ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of High Altitude Hypoxia on the Knee Menisci of Rat

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Manuscript Info

Manuscript History:

Received: 12 December 2013
Final Accepted: 22 January 2014
Published Online: February 2014

Key words:
high altitude hypoxia and knee menisci.
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Abstract

BACKGROUND: Hypoxia is acondition in which the percentage saturation of hemoglobin with oxygen is decreased in blood. The body functions are suppressed at high altitude (1,500 to 3,500 meters), resulting in diminished inspiratory oxygen pressure and decline arterial oxygen saturation leading to increase in the ventilation, heartbeat, blood pressure, and decreased exercise performance. The knee menisci are wedge-shaped semilunar structures that lie between the femur and tibia and function to transmit and distribute load and it have both fibrous and cartilaginous tissues. Damage to the meniscus is common and disruption of tissue structure and function result in erosion of the underlying articular cartilage. The healing process of meniscal damage depending on the degree of vascularity so the periphery meniscal damage spontaneous heal, but damage of the inner region is poor to heal. This work is designed to report the structural alteration of the knee menisci of the rat under effect of the high altitude hypoxia. *MATERIAL AND METHODS*: Forty healthy young male albino rats, having average weight of 200 grams for each, were used in this study. The rats were dividing into four groups (10 rats for each). The control group was kept in normal environment for one month, the second, third and fours groups admitted in high altitude environment for ten days, twenty days and one month respectively. At the end of the above periods the blood gases were investigated and the knee menisci were dissected and prepared for light and scanning electron microscopic examination. *Results:* The knee menisci were affected by the high altitude hypoxia, where the chondrocytes atrophy with pyknotic nuclei and shrunken cytoplasm, disorganization of the collagenous fibers and areas of degeneration. Also In our work the direct measurement of oxygen Pressure (PO2) values in the blood were reveled significant decline.

Introduction

Hypoxia is disproportion between the amount of oxygen supplied to the tissue and the amount actually required and it occurs when the oxygen tension is lower than physiological levels (Neckar et al., 2002and Patterson & Zhang, 2010). There are three altitude regions that reflect the lowered amount of oxygen in the atmosphere (high altitude 1,500-3,500 meters, very high altitude 3,500-5,500 meters and extreme altitude above 5,500 meters), (West. 2002, Zubieta et al., 2008 and Brenner et al., 2011).

In synovial joints, oxygen supply to articular chondrocytes is very limited and depends on the oxygen binding capacity of synovial fluids and its flow during joint motion (Schneider et al 1996 & Lee et al. 2007). The meniscus is a specialized articular cartilage located in the knee joint where it functions to aid joint stability, protection, absorb shock and transmit load and it has a poor repair capacity, especially when injury is located in the a vascular region. The meniscial cartilage comprised of chondrocytes surrounded by a dense extracellular matrix and collagen fibers forms the structural skeleton of the tissue, enclosing a hydrated proteoglycan gel that play important role in the joint pressure (Urban et al., 1979; Basser et al., 1998 and Bank et al., 2000). Oxygen concentration is a critical parameter

proposed to modulate the functions and metabolic activity of chondrocytes as well as in damaged joints (Malda et al., 2003and Ströbel et al., 2010). Today, research on articular cartilage is even more important due to the rising numbers of people suffering from the articular diseases such as osteoarthritis and rheumatoid arthritis (Hughes et al., 2005). In this study, we will explore the effect of effect of high altitude hypoxia on the chondrocytes of knee menisci of rat.

Materials and Methods

Forty healthy young male albino rats, having average weight of 200 grams each, were used in this study. The animals obtained from the animal house of Gazan University Kingdom Saudi Arabia at sea level where they were fed with standard feed and allowed free water excess. The rats were divided into four groups (10 rats for each). The control group admitted in normal environment in Darb city Kingdom Saudi Arabia, at sea level for one month, where the animals housed in open mesh-wire cages in temperature-controlled room at 22-24°C and 50-60 % relative humidity with 12-hr light dark cycle **(**Soldani et al**.,** 1997**)**. The second, third and fourth groups admitted in high altitude environment (in the animal house of Collage of Medicine King Khaled University Abha city Kingdom Saudi Arabia, 2200 meters above sea level) for ten days, twenty days and thirty days respectively where they were fed with standard feed and allowed free water excess. At the end of the previous mentioned periods the rats were anesthetized by inhalation of ether solution, the knee joint were took and dissected and the medial menisci were prepared for light and scanning electron microscopy examination (Grogan et al., 2003 and Stolz et al., 2009). Also, the arterial blood samples were taken and investigated for blood gases in Aseer university hospital to determine the degree of hypoxia.

Results

The arterial blood Oxygen:

In first group (control) where the animals were kept in normal environment in Darb city at sea level for one month have mean arterial blood oxygen Pressure (PO2) was 94.955 / mmHg. Table (1). The second group animals were kept at high altitude environment (Abha city 2200 meter above sea level) for ten days, showed the mean arterial blood oxygen was significantly declined to about 18.646 /mmHg than the control group Table (1). The third group animals were kept at high altitude environment for twenty day and the mean arterial blood oxygen was significantly declined to 15.964 /mmHg than the control group Table (1). The fourth group animals were kept at high altitude environment for one month and their mean arterial blood oxygen revealed significantly declined to 12.21 /mmHg than the control group Table (1).

Haematoxylene and eosin stains:

In first group (control group) the chondrocytes one arranged in rows between the cartilaginous matrix and it has centrally located nuclei lying within granular vacuolated cytoplasm and surrounded by lacunae (Figs.1and 2). The second group showed that disorganization and vacuolation of the cartilaginous matrix with narrowing of intracellular spaces, the chondrocytes swelling and vacuolated cytoplasm, with areas of hemorrhages (Figs. 3 and 4). The third group showed that the chondrocytes reduced in number, diminished in size with its disrupted lacunae, vacuolated cytoplasm and increased intracellular spaces. (Figs. 5 and 6). Lastly, the fourth group showed atrophy of the chondrocytes with pyknotic nuclei with no clear lacunae and the cells appeared with shrunken cytoplasm many vacuoles and they were surrounded by faint cartilage matrix (Figs. 7 and 8) with areas of degeneration (Fig. 9).

Scanning electron microscopy:

First group showed that the chondrocytes arranged in groups between rows of the collagen fibrils and it were spherical in shape with fine microvilli on their surface. The collagen fibrils were organized in rows (Figs.10, 11 and12). The second group showed swelling of the chondrocytes with abnormal microvilli on their surface warped around the collagen fibrils **(**Fig**.** 15**),** the collagen fibrils were disorganized, thicker than the control group and created a dense network (Figs. 13 and14).In the third group the collagen fibrils were disorganized**,** reduced in thickness and increase in cavities in the cartilaginous matrix **(**Fig**.** 16**),** the chondrocytes were reduced in number, diminished in size with abnormal enlarged microvilli and some them were distracted (Figs. 17 and18). Lastly in the fourth group the chondrocytes were destructed and atrophied in some areas with disrupted microvilli on their surface with elevations containing aligned hydroxyl apatite crystallites projected. The collagen fibrils were atrophied and disorganized with widening of the intracellular spaces **(**Figs**.**19, 20 and 21**)**.

Animals	The blood samples			
	PO2 / mmHg group one	PO2 / mmHg group Two	PO2 / mmHg group Three	PO2 / mmHg group Four
1	98.53	78.55	78.90	82.35
2	95.25	75.55	79.58	83.50
3	91.41	81.48	80.66	84.90
4	96.75	76.75	77.25	81.80
5	96.64	68.74	77.94	83.60
6	93.52	80.42	80.50	83.40
7	90.28	80.25	78.59	82.20
8	95.81	75.80	79.85	81.90
9	93.65	67.85	76.85	81.80
10	97.71	77.70	79.79	82.00
Mean	94.955	76.309	78.991	82.745
S-D	2.696394	4.6666	1.319701	1.0478

Table (1) The blood gasses of the animals.

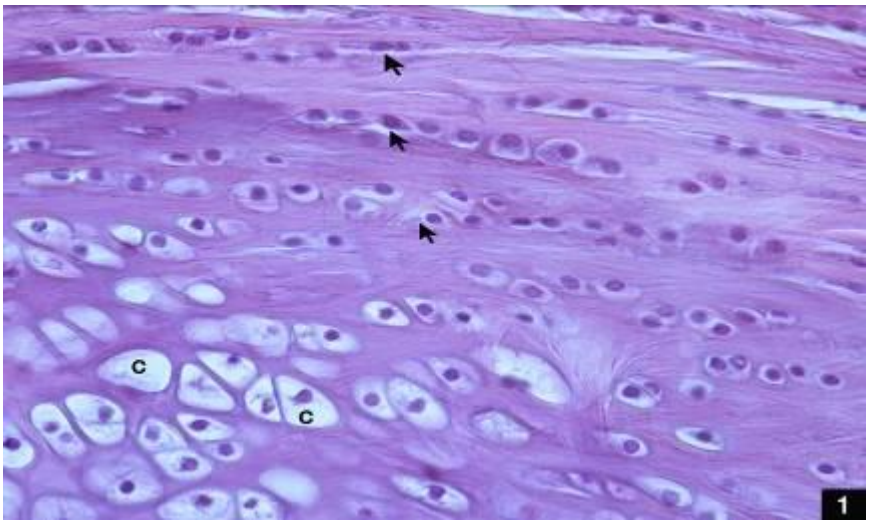

Fig. (1) A photomicrograph of a section from the medial meniscus of an adult rat (control group) showing chondrocytes (C) arranged in rows (arrows) in the cartilaginous matrix (H@E X 400).

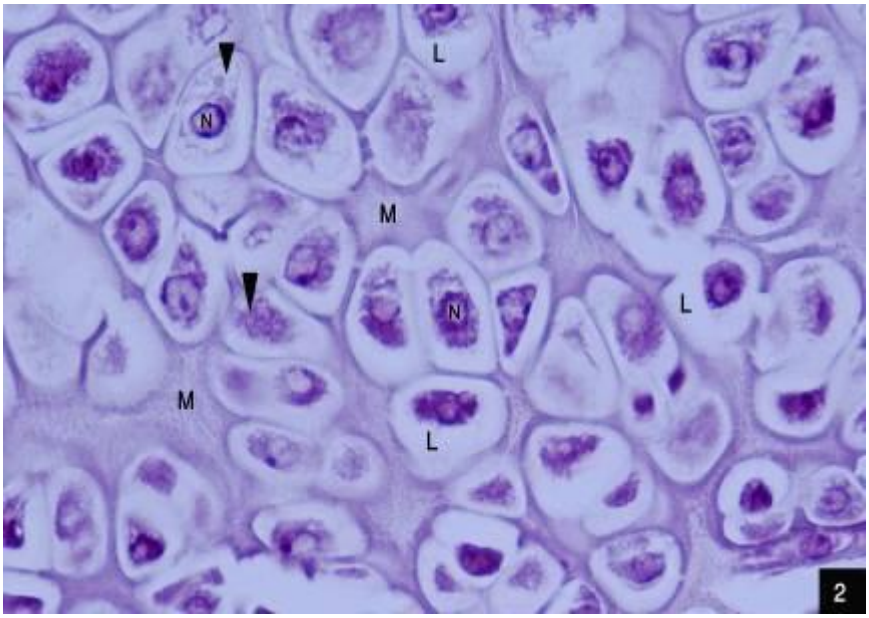

Fig. (2) A photomicrograph of a section from the medial meniscus of adult rat (control group) showing groups of chondrocytes in the cartilaginous matrix (M), it has centrally located nuclei (N) lying within granular vacuolated cytoplasm (arrows head) and surrounded by lacunae (L) (H@E X 1000).

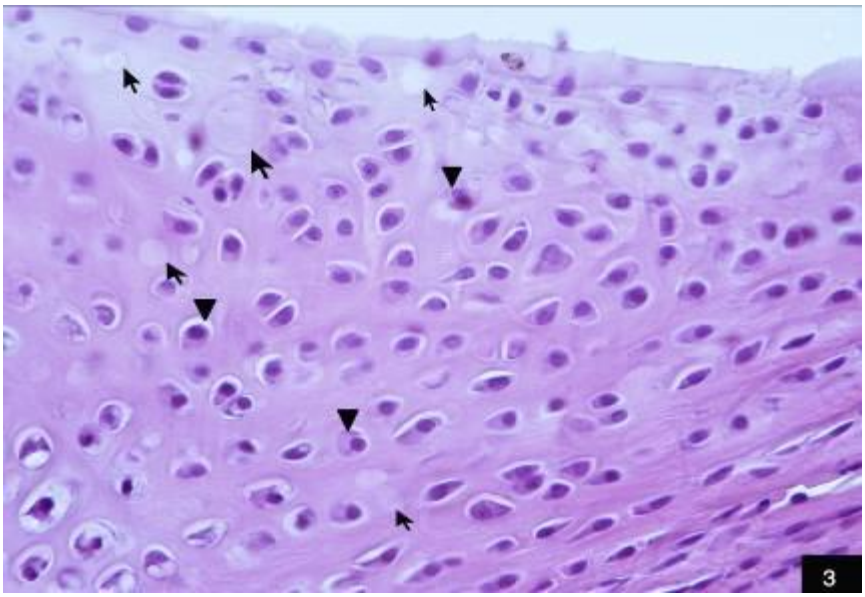

Fig. (3) A photomicrograph of a section from the medial meniscus of an adult rat (second group) showing vacuolation in the cartilaginous matrix (arrows) and disorganization of chondrocytes (arrows head) (H@E X 400).

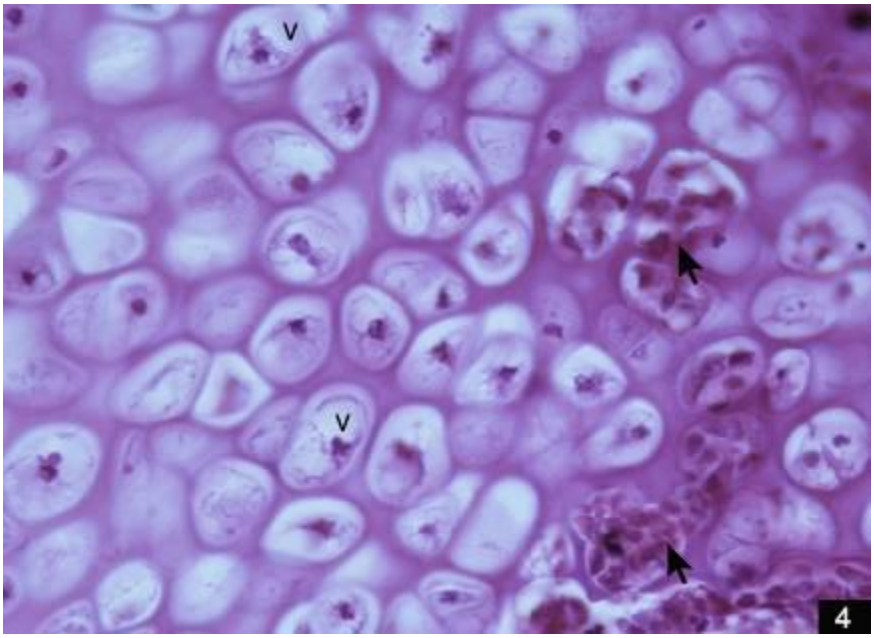

Fig. (4) A photomicrograph of a section from the medial meniscus of an adult rat (second group) showing swelling of the chondrocytes within vacuolated cytoplasm (V), narrow intracellular spaces and areas of hemorrhage (arrows) (H@E X 1000).

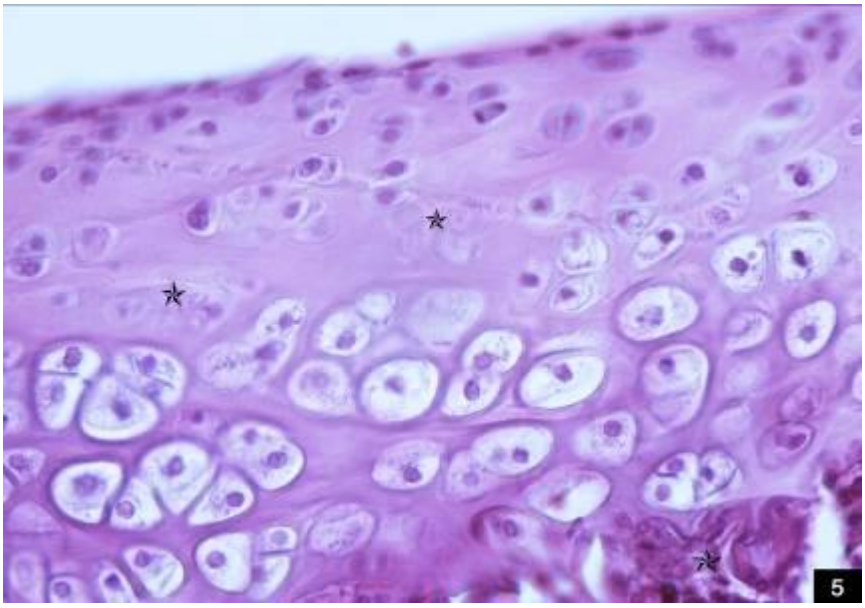

Fig. (5) A photomicrograph of a section from the medial meniscus of an adult rat (third group) showing disorganization of chondrocytes, some of them were degenerated (*) and more spaces between the chondrocytes are clearly seen (H@E X 400).

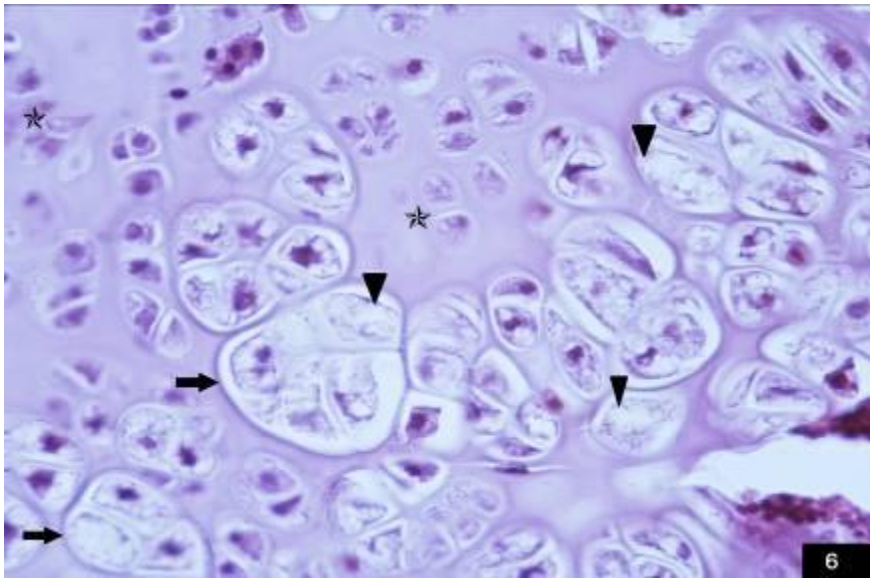

Fig. (6) A photomicrograph of a section from the medial meniscus of an adult rat (third group) showing degenerated chondrocytes (*) with pyknotic nuclei and some chondrocytes were found in groups (arrows) and it has pale vacuolated cytoplasm (arrows head) (H @E X 1000).

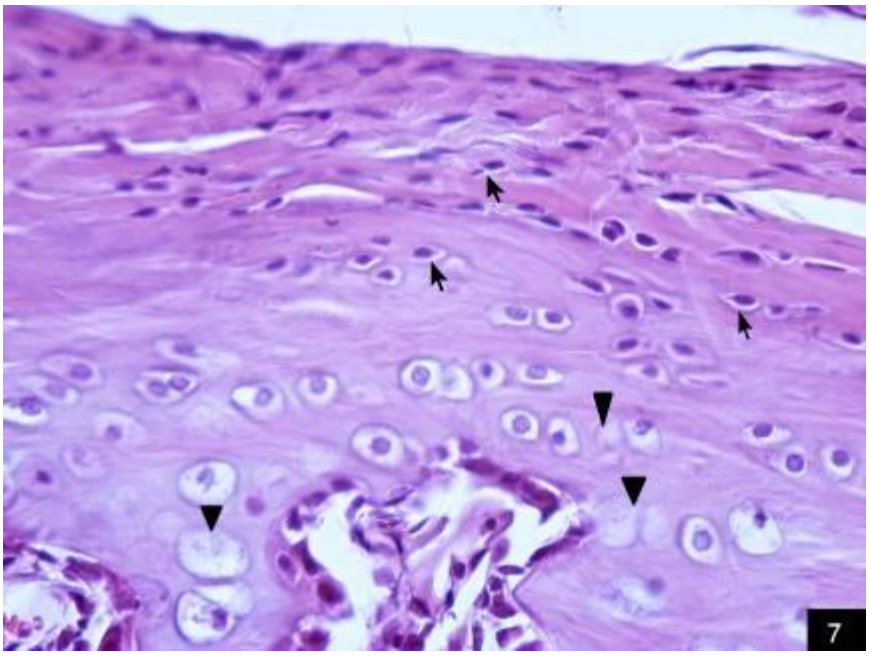

Fig. (7) A photomicrograph of a section from the medial meniscus of an adult rat (fourth group) showing atrophy and disorganization of chondrocytes (arrows) and some of them were degenerated (arrows head), also the intracellular spaces were wide (H@E X 400).

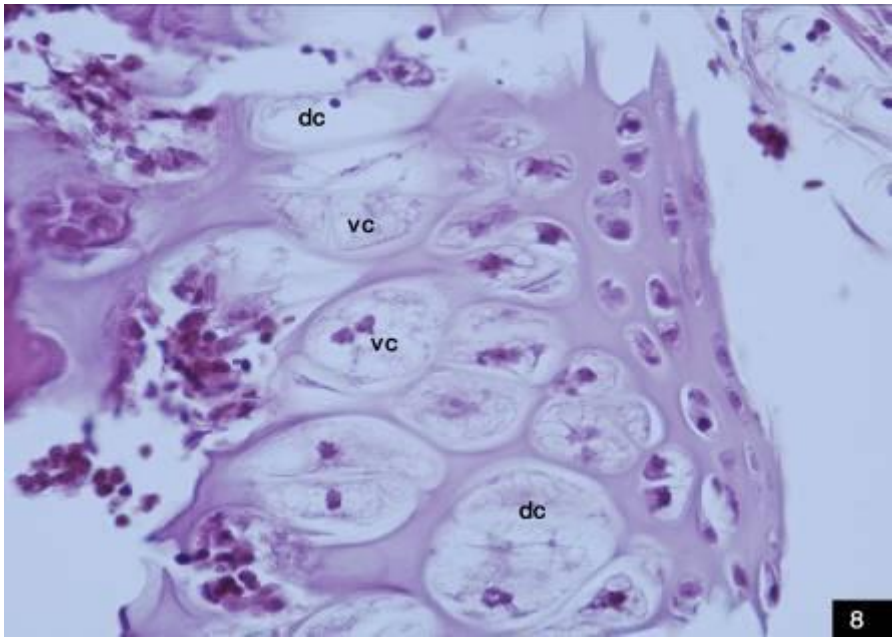

Fig. (8) A photomicrograph of a section from the medial meniscus of an adult rat (fourth group) showing vacuolated chondrocytes (vc) with pyknotic nuclei and some of them were degenerated (dc) (H@E X 1000).

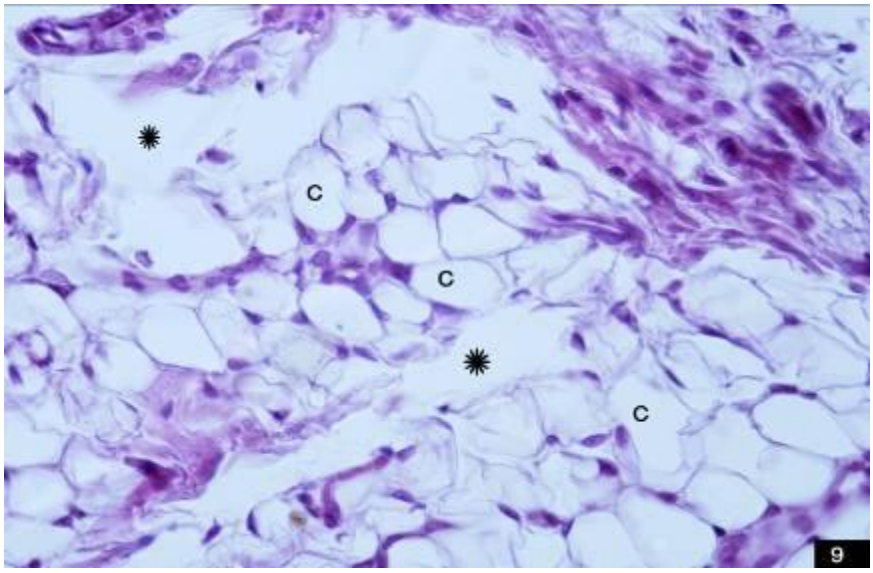

Fig. (9) A photomicrograph of a section from the medial meniscus of an adult rat (fourth group) showing degenerated chondrocytes (c) with pyknotic nuclei and variable shape cavities in the cartilage matrix (*) (H@E X 1000).

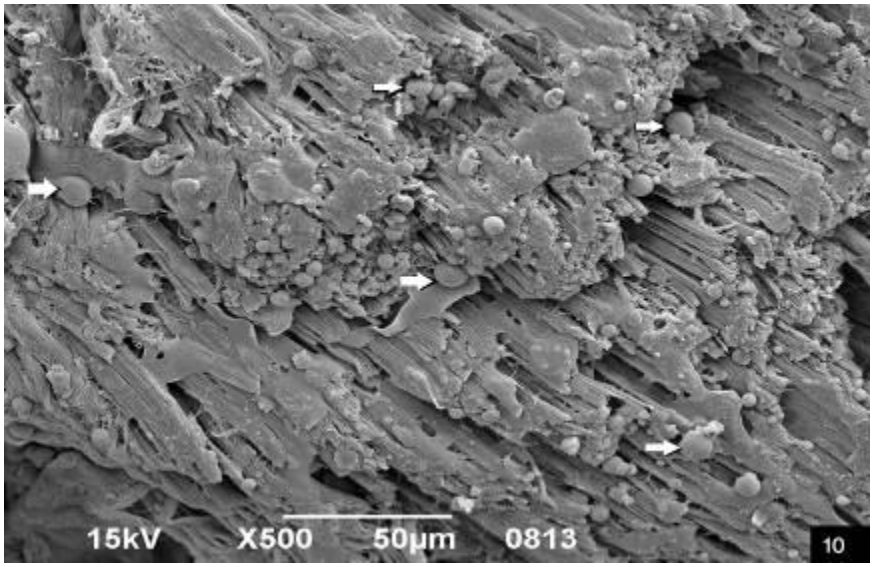

Fig. (10) Scanning electron micrograph of the medial meniscus of an adult rat (control group) showing the chondrocytes (arrows) which are spherical in shape and arranged in rows between the bundles of collagen fibrils.

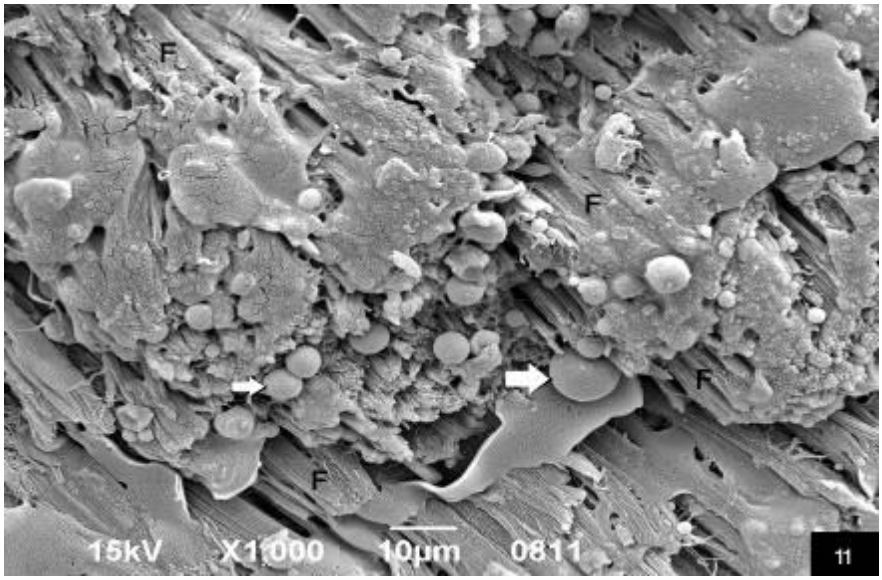

Fig. (11) Scanning electron micrograph of the medial meniscus of an adult rat (control group) showing the bundles of collagen fibrils (F) and the chondrocytes (arrows) among them having microvilli on their surface.

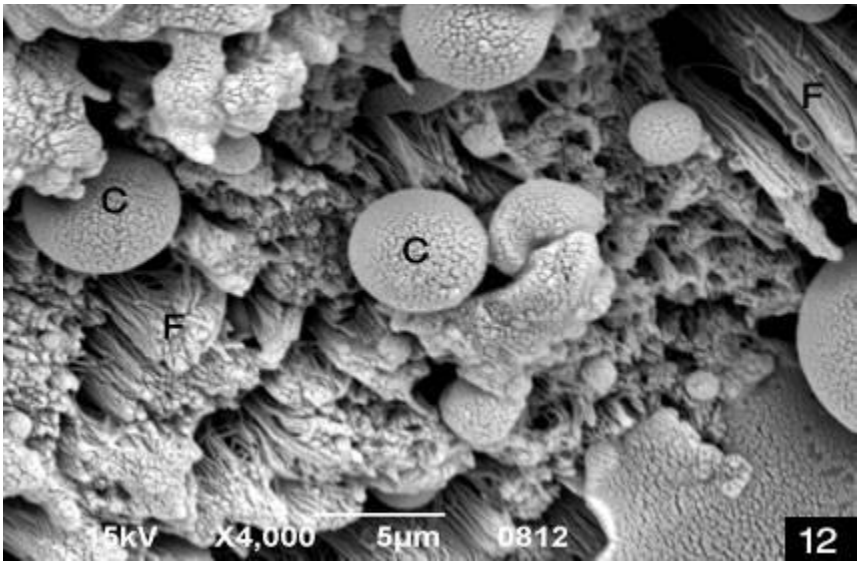

Fig. (12) Higher magnification of the previous section showing the bundles of collagen fibrils (F) and chondrocytes (c) having microvilli over their surface.

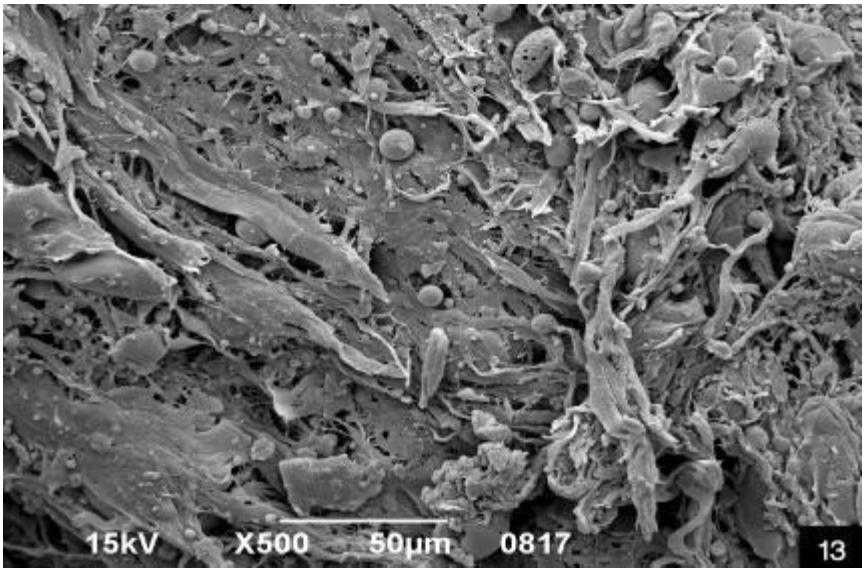

Fig. (13) Scanning electron micrograph of the medial meniscus of an adult rat (second group) showing disorganization of collagen fibrils.

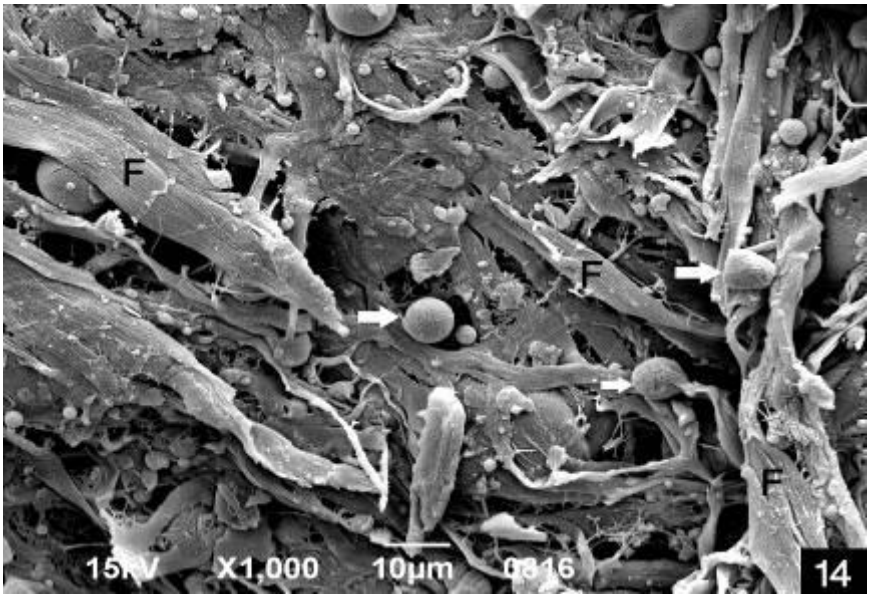

Fig. (14) Higher magnification of the previous section showing disorganization of collagen fibrils (F) which one abundant and disrupted extending to all areas around chondrocyte cell (arrows) creating a dense network.

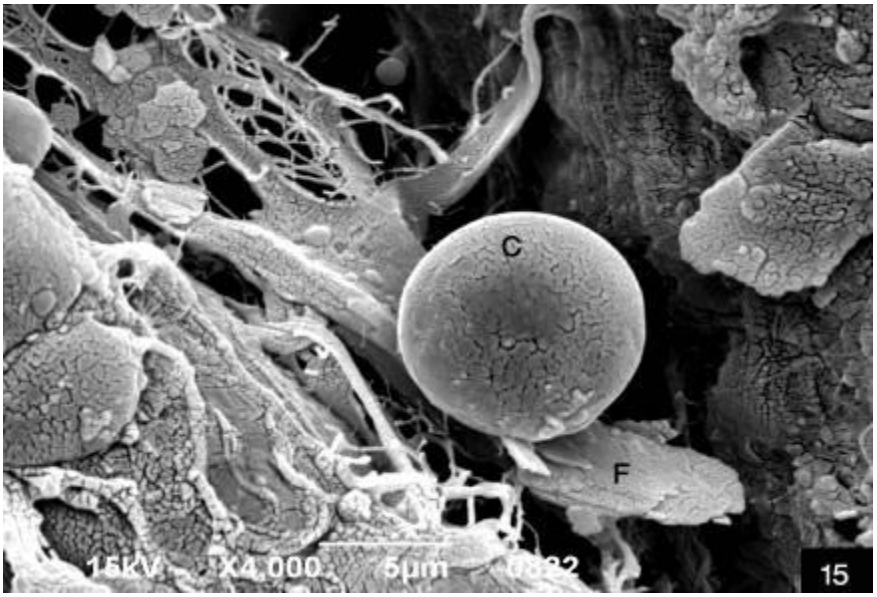

Fig. (15) Scanning electron micrograph of the medial meniscus of an adult rat (second group) showing swelling of the chondrocytes (C) with abnormal microvilli on their surface which wrap around the collagen fibrils.

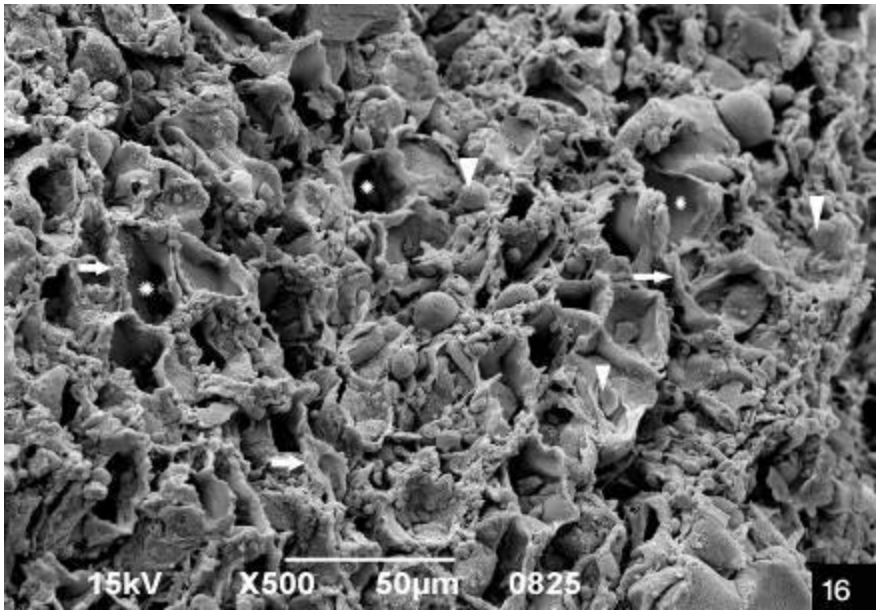

Fig. (16) Scanning electron micrograph of the medial meniscus of an adult rat (third group) showing more disorganization of collagen fibrils (arrows), abnormal shaped chondrocytes (arrows head) and variable cavities (*) in the cartilaginous matrix.

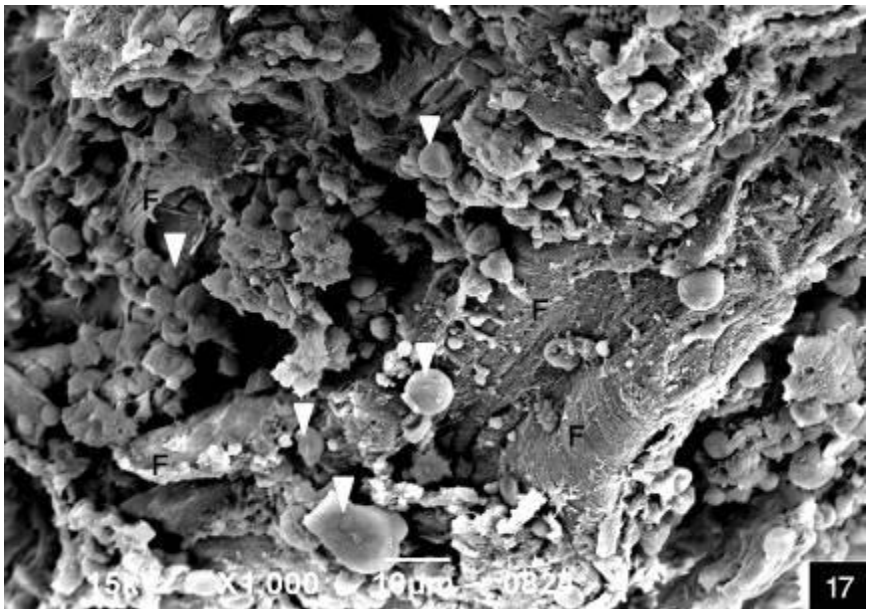

Fig. (17) Scanning electron micrograph of the medial meniscus of an adult rat (third group) showing more disorganization of collagen fibrils (F), abnormal shaped chondrocytes (arrows head).

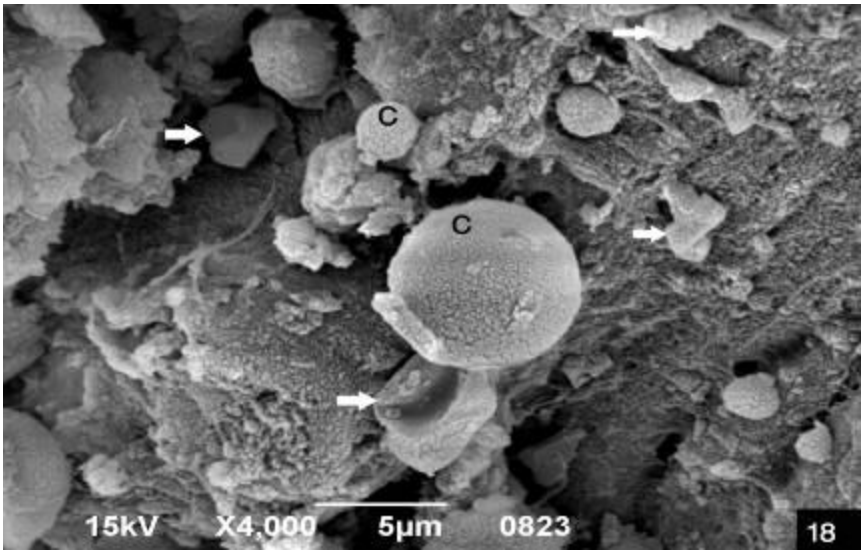

Fig. (18) Higher magnification of the previous section showing the chondrocytes (C) covered by abnormal microvilli and has hydroxyapatite crystallites projected prominently above the level of microvilli, also some chondrocytes were distracted (arrows).

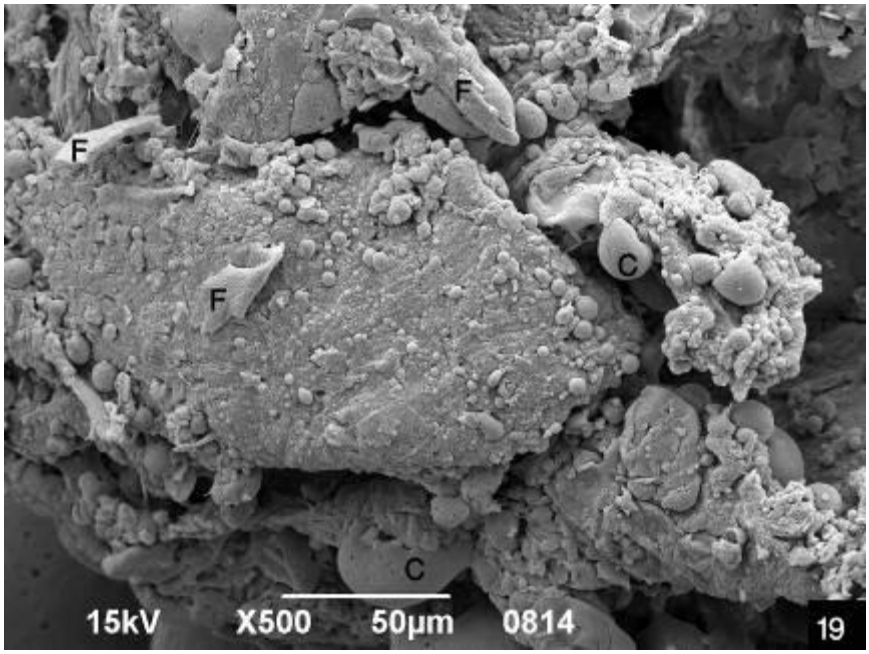

Fig. (19) Scanning electron micrograph of the medial meniscus of an adult rat (fourth group) showing more disorganization of collagen fibrils (F), atrophy of the chondrocytes and some of them were abnormal shaped (C).

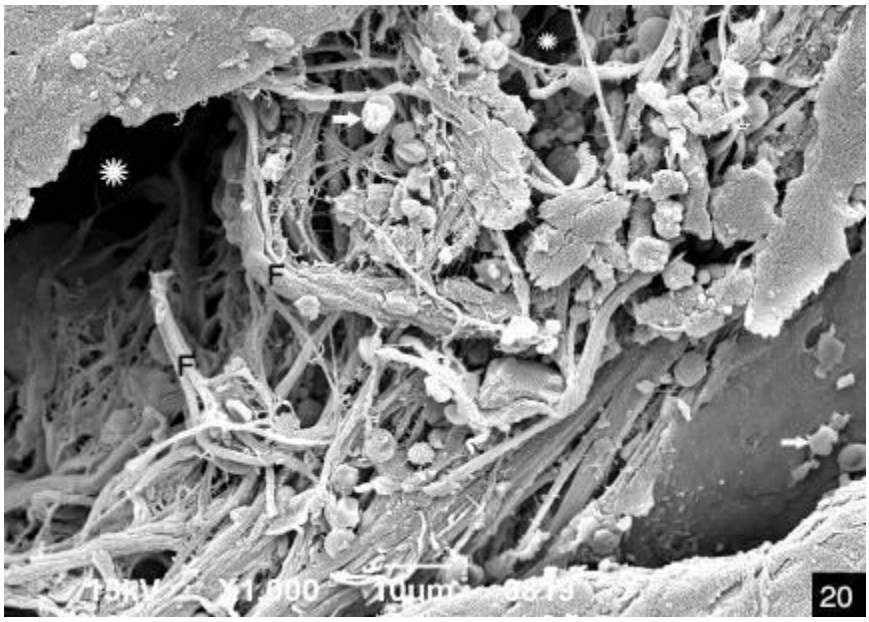

Fig. (20) Scanning electron micrograph of the medial meniscus of an adult rat (fourth group) showing more disorganization and atrophy of collagen fibrils (F) atrophy and distraction of the chondrocytes (arrows) and wide cavities (*) in the cartilaginous matrix.

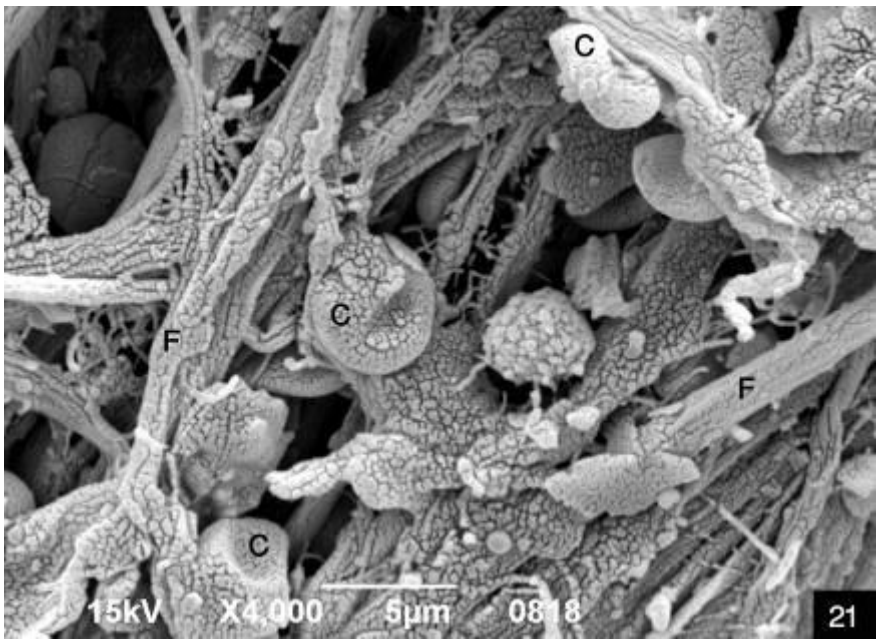

Fig. (21) Higher magnification of the previous section showing atrophy and distraction of the chondrocytes (C) with abnormal microvilli and has hydroxyapatite crystallites projection prominently above the level of microvilli. Also, the atrophy of collagen fibrils (F) was clarified.

Discussion

Hypoxia is known to have critical effects on the vitality of living bodies and hence we designed our work to evaluate alterations of menscial structure under effect of high altitude hypoxia 2200 meters above the sea level at Abha city kingdom Saudi Arabia in which about 200,000 persons live. About 140 million people live at high altitude environment worldwide and high altitude is considered to be elevations above 2500 meters (Moore et al; 1998). In our work, the direct measurement revealed significant decline of pO_2 values in the arterial blood to 18.646 mmHg,

15.964 mmHg and 12.21 mmHg second, third , and fourth groups respectevely. These resultes are in agrement with previous work (Gross, et al. 1995, Raleigh et al. 1998, Flueck. 2009 and Ivanovic. 2009) which reported that the hypoxia identified by direct measurement of pO_2 values and/or significant induction of hypoxia-inducible genes, suggest oxygen tension between 1% (-7 mm Hg) and 3% (-21 mm Hg) as physiological hypoxia. The animals are typically subjected to between 8% and 12% oxygen in order to significantly reduce arterial pO_2 to comparable values Fisher, et al. (2007). In our work the gradually decrease of the arterial pO_2 values among the treated groups and control one could be explained by stimulation of erythropoiesis.

Present study revealed that progressive degeneration of the cartilaginous tissues of knee menisci according to the duration of high altitude hypoxia exposure, where the first group (control group) showed the chondrocytes arranged in rows in the cartilaginous matrix and it has centrally located nuclei lying within granular cytoplasm and surrounded by lacunae These resultes are in agrement with *McGraw-Hill, (2001)*. Scanning electron microscopy showed the chondrocytes arranged in groups between rows of the collagen fibrils which were spherical in shape and had fine microvilli on their surface. The collagen fibrils were organized in rows.

The second group showed disorganization and vacuolation of the cartilaginous matrix with narrowing of intracellular spaces, the chondrocytes were swollen and has vacuolated cytoplasm. Also areas of hemorrhage were found and the scanning electron microscopy revealed swollen chondrocytes with abnormal microvilli on their surface with wrap around the collagen fibrils**,** the collagen fibrils were disorganized, thicker than the control group and created a dense network. These result could be explained by inflammatory process of the knee joint according to the study done by *Ströbel et al. (2010)* which found that at low, more physiological (5%) oxygen percentage has role in articular cartilage metabolism, enhance the proteoglycan and collagen synthesis and at the same time to reduce the activity of catabolic enzymes involved in cartilage breakdown and leading to structural features similar to those found in osteoarthritic tissue. In addition, when combined with intermittent dynamic compression, oxygen tension has been shown to have a significant effect on the induction of inflammatory mediators in cartilage explants such as nitric oxide and prostaglandin E2 *(Fermor et al., 2005).*

In the third group the chondrocytes were reduced in number, diminished in size within its disrupted lacunae having vacuolated cytoplasm and increased intracellular spaces. By scanning electron microscopy it was found the collagen fibrils were disorganized**,** reduction in there thickens and increase in the cavities between the cartilaginous matrix and abnormally enlarged microvilli, these meaning that the degenerative processes started by continuity of high altitude hypoxia and are in agrement with *Pfandera and Gelse, (2007):* who mention that the hypoxia is important factors in cell survival, energy generation and matrix synthesis by chondrocytes. The fourth group showed atrophy of the chondrocytes with pyknotic nuclei without clear lacunae and the cells appeared with shrunken cytoplasm with many vacuoles and were surrounded by a faint cartilage matrix and areas of degeneration were found. By scanning electron microscopy it was found that the chondrocytes atrophied and destruction in some areas with disrupted microvilli on their surface with elevations containing aligned hydroxyl apatite crystallites projected. The collagen fibrils were atrophied and disorganized with widening of the intracellular spaces. In the current study the chondrocytes cultured revealed that the time and dose dependent of oxygen tension on vitality of the culture, where the destruction of collagenous fibers around most of the chondrocytes increased by more concentrations of hypoxia *(Malda et al., 2004).* In osteoarthritis, the slow progressive degeneration of articular cartilage is the most common feature of the joint disease. It may be caused by persistent and abnormally high loads on the joint surfaces which initially result in the loss of proteoglycans and chondrocytes from the articulating surface of the cartilage. Subsequently, the cartilage may crack, erode and expose the underlying bone. The osteoarthritic chondrocytes were characterized by an increased synthesis of matrix destructive enzymes; the cells appeared with shrunken cytoplasm with many vacuoles and were surrounded by a faint cartilage matrix *(Aigner et al., 2001).*

Recommendations:

The menscial structure of the knee joint affected by the high altitude hypoxia, in which the intracellular spaces are increased, atrophy of the chondrocytes and disorganization of the collagenous fibers with areas of degeneration. So we invite more researches on the high altitude areas in the world.

Acknowledgements:

I would like to express my deepest thanks to all persons help me in this work.

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