholglcal changes in the groups that treated with Ibuprofen +water extract of lipidium sativum seeds (50,100,150)Mg/kg compared with control group. As well as the group that was treated with water extract of Lepidium sativum showed cellular activity. From this study, it was concluded that Ibuprofen drug has toxic effects in the osteocytes that was affected in the bone tissue and bone formation for long term while the concentration aqueous extract of Lepidium sativum seeds has prevented the uncertain effects of drug. This stuy showed the important role of lepidium sativum as food supplements rich with vitamins and minerals on growth of bones.*

**Key-words:** Sativum, Seeds, Histopatholglcal, Lepidium.

## **1. Introduction**

The moving part of the skeleton ,which includes the upper and lower limbs with its girdles ,is known as the appendicular terminal structure which is derived from the Latin word appendix as it represents the prominent part of vertebrates that share the skeleton, which in turn provides structure and facilitates movement (1). The appendicular terminal skeleton is the second major group in the skeleton after the axial skeleton, which is attributed to the path of appendages represented by the limbs attached to a of joints and ligaments that allow movement and more movement than in the axial structure (2) The complex structure of the appendicular skeletal system has contributed to its participation in most of the vital functions, as the 126 bones in this type of structure play an important role in making all mechanisms work properly, taking into account the diversity of life patterns used by mammals among other groups despite the slight difference in The number of bones where coordination and structure remain following one basic plan for the same goal which is support and backing (3)The critical role of bones, which are the main component of the peripheral skeletal system, is evident in providing the skeletal support as a home for blood cell formation by providing an environment for blood formation within the marrow spaces (4) and a reservoir for minerals such as phosphate and calcium in addition to the mechanical role that gives regularity on a wider scale in generating movement It is possible for anyone to use NSAIDs as a pain reliever or reduce inflammation, among the drugs of this group is Ibuprofen which the most commonly used drugs, and the action of these antibiotics is evident through their effect on the prostaglandin and thus inhibition

COX enzyme(5), The activity of ibuprofen was discovered by (Stuart Adams and colleagues), at the end of the 1960s Ibuprofen is one of the most common drugs of this group as it has a prominent role in relieving pain that ranges from mild to moderate (6). Although Ibu has a short history, it has a popular effect and success in skeletal pain, bone healing after orthopedic surgery and Rheumatoid arthritis (7) NSAIDs drugs, including ibuprofen, are often taken without medical supervision, being available without a prescription and thus may exceed the recommended dose, as it was found that 15% of adult ibuprofen users exceed the required dose does not prevent its excessive and protracted use(8), as the researchers recommended the use of the lowest dose and the shortest duration in cases of people with COVID-19, as its excessive use may lead to a more d use may lead to a more dangerous path.(9)

Medicinal plants were and still are herbal medicines commonly used as an alternative to chemical medicines as of the important sources of active ingredients with curative (10) and preventive effects that have taken up a large space in our daily life with the alternative in the uses of these medicinal plants (11).

Recently, studies specializing in medicinal plants have appeared after their medicinal status was revealed, as the World Health Organization (WHO) has waged wide attention to the adoption of plant-based medicines as a replacement for chemical drugs.

The cress plant *Lepidium sativum* or locally known with Hab el-Rashad, which is also called the garden cress, is included among the common herbal medicines and belongs to the cruciferous family and is characterized by being an annual herbal plant with many leaves that contains many effective compounds used in medicinal treatments, as the cress plant represented by the roots, leaves and seeds used the treatment of diseases and in controlling various medical problems, including asthma, high blood pressure and kidney diseases, in addition to its role as a good mediator in the field of bone fractures, in addition to the widespread adoption of the *Lepidium* plant as an object in examining levels of toxicity and environmental pollutants in experimental studies that assess the ca of various diseases(12)(13).

## **2. Material and Methods**

### **Preparation of Laboratory Animals**

The use of male white rat animals of the type (Sprague Dawley) by 80 rats with an age (16-20) weeks and with an average weight of (200-250) g. They were transferred from the College of Science / University of Kufa and left for acclimatization in the animal house of the College of

Education for Girls before the start of the study after housing them in cages good ventilation and access to feed and water throughout the experiment, taking into account the temperature degrees between (22-28) C.

### **Medicinal Doses**

The body weight of the laboratory animal used in the study was used in determining the drug according to the pharmacokinetic constitutions and the dose of ibuprofen that was determined in the current study was 400 mg / kg(13) Note that the half lethal dose (Lethal dose) is considered a safe dose of up to 1600 mg / kg according to (14).

### **Preparation Aqueous Extract of LS**

The seeds of *Lepidium Sativum* used in the current study were obtained from the local market of the city of Kufa. The seeds were manually sifted to remove the bad seeds. They were dried and milled in preparation for preparing the aqueous extract. The method included taking (2) g of seed powder and adding 200 mg/kg of distilled water and then leaving it for 24 hours then the mixture was boiled for 10 minute, the extract was left to cool then filtrated by gauze (15).

### **Groups of Study**

The use of 80 male white rat, which was divided into eight groups.

**Group No. (1):** Included 10 male white rats, that were dosed orally with distilled water from the first day of the beginning of the experiment until the end of the experiment, which were sacrificed during periods of 30 days and 45 days and approved as a control group

**Group No. (2):** It included 10 white males that were dosed orally with ibuprofen at a concentration 400mg / kg from the first day of the beginning of the experiment until the end of the experiment, which were sacrificed during two periods of 30 day and 45 days.

**Group No. (3):** It included 10 white males that were dosed orally with ibuprofen at a concentration of 400 mg / kg plus a aqueous extract of LS seeds at a concentration of 50 mg / kg from the first day of the beginning of the experiment until the end of the experiment, which were sacrificed during two periods of 30 day and 45.

**Group No. (4):** It included 10 white males that were dosed orally with ibuprofen at a concentration of 400 mg / kg plus aqueous extract of LS seeds at a concentration of 100 mg / kg from

the first day of the beginning of the experiment until the end of the experiment, which were sacrificed during two periods of 30 day and 45.

**Group No (5):** Included 10 white rat males who were verbally ingested with ibuprofen , which is aqueous extract with LS seeds and a concentration of 150ml/kg from the first day of the beginning of the experiment until the end of the experiment ,which was sacrificed during the periods of 30 days.

**Group No. (6):** Included 10 white rat males that were dosed orally with cold aqueous extract of LS seeds at a concentration of 50 mg / kg from the first day of the beginning of the experiment until the end of the experiment, which were sacrificed during periods of 30 days and 45 days.

**Group No. (7):** Included 10 males of the White Rat, that were dosed orally with aqueous extract of LS seeds at a concentration of 100 mg / kg from the first day of the beginning of the experiment until the end of the experiment, which were sacrificed during periods of 30 days and 45 days.

**Group No (8):** It included 10 white rat males that were orally ingested with the aqueous extract of LS seeds and focused on 150 mg/kg from the first day of the beginning of the experiment until the end of the experiment ,which was sacrificed during the 30-day and 45-day periods.

### **Preparation of the Terminal Structure**

After the autopsy process that followed the euthanasia for the current study animals, then extracting the terminal structure represented by the front and hind limbs Then it was placed in a physiological solution until the cleaning process began and the limbs were stripped of the muscles (16); and the adjacent soft tissues, then the limbs and girdles were immersed in sodium hydroxide (3%) for 24 hours in order to remove the soft tissues and muscles surrounding the limbs using. Vernia and sensitive balance and for the purpose of preparing the structures for the morphological study that includes measuring the weight of each bone, and then preparing the histological sections for the current study.

### **The Histological Study**

A histological study that sheds light on the tissue components of bone such as cells and bone marrow or diagnosing tumors and infections requires the use of decalcified sections (17), where a clear procedure is adopted in tissue technique as strong acids such as Nitric acid are the fastest in

making tissue sections of bone. Decalcified with accurate identification of the end point. in the current study the samples were prepared for the decalcification process which took place by immersing the bones in nitric acid at a concentration of (10%) with continuous monitoring to reach the required end of its treatment wherein the bone samples were preserved in formaldehyde in preparation the required end of its treatment was performed a series of steps, depending on the method (18).

### **The Statistical Analysis of Study**

The data of the current study was performed by using the SPSS program by testing contrast analysis and extracting LSD value either graphs has been made by Microsoft Excel program and data has been compared to a more than 0.05(19).

### **3. Results**

#### **The Effect of Treatment with Ibuprofen and the Aqueous Extract LS on the Weights of Body for Periods of 30 and 45 Days**

The result of current study according to the data was significant ( $p < 0.05$ ) in the relation weight of body that orally administrated of ibuprofen (400)mg\ kg and the group that dosed the ibuprofen + aqueous extract of LS at (50)mg kg for (30) and (45)day when compared with others group; Otherwise, the group treated with ibuprofen plus the aqueous extract of LS seeds at a concentration of 150 mg/kg showed a moral gain in weight at the level ( $P < 0.05$ ) compared to the control group and other groups and this Applies to the rest of the remaining trial transactions treated with the aqueous extract of (LS) seeds (150,100.50) mg/kg during the study periods when compared with the control group and with other groups. As in the figure (1).

#### **The Effect of Treatment with Ibuprofen and the Aqueous Extract of LS Seeds on the Weights in the Front and Hind Limbs for Periods of 30 and 45 Days**

A significant decrease was observed at ( $P < 0.05$ ) in the weights of the front and hind limbs during the 30- and 45-day dosing periods for the treatment of ibuprofen as in Figure (2) and Figure (3). The front limbs did not show significant differences for the drug treatments, plus the aqueous extract of LS at a concentration of 150,100 mg / kg for two periods 45,30 days.

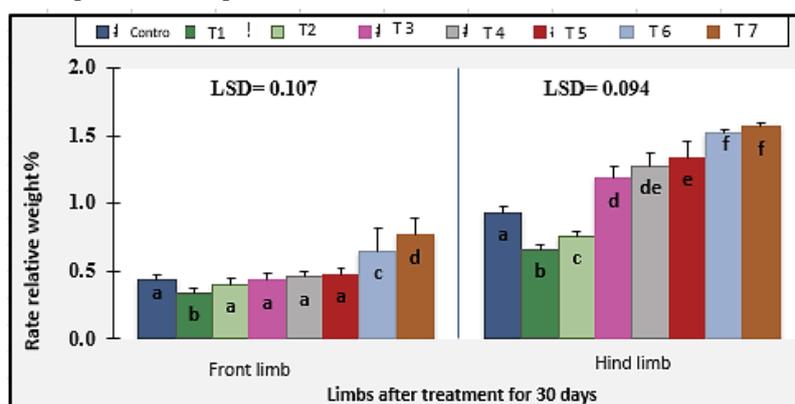
The results of Figure (2) and (3) showed a significant increase in the weights of the front and hind limbs during the two dosing periods in the current study of the aqueous extract of LS seeds at concentrations of (150,100.50) mg / kg.

Fig. 1- Effect of Ibuprofen and Aqueous Extract of LS Seed on the Body Weight for 30 and 45 Day



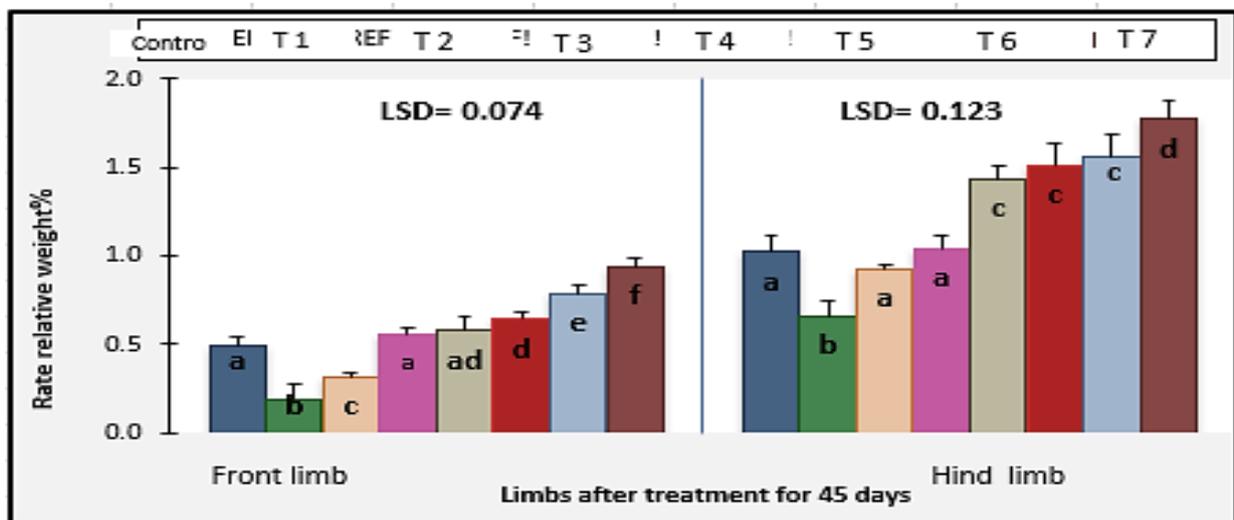
- Number of animals (10) animals per treatment.
- Control dosed with distilled water.
- T1: ibuprofen at 400 mg/kg.
- T2: ibuprofen at 400 mg/kg + aqueous extract of LS seeds at of 50ml/kg.
- T3: ibuprofen at a concentration of 400 mg/kg +aqueous LS seeds at 100ml/kg.
- T4: ibuprofen at 400 mg/kg + aqueous extract for the seeds of LS 150ml/kg.
- T5: aqueous extract LS seeds at 50 mg/kg.
- T6: aqueous extract LS seeds at 100ml/kg.
- T7: aqueous extract seeds at of 150 mg/kg.

Fig. 2- Effect of Ibuprofen and Aqueous Extract of LS Seed on the Front and Hind Limbs for 30 Days



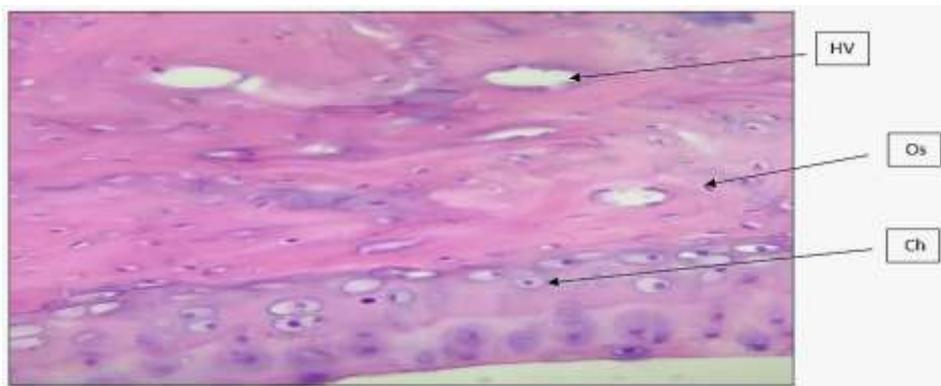
- Number of animals (10) animals per treatment.
- Control: dosed with distilled water.
- T1: ibuprofen at 400 mg/kg.
- T2: ibuprofen at 400 mg/kg + aqueous extract of LS seeds at 50ml/kg.
- T3: ibuprofen at a concentration of 400 mg/kg +aqueous LS seeds at 100ml/kg.
- T4: ibuprofen at 400 mg/kg + aqueous extract for the seeds at LS 150ml/kg.
- T5: aqueous extract LS seeds at 50 mg/kg.
- T6: aqueous extract LS seeds at 100ml/kg.
- T7: aqueous extract seeds at 150 mg/kg.

Fig. 3- Effect of Ibuprofen and Aqueous Extract of LS Seed on the Front and Hind Limbs for 45 Days



- Number of animals (10) animals per treatment.
- Control: dosed with distilled water.
- T1: ibuprofen at 400 mg/kg.
- T2: ibuprofen at 400 mg/kg + aqueous extract of LS seeds at 50ml/kg.
- T3: ibuprofen at a concentration of 400 mg/kg +aqueous LS seeds at 100ml/kg.
- T4: ibuprofen at 400 mg/kg + aqueous extract for the seeds of LS at 150ml/kg.
- T5: aqueous extract LS seeds at 50 mg/kg.
- T6: aqueous extract LS seeds at 100ml/kg.
- T7: aqueous extract seeds at 150 mg/kg.

Figure 4- Section in the Declassified Tibia Bone of the Control Group for 30 Days and Shows the Lamellar Bone in which the Lamellae Around the Havris Canal (Hv) havris canal (Os): Osteocyte Inside the Lacuna (Ch) Chondrocyte Inside the Cartilage Lacuna and appear as Cellular Nests Staining Hematoxylin-eosin (400x)



**Different Letters Moral differences between Transactions below the Probability Level of less tha05**

Figure 5- Section in the Declassified Humerus Bone of Ibuprofen Group for 30 days in which the Cortical Bone Appears, (Hv) Havris Canal (P): Periosteum (At): the Adipose Tissue (M): Bone Marrow Staining: Hematoxlin-eosin100x

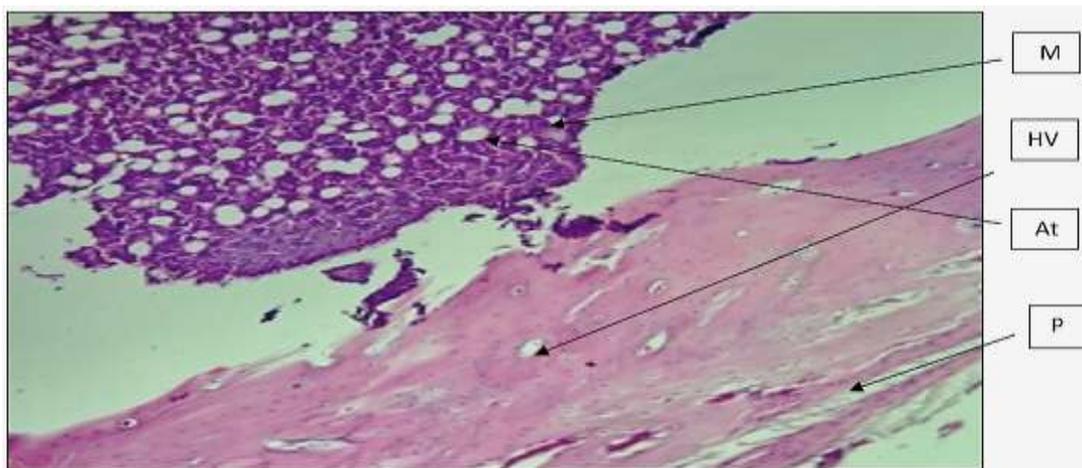


Figure 6- Section in the Declassified Humerus Bone of the Ibuprofen and Aqueous extract of LS at 50 mg \kg Group after 45 Days shows the Natural Tissue Composition of the Bone and Appears(Os): Osteocyte with Dark Nuclei(L)Lacuna (M) Bone Marrow with Active Cellular Content Staining: Hematoxylin-eosin(400x)

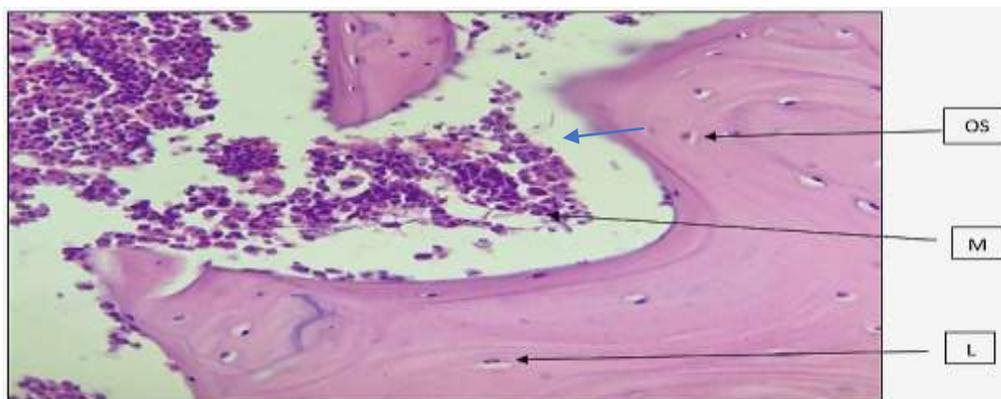


Figure 7- Section in the Declassified Foot Bone of Ibuprofen Group for 30 Days shows:(Tb): Trabecular Bone (Bm): Marrow of the Bone between the Bone Trabecula ,the Cortical Bone with a Natural Content of Bone Cells between the Matrix of the Bone (ch): Chondrocyte Appears Active Staining: Hematoxylin-eosin(100x)

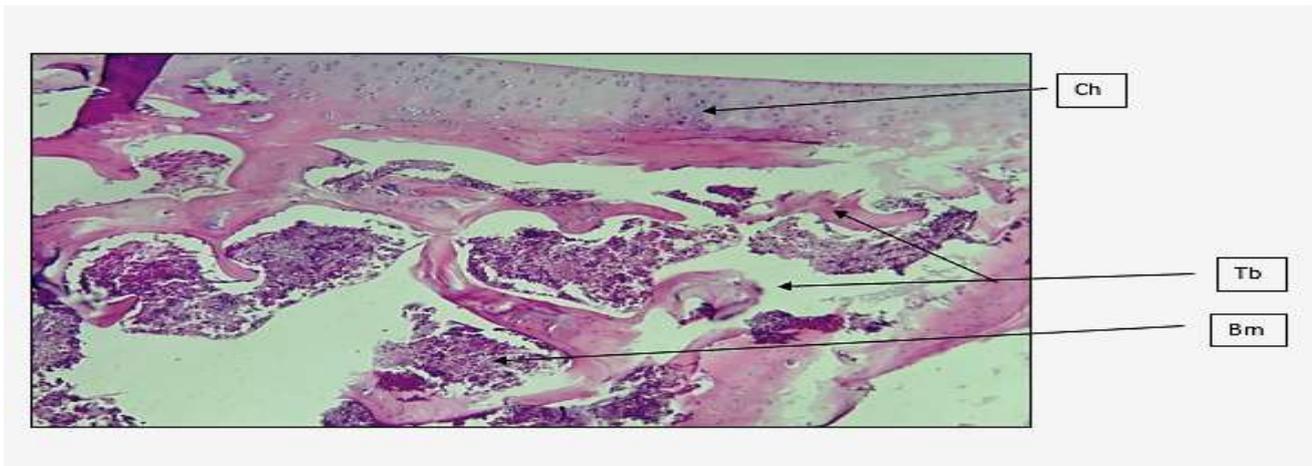


Figure 8- Section in the Declassified Femur Bone of Aqueous Extract LS at 150 mg \kg Group after 30 Days shows Bone Marrow with Hematological Content Showing(Wb): Blood Cells Staining: Hematoxylin-eosin (400x)

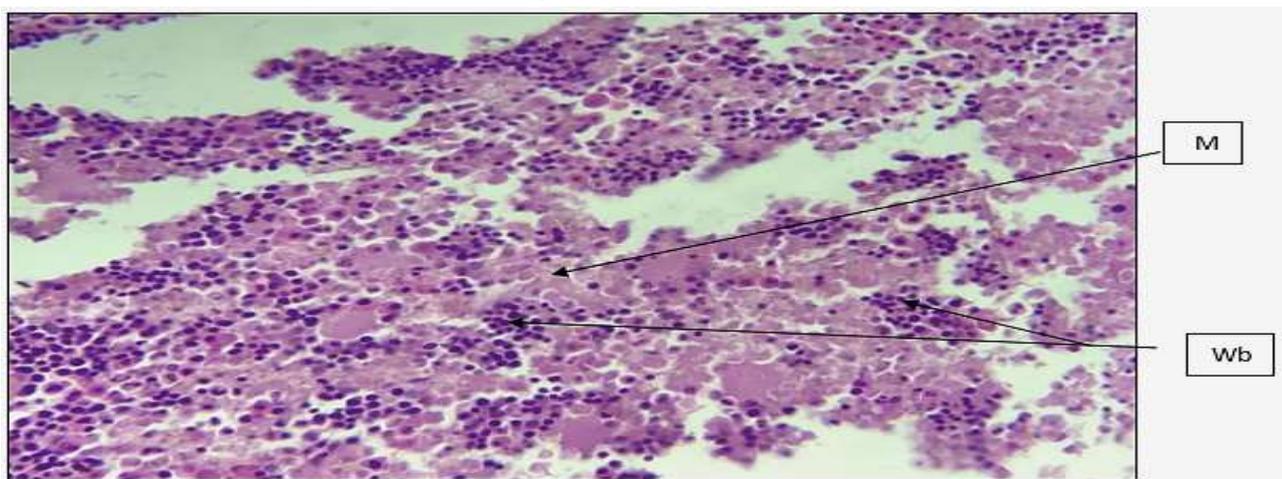
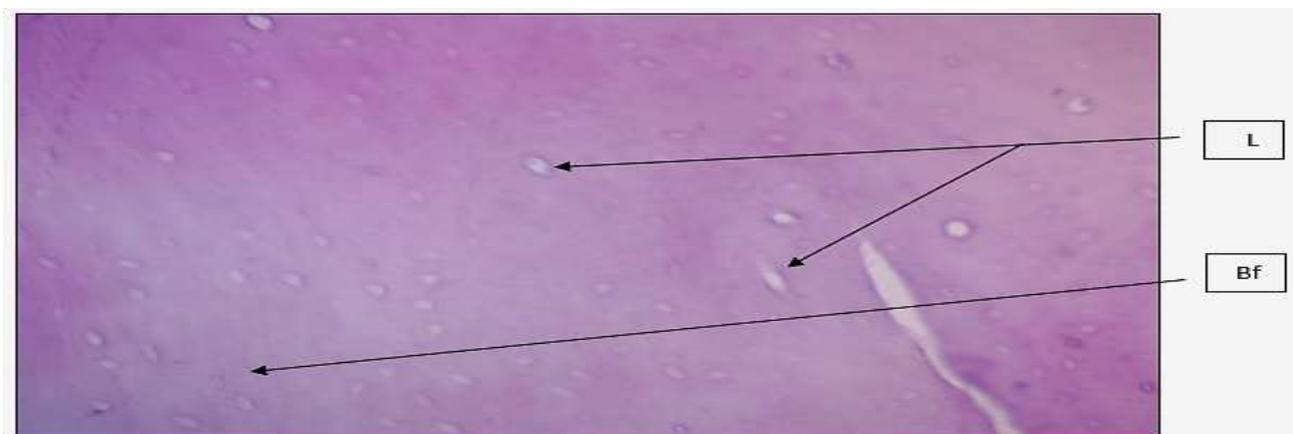


Figure 9- Section in the Declassified Femur of Ibuprofen Group for a Period of 45 Days Shows (L): Lamellae Bone Influenced by Cellular Content and Explains the Interconnection Content of the Bone Tissue Empty Bone Lamella of Osteocyte (Bf): Fibers of Bone Staining: Hematoxylin-eosin(400x)



## 4. Discussion

### Effect of Ibuprofen and Aqueous Extract of LS in the Body Weights

The current study recorded a significant decrease in the body weights of animal that were treated with ibuprofen during the two trial periods, as continued dosing of the drug led to a significant decrease at ( $P < 0.05$ ). The current study agreed with the researcher (20). Who noticed a clear decrease in the body weight of the rats used in his study, while the current study differed with the researcher (21), who studied several groups and concentrations of non-steroidal anti-inflammatory drugs, as the ibuprofen group was one of the groups that maintained their weight or gained weight. Weight loss may be due to drug reactions resulting from the use of ibuprofen, including gastric mucosal ulcers and bleeding, as indicated by (22) or it is possible to explain the weight loss to the negative effect of the drug that led It is due to the consumption of body fat and thus compensation for the destruction processes, or the weight loss may be due to the effect of the liver tissues that have been damaged by the daily dose of the drug, as the liver is one of the most important organs that contribute to the body's metabolism. The decrease in body weight may be due to the effect of ibuprofen on inhibiting the activity of the oxidative enzyme (Cox), which affects the synthesis of prostaglandin fats, which is the basis of the drug's mechanism of action, as prostaglandins have a role in regulating the functions of the digestive system, kidneys and blood vessels(23) and this explains the prevention of non-steroidal drugs for those important functions within the body, as treating pain, fever and inflammation negatively affects other critical functions by masking valuable signals of a problem occurring, which allows pain to be overcome repeatedly and thus a greater injury. The weight of laboratory animals used in the experiment indicated clear changes diagnosed during the study period, including general weakness, lack of movement and loss of appetite, especially with regard to the second dose period, that is, after 45 days., The current study showed a moral difference in the weights of the body during the two periods of dose in the treatment of the drug plus the water extract of LS seeds with a concentration of 50 mg/kg, which confirms the continued negative impact of the drug on the weights of the body for this treatment, as the decrease in body weight continued with the treatment that was taken for the drug+ aqueous extract of LS seeds at a concentration 100ml/kg during the 30-day period while the weights of the same treatment increased during the second period of the potion when compared with the control group and this can be explained to the role of preventive water extract, which was evident with increased concentration and length of the potion period. The study showed a marked increase in body weight, particularly in the treatment of ibuprofen plus the aqueous extract of LS seeds at a concentration of 150 mg/kg and in a period of

45 days where the role of preventive water extract was shown and inhibited the negative role of the drug on the efficiency of the digestive system and thus improved the work of organs affected by the drug. A moral increase in the weights of the treatment that dosed of aqueous extract and all the concentrations used in the current study was detected during the two periods of study with the weighting of the water extract at a concentration of 150 mm/kg and for the period 45 days and this is consistent with the study(24) which referred to the role of LS seeds in weight gain but disagreed with the study (25) where the researchers showed the decrease in the weight of rats after weeks of feeding in a water suspension of LS seeds and various doses where they pointed out the effect of the high dose negatively on weight gain. The explanation for increased body weight in the current study is due to the inclusion LS seeds in nutrients with a positive effect in improving appetite where they stimulated appetite centers in brain cells that increased the rates of water and food intake and this is observed in the animals of the current study it is possible that weight gain occurred due to the ability of seeds of the grain of the container LS Anti-oxidants in providing protection for nutrients from oxidation within the digestive tract and thus reducing the risk of digestive disorders, chemical seed elements such as fatty acids and proteins may play an active role through their activity or by influencing similar biological activities and this is consistent with (26).

### **Effect of Ibuprofen and Aqueous Extract of LS in the Bones Front and Hind Limbs**

The current study regarding the effect of the drug on the group of frontal and posterior limb bones decreased in the weights of those bones ,as the relative rate of weight decreased significantly during the two periods of potion conducted in the current study ,and this is consistent with (27) which concluded the lack of bone mass by increasing the period of ibuprofen treatment in human related mice .This can be explained by the decrease in the weights of laboratory animals treated with the drug ,which has led to a decrease in the weights of the bones of the limbs and may be due to the explanation of the above to the role of bone metabolism based on pathological and physiological conditions .Co x-2 inhibitors are likely to play an influential role in bon , thickness thus inhibiting bone formation and an increase in bone recovery without loss ,which is in line with(28) While the researcher and his group) (29)who emphasized in his study on the bones of the forearm in mice the ability of ibuprofen to improve bone quality by reducing bone and inflammatory cytokines of bones, where agreed with(30) the role of NSAIDs in bone health through Modifying the inflammatory response .The low weight of limb bones may be caused by poor vascular formation as inhibition of Cox-2 ring oxidant enzymes is known to reduce prostaglandin throbbing ,which has a role in the

formation of blood vessels necessary for ossification within(31) A moral decrease in the weights of the front limbs from the hind limbs was observed during the two periods of introspection ,which can be due to the adjustment of the skeleton with physical pregnancy ,as the lack of motor activity observed in the animals of the current study led to the loss of bone due to increased area of formation , thus reducing the thickness of the bone pore or may be due to the loss of negative bone balance ,that means the amount of bone formed exceeds the amount of bones absorbed. Inhibition of new bone formation may be due to the effect of the drug on bone-forming cells ,which is consistent with (32) . Who studied the broken and non-broken leg stubble within four to ten weeks of ala J ibuprofen and may be likely to be affected by cartilage differentiation ,which is important in bone formation led to a decrease in the weights of the bones of the limbs.

### **The Histological Study of the Bones Front and Hind Limb**

The tissue sections did not indicate an abnormal effect of the treatments treated with the drug and the aqueous extract of LS seeds ,especially in the high concentrations of aqueous extract ,which are (100, 150 mg/kg ,where a microscopic examination of the sections showed that the tissue components of the bones of the front and hind limbs are not affected ,which outweighs the protective characteristics For the water extract ,which has proven its efficiency in ridding living cells of physiological effects and thus gave protection to bone tissues through rebalancing and positive addition in the performance of cells and tissues ,this indicates the ability of the extract and its preventive effectiveness against the effect of the drug on the cell cycle and thus modify the course of the cell and reduce the toxicity caused by the drug and this confirms the role of the plant as an antioxidant ) While the tissue sections of the treatment of the drug plus the water extract of the seeds of LS and the concentration of 50 mg/kg showed a clear tissue effect on the structure of the bone marrow ,which was shown through the lack of marrow tissue in the bone trabecula replaced by adipose tissues ,which reduces the continued effect of the drug on bone tissue through lack of blood tissue for different areas of the bones.

The front and hind limbs of the animals ,showed the treatment of the aqueous extract of LS seeds at 50, 100, 150 mg/kg and during the 45.30 days ,there were no pathological appeared change in the bone cells that looks with clear nuclei and did not notice any necrosis of the cells The tissue sections, which were made for different areas of the bones of the front and back limbs of the animals, showed the treatment of the water extract of LS seeds and 150,100.50 mg/kg and during the 45.30

days of in trophy, there were no pathological changes as the bone cells appeared to be of clear nuclei and did not notice any necrosis when compared to the control group.

The cartilage tissue appeared active in more than one of tissue section, which confirms the stages of bone growth properly, as observed the natural tissue and fatty tissues of the sections in which the bone marrow appeared, confirming the differentiation and activity of the cellular content of the bones due to the natural characteristics they possess. The ingredients of the plant extract that acted as antioxidants(31) and this was positively reflected in the tissue content of the bones, which confirms the role of the active substances of the aqueous extract of LS seeds such as vitamins and minerals that have already shown efficiency on the tissues of bones limbs , especially high concentrations of plant extract. The aqueous extract has increased calcium levels in the blood, which have a stimulating and stimulating role for calcification, as well as the role of phosphorus found in the *Lepidium* intermediate stem cells that stimulate bone cell differentiation and reproduction (32). (33)

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