التأثير الواقي لخلاصة الشاي الأخضر كمضاد لضغط الأكسدة في التهاب المفاصل التجريبي عند الجرذان

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الملخص

خلفية وهدف البحث: من خصائص الشاي الأخضر أن له تأثيرات مضادة للأكسدة وإزالة الجذور الحرة. في هذه الدراسة درسنا تأثيرات خلاصة الشاي الأخضر على ضغط الأكسدة في التهاب المفاصل عند الجرذان(المحدث تجريبياً بالحقن داخل المفصلي ل 0.1 مل من الكاراجينان وبتركيز 1%).

المواد والطرائق: كان لدينا ثلاث مجموعات كل مجموعة تحوي 10 جرذان. مجموعة الشاهد،ومجموعة التهاب المفاصل، ومجموعة التهاب المفاصل مع خلاصة الشاي الأخضر. بعد أسبوع من إعطاء خلاصة الشاي الأخضر، قيست مستويات الأكسدة الفائقة للدسم و أوكسيد النتريك والثيول الكلي في البلازما، أيضاً أُجْرِيَتْ دراسة نسيجية للمفاصل في مختلف المجموعات.

النتائج: في مجموعة التهاب المفاصل لاحظنا زيادة ذات دلالة إحصائية في مستوى أوكسيد النتريك والأكسدة الفائقة للدسم بالمقارنة مع مجموعة الشاهد.

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في المجموعة التي أعطيت الشاي الأخضر لم نلاحظ أي تبدل ذي دلالة إحصائية في مستويات الأكسدة الفائقة للدسم و أوكسيد النيتريك عن مجموعة الشاهد، بينما كانت هناك زيادة ذات مدلول أحصائي في مستوى الثيول الكلي في البلازما بالمقارنة مع مجموعة الشاهد ومجموعة التهاب المفاصل.

أظهر الفحص التشريحي المرضي عند الجرذان المحقونة بالكاراجينان أن لديها تنكساً شديداً فضلاً عن تبدلات تنخرية في المفاصل، في حين التي أعطيت الشاي الأخضر بعد الكاراجينان ظهرت عندها تبدلات تنكسية معتدلة مع نقص واضح في عدد الخلايا الالتهابية المندخلة في الغشاء الزليلي للمفصل بالمقارنة مع التي أعطيت الكاراجينان فقط. وأيضاً لم يكن هناك أي تخرب عظمي أو غضروفي إلى جانب عدم وجود نقص تمعدن وعدم، وجود نقص في العناصر اللمفاوية في نقي العظام.

الاستنتاج: هذه النتائج تشير إلى أن للشاي الأخضر تأثيراً واقياً كمضاد لالتهاب المفاصل عند الجرذان.

هذه النتيجة تقترح أن للشاي الأخضر فعل مضاد للأكسدة الطبيعية الناقصة في التهاب المفاصل. هذه التأثيرات المضادة للأكسدة يمكن أن تفيد في حالات التهاب المفاصل بسبب عدم التوازن بين إنتاج الجذور الحرة ومضادات الأكسدة.

The Protective Effect of Green Tea Extract Against The Oxidative Stress Of the Experimental Arthritic Rats

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Abstract

Background and Objective: Green tea extract (GTE) displays antioxidants and free radical scavenger properties.

In the present study, the effect of GTE on the oxidative stress in arthritic rats (using intra-articular injection of 0.1ml of 1% carrageenan solution) was investigated.

Material and Methods:Three groups of 10 rats each were used- controls, arthritic group, and arthritic +GTE group. After one week of treatment with GTE, the plasma levels of lipid peroxides, nitric oxide and total thiols were measured. The histopathological examinations of joints in different groups were performed also.

Results:In arthritic rats, the plasma levels of nitric oxide and lipid peroxides were significantly higher than their corresponding levels in control group.

In rats received GTE, the changes in the plasma levels of lipid peroxides and nitric oxide were not statistically significant compared to controls, while, the plasma levels of total thiols were significantly increased in comparison with arthritic and control groups.

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The histopathological examination revealed that cases treated with carageneen alone showed severe degenerative as well as necrotic changes in the arthritic rat joint, while cases treated with carageneen and GTE showed mild degenerative changes represented by a marked reduction in the numbers of the inflammatory cells infiltrating the synovial membrane comparing to that one treated with carageneen only. Also no cartilage as well as bone erosion showed, besides mild mineralization and lymphoid element depletion of the bone marrow.

These results indicate the protective effects of the GTE against arthritis in rats.

Conclusion:The result indicates that GTE has a protective effect against arthritis in rats ,this results suggests that GTE may effectively normalize the impaired antioxidants status in arthritic rats. The effects of this antioxidant may be useful in osteoarthritis due to imbalance between free radicals and antioxidant systems.

Keywords: Carrageenan; Green tea extract; Oxidative stress; arthritic rats; Total thiols; Lipid peroxides; Nitric oxide

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Introduction

Osteoarthritis (OA) is a degenerative joint disease of complex etiologies that results in loss of normal function due to breakdown of articular cartilage. It is resulted of both mechanical and biologic events that destabilize the normal coupling of degeneration and synthesis of articular cartilage chondrocytes and extracellular matrix, and subchondral bone ,if damaged that tissues will contribute to accelerated cartilage degradation [(1)]. The components of articular cartilage include type II and other collagens, proteoglycans and chondrocytes [(2)].

Oxidative damage has been demonstrated within the inflamed human joint by a radical mediated mechanism which is termed "hypoxic reperfusion injury." This mechanism involves the production of reactive oxygen species causing tissue damage and subsequent persistence of the inflammation of the synovial lining the joint. Assessment of reactive oxygen species and lipid peroxidation is possible in human joint fluid (effusion) by the identification of both known intermediates and end products of these reactions [(3&4)].

Nitric Oxide is a short acting signaling molecule with multiple important physiologic and pathologic functions [(5&6)]. In the presence of oxygen, NO is converted rapidly to nitrite and nitrate, substances which generally are not bioactive [(7)]. However, on reacting with O^{2-}, NO may form peroxynitrite ($OONO^-$), a toxic and reactive molecule. Nitric Oxide is formed during the conversion of arginine to citrulline by the nitric oxide synthase (NOS) enzymes. There are three forms of NOS encoded by three different genes NOS-1, NOS-2, & NOS-3. Inducible NOS was described initially in mononuclear phagocytes, and seems to be the primary NOS enzyme in chondrocytes [(8)]. Nitric oxide synthase 2 can produce high levels of NO and most commonly is associated with inflammation in arthritic disorders. Inhibition of NO production using NOS2 specific inhibitors can considerably reduce disease progression in animal models of OA [(9)]. Chondrocytes obtained from patients with osteoarthritis produce different inflammatory mediators including nitric oxide (NO). Since chondrocytes live in a milieu that is avascular and aneural, these mediators may not produce the classical signs of inflammation, but nevertheless they are involved in degradation of cartilage [(10)].

Lipid peroxidation is a reactive oxygen species mediated pathophysiologic process which leads to cell membrane damage which in turn results in cellular dysfunction or cell death. This process is initiated by the more potent free radicals such as alkoxyl, peroxyl and hydroxyl radicals. This lipid peroxidation can lead to an autocatalytic process, since these radicals, particularly peroxyl, have the ability both to initiate and to propagate lipid peroxidation[10].

Small amounts of free radicals, particularly the hydroxyl radical, may trigger this pathologic process. Also, the generation of superoxide radicals by any source, in the presence of iron ions further leads to formation of hydroxyl radicals with consequent start of the lipid peroxidation reaction [11]. Such iron has been detected in human synovial fluid and has also been shown to initiate this peroxidation within the joint. The arthritic joint as a site of oxidative stress, an overproduction of free radicals, exceeds the inherent, local antioxidant capacity thereby causing damage [11].

The importance of reduced glutathione (GSH) in protecting cells and aerobic organisms against oxidative stress by its role as an antioxidant has been well established. However, GSH in this role is itself oxidized (GSSG). Thus, glutathione metabolism needs to act in combination with other enzyme systems in order to again reduce the glutathione molecule to GSH so it may renew its role as a free radical scavenger. GSH functions coordinately with the enzyme glutathione peroxidase to break down hydrogen peroxide and lipid hydroperoxides [12].

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease [13]. More attention has been paid to the protective effects of natural antioxidants against compounds-induced toxicities especially whenever free radical generation is involved [14]. Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress [15].Flavonoids are group of polyphenolic compounds that occur widely in fruit, vegetables, tea and cocoas [16].

Tea is second only to water in popularity as a beverage., lollipops and ice. Fresh tea leaves are rich in flavanol monomers known as catechins such as epicatechins, which are 13.6 g/100 g in green tea and 4.2 g/100 gm dry weight in black tea [17]. Catechins have beneficial effects in prevention of cardiovascular diseases including LDL oxidative susceptibility, serum

lipids and lipoprotein concentrations [18]. In animal studies, some authors [4] revealed that green tea may protect liver and brain cells against of oxidative stress induced by ethanol intoxication. In a recent study, supplementation of green tea extract (GTE) attenuates cyclosporine A-induced oxidative stress in rats[19] . Also, it can be reduce the risk of colorectal inflammatory disease and muscle necrosis [20]. On the other hand, Chantre et al [21] revealed GTE can be effective for treatment of obesity. Some authors [22&23] suggested that the development of chewing gum fortified with green tea extract will be effective to oral cavity and periodontal disease**.**Some authors [24] recommended daily consumption of 10 cups of GTE (about 25 g green tea).

The aim of the present investigation is to determine the possible protective effect of green tea extract against oxidative stress inducing arthritis in rats. *The contribution of oxidative stress in a carrageenan - induced rat model of arthritis and the effect of GTE were assessed by histopathology and biochemically measuring the plasma nitric oxide, lipid peroxides and total thiols.*

Materials and methods

1-Chemicals:

Thiobarbituric acid, reduce glutathione naphthylenediamine dihydrochloride, sulphanilamide, sodium nitrite and all other chemicals were fine grade and obtained from Sigma (St. Louis, M.O., USA).

Green tea extract: The GTE was made by soaking 15 g of instant green tea powder in 1 L of boiling distilled water for 5 minutes[25]. The solution was filtered to make 1.5% green tea extract (GTE). This solution was provided to rats as their sole source of drinking water.

2-**Animals and experimental design**: The experiment was performed with 30 male albino rats weighing about 150-200 g. The animals were housed in plastic cages and provided with ad lib standard laboratory animal feed, and water.

They were randomly classified into three groups (10 rats per each) as the following:-

Group I (*Control*) received distilled water as sole drinking source for 3 weeks.

Group II *(arthritic group)* served as positive control with intraarticular injection of 0.1ml of 1% carrageenan solution for one week.

Group III (*-arthritic group + GTE group)* also injected with of 0.1ml of 1% carrageenan solution for one week and followed by green tea extract as sole drinking source during one week.

Animals of different groups were sacrificed under light anesthesia 1 day after the end of the treatment after one week. The blood sample were collected in heparinised centrifuge tubes and centrifuged to obtain plasma. The joints were excised immediately and washed in saline for histological examination.

3-Histopathological estimation:-

Specimens from the arthritic joints (knee)red, edematous and swollen ones were collected , fixed in 10% neutral buffered formalin solution. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin (H&E) stain.

4-Biochemical estimation

Lipid peroxide: Measurement of malondialdehyde (MDA) continues to be a useful method for determination of extent of lipid peroxidation, as it is the most abundant aldehyde formed as a by product during this process. The plasma levels of LPO were estimated as described else where [(26)].

Nitric oxide: Plasma Nitric oxide was determined as nitrite concentration after reduction of nitrate to nitrite. The reaction was performed at 22 °C for 20 min and the absorbance at 546 nm was measured using NaNO3 solution as standard [(27)].

Total Thiol: plasma total thiols was determined chemically [(28)].

5-Statistical analysis: The data were statistically analyzed using one way analysis of variance to compare the means of different treatment groups with that of control lead-exposed groups.

Results:

1-The plasma levels of lipid peroxides were significantly increased in group II(arthritic group) as compared to control group($p<0.001$).Concomitantly, there was no significant difference in plasma lipid peroxides between group III (green tea +arthritic group)and control group. The plasma nitric Oxide (NO) levels were significantly increased in the plasma in group II as compared to both groups I($p<0.05$) ,and group III ($p<0.05$).Non significance difference was found between group III and control one.

2 –Anti oxidant markers (Total thiol):
There were significantly increased in group III as matched to both groups II&I ($p<0.05$ and $p<0.02$ respectively).In comparison between group I and group II, there was no insignificant difference.
Table (1):Plasma levels of lipid peroxides, nitric oxide, total thiols in all studied groups.

Parameters	Control group (n=10)	Arthritic group (n=10)	Arthritic+ GTE group (N=10)	P-Value
Lipid peroxides (mol/mL)	0.758±0.118[(a)]	3.820±0.563[(b)]	1.995±0.676[(c)]	a versus b (p< 0.001) a vs c (NS) b vs c (NS)
Nitric Oxide (nmol/mL)	5.220±0.130[(a)]	9.116±1.402[(b)]	5.170±0.375[(c)]	a vs b (p< 0.05) a vs c (NS) b vs c (p < 0.05)
Total thiols (mol/L)	1.66±0.208[(a)]	1.897±0.318[(b)]	2.94±0.355[(c)]	a vs b (NS) a vs c (p< 0.02) b vs c (p < 0.05)

GTE: Green tea extract; NS: Non significant

3-Histopathologically, the cases treated with: Carrageenan alone showed sever degenerative as well as necrotic changes" Zene Ker's necrosis"of the underlying skeletal muscle of the arthritic joint:
that represented by cytoplasmic swollen,hypereosinophilia,nuclear pyknosis and mononuclear inflammatory cells infiltration besides congestion and edema.(Fig.1).
Marked synovial membrane hypertrophy as well as hyperplasia with formation of villi showed(Fig.2).Also the synovium showed edematous congestion and contains scattered lymphocytes, plasma cells, vesicular-nucleated-macrophages as well as hemosiderin-laden macrophages and multinucleate cells.Necrotic synovitis detected by nuclear pyknosis,cytoplasmic hypereosionphilia as well as nuclear mitosis.(Fig.3).

The articular cartilage (hyaline cartilage)of the arthritic joint suffered severe degenerative changes, erosive necrosis,congestion,mononuclear leucocytic cells infiltration as well as osteoclasts.The palasading arrangements was completely lost,eburnation and mineralization also seen(Fig4).Subchondral granuloma like(Nodule-like)structure also noticed.(Fig.5).While the cases treated with **Carrageenan plus green tea extract** showed mild degenerative changes represented by the following:-
1-Marked reduction in the number of the inflammatory cells infiltration in the synovial membrane comparing to that one treated with carrageenan only.
2-No cartilage as well as bone erosion ,mild mineralization or bone marrow lymphoid elements depletion as it showed near normal ones.(Fig.6&7).In the opposite ,the reactive osteocytes hyperactivation showed indicates a starting of the regeneration process of the arthritic joint. Finally,the control cases of the knee joint showed normally by naked eye examination as well as histologically. The all layers of the joints appeared normal in comparing to the cases treated with carrageenan or that carrageenan with green tea extract.

Discussion:

Oxidative stress has been implicated in the development of many diseases such as osteoarthritis. In the present study, the levels of oxidative stress indices (NO and lipid peroxides) were significantly elevated in arthritic rats as compared to control group, this results are in accordance with the view that the onset of arthritis is associated with an enhanced release of inflammatory mediator substances free radical like NO[(3)]. Moreover, some authors [(5)] suggest that the inflammation is known to result in increased production of NO and other oxidative stress indices & they reported that the NO has proinflammatory role in acute joint inflammation[(6)] . In addition, Some authors [(29)] observed that NO is produced in synovial fluid from arthritic joints, and treatments that block NO or NO synthetase may be useful in the treatment of osteoarthritis.
In this respect, the anthers noticed that the NO is implicated in the development of both acute &chronic inflammation and that NOs inhibitors have potential anti-inflammatory activity[(4)].
On the other hand , Some authors noticed that the plasma levels of lipid peroxides significantly increased in arthritic animal model, and they suggested that inflammation might cause enhanced membrane fragility in

arthritic rats , and they observed the development of clinical arthritis(as swelling&redness) [30]. Another authors [6], found that rats given carrageenan developed severe arthritis after one week. The results in our study are in accord with the view that the onset of arthritis is associated with an enhanced release of inflammatory mediator substances and free radical like NO and lipid peroxides.

In arthritic group, we observed all characters of arthritis as skeletal muscle necrosis, the joint capsule of the arthritic joints showed multifocal fat cysts infiltration, necrisis, congestion, perivascular edema with mononuclear cells infiltration. In adition, collagenalysis as well as fibroplastic proliferation also found.

Marked synovial hypertrophy with formation of villi showed, besides macrophages and multinucleate cells infiltration.Nicrotic synovitis also showed.

Some other cases showed complete destructive changes of the synovium The articular cartilage (hyaline cartilage) of the arthritic joint suffered severe degenerative changes, erosive necrosis,congestion,mononuclear leucocytic cells infiltration as well as osteoclasts.The palasading arrangements was completely lost,eburnation and mineralization also seen .Subchondral granuloma like(Nodule-like)structure also noticed. The previous mentioned results were in parallel lines with many investigators [31,32,33&34].

Similarly, previous studies [6] demonstrated that the histopathologic examination of some of the arthritic joints of mice revealed extensive cartilage and bone erosions with massive infiltration of mononuclear cells and fibroblasts. Furthermore, osteocytic necrosis, and mineralization as well as lymphoid element depletion of bone marrow also noticed..

This histological changes have been showed by some others [35], they suggested that the carrageenan increased edema, and proliferation of granulation tissue in acute arthritis.Moreover, the other [36], noticed that the carrageenan arthritis is associated with high turnover bone loss ,,in addition osteoclast number and eroded perimeters remained abnormally high. Our findings are supported by more recent studies [6] which showed cartilage damage with cellular filtration .As regards the histological examination in this group we observed marked reduction in the number of the inflammatory cells infiltration in the synovial membrane comparing to that one treated with carrageenan only, no cartilage as well

as bone erosion ,mild mineralization or bone marrow lymphoid elements depletion as it showed nearly to the normal ones. In the opposite, the reactive osteocytes hyperactivation showed indicates a starting of the regeneration process of the arthritic joint.

These results supported by many investigators [37&38] that suggested the green tea are chondroprotective and that consumption of green tea may be prophylactic for arthritis may benefit the arthritis patient by reducing inflammation and slowing cartilage breakdown. In addition some authors [39] demonstrated that the green tea inhibits the development of inflammation and cartilage damage in an animal model of arthritis. In accord with the view that the green tea appear to attenuate the severity of arthritis by way of decreasing the cellular infiltration of the joints and the concentration of both free radicals and their rate of degradation in the synovium. [40]

Furthermore, we observed the development of clinical arthritis (swelling,edamatous &redness)these results indicate that rats given carrageenan developed severe arthritis after one week. This results were supported by some investigators that found carrageenan induced rabbits models of arthritis[41].

In our study,-Arthritic group treated with green tea(Group III):showed that the levels of antioxidants as a total thiol were significantly increased as matched to both arthritic and control groups. An antioxidant rich polyphenolic fraction isolated from green tea has been shown to possess anti-inflammatory effects in experimental animals.[37] .In addition the others [38] suggested that the green tea has antioxidants and scavenge free radicals actions. Moreover, recent studies have demonstrated that green tea possess both anti-inflammatory and antiapoptotic properties in normal human cells. Some authors [38&42] demonstrated that the green tea may play a role in reducing stress and has antioxidant role. On the other hand the others[43] noticed that the green tea inhibits production of NO in human chondrocytes .Furthermore, green tea have been shown to reduce inflammation in arthritis and is rich in antioxidants may be useful in the prevention of onset and severity of arthritis [37&43].

In conclusion ,our results suggest that green tea rich in antioxidants reduce the frequency of injurious cells in the affected joints. These same joints also had significantly lower concentrations of oxidative stress markers . Our data thus provide documentation that the antioxidant of

green tea reduces the incidence and severity of carrageenan -induced arthritis in rats . Based on our data it is tempting to suggest that green tea in general may prove to be a useful supplement/addition with other agents for the treatment of arthritis .

Figures description:

Fig.(1):The underlying skeletal muscle, of the rat treated with carrageenan alone,showing:Zene Ker's necrosis and congestion.HάE-X.400.

Fig.(2):Synovial membrane, of the rat treated with carrageenan alone,showing:synovial hypertrophy and hyperplasia with formation of villous HάE-X.1 00.

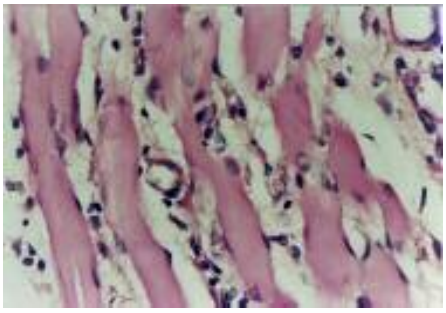
Fig.(3): Synovial membrane, of the rat treated with carrageenan alone,showing:scattered inflammatory cells infiltration, mostly lymphocytes,plasma cells,macrophages,siderocytes as well as multinucleated cells infiltration ,edema and congestion. HάE-X.400.

Fig.(4):Articular cartilage of treated rat with carrageenan alone, showing:erosive necrosis,chondrodysplasia,congestion,edema,mineralization and multinucleated giant cells infiltration. HάE-X.400.

Fig.(5):Arthritic joint, of rat treated with carrageenan alone, showing:granuloma like structure. HάE-X.400.

Fig.(6):Synocium,of rat treated with carrageenan plus green tea, showing; mild degenerative changes with mild inflammatory cells infiltration. HάE-X.2 00.

Fig.(7):Bone marrow,of rats treated with carrageenan plus green tea, showing;mild congestion and nearly bone marrow lymphoid elements. HάE-X.400.



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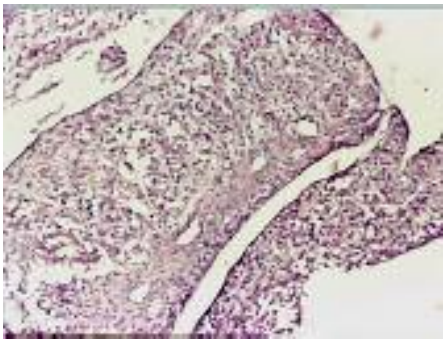

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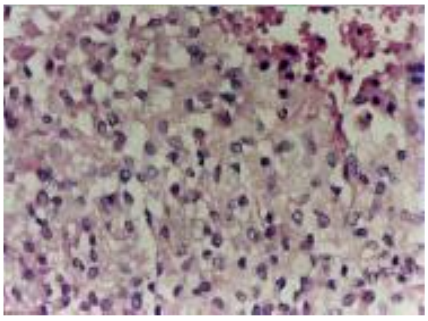

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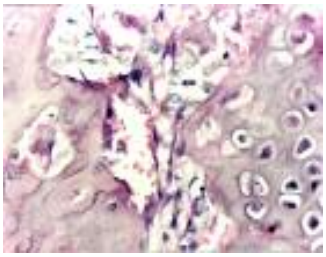

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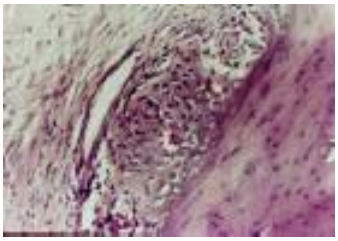

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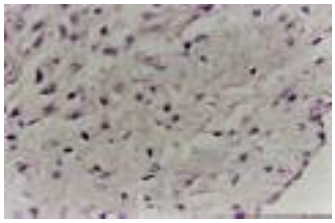

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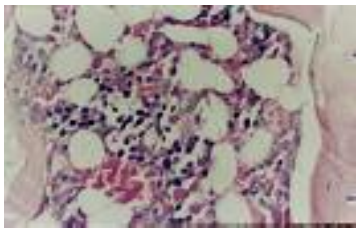

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