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Histological Study of Socket Healing after Tooth Extraction in Rabbits Treated with Short Term Diclofenac Sodium (Expermintal Study)

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الخلاصة

الأهداف: الغرض من الدراسة الحالية لبحث أذا كان إعطاء دواء دايكلوفيناك يتعارض مع أوقات تقدم التئام الجرح السنخي في الأرانب. **المواد وطرائق العمل:** العينة احتوت على اثنا عشر أرنب،ستة من الأرانب تلقوا حقن عضلي بدايكلوفيناك صوديوم (١٠ ملغ م/كيلوغرام/يوم)قبل القلع بيوم،وفي يوم القلع وبعد أربعة أيام من قلع القاطع السفلي الأيمن وستة أرانب تلقوا حقن عضلي بالمحلول الملحي طبيعي ذبحت لكل مجموعة بعد ٧ ،١٤، ٢١ يوم من قلع السن. **النتائج:** الدراسة الحالية تظهر إن تأثير العلاج بدايكلوفيناك صوديوم سبب تأثير غير معنوي في نقصان تكوين العظم السنخي عند الارانب. **الاستنتاجات:** الدراسة النسيجية للعلاج بدايكلوفيناك لمدة قصيرة بعد قلع السن سببت نقصان غير معنوي في تكوين العظم لالتئام السنخي في الأرانب.

ABSTRACT

Aims: The aim of the present study is to investigate if short term therapy with diclofenac sodium of interferes with the time course of alveolar wound healing in rabbits. **Materials and Methods:** The sample included twelve rabbits; 6 rabbits received interamuscular injections of diclofenac sodium (10 mg/kg/day), one day before extraction, at a day of extraction and 4 days after extraction of the right mandibular incisors and 6 rabbits received interamuscular injections of normal saline. The animals were sacrificed in each group at 7, 14 and 21 days after tooth extraction. **Results:** The present results showed that the effect of diclofenac treatment caused no signifcant decrease in bone formation of socket healing. **Conclusion:** Histological study of short term therapy with diclofenac sodium after tooth extraction caused non-significant decrease in bone formation of extraction sockets healing in rabbits.
Keywords: Diclofenac sodium , tooth socket, healing

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INTRODUCTION

Mandible anatomy: The mandible, "jawbone" forms the lower jaw and holds the lower teeth in place. Nerves supply**;** Inferior alveolar nerve, branch of the mandibular division of Trigeminal (V) nerve. The nerve divides into two terminal branches; incisive and mental nerves. The incisive nerve runs forward in the mandible and supplies the anterior teeth.[(1)]

Alveolar Process: is that bone of the jaws containing the sockets(alveoli) for the teeth. It consists of an outer(buccal and lingual) cortical bone, a central spongiosa, and bone lining the alveolus(alveolar bone).[(2)]

Bone Anatomy and Histology: Bone is a dynamic biological tissue compose of metabolically active cells that are integrated into a rigid framework. The cellular components of bone consist of osteogenic precursor cells, osteoblasts, osteoclasts, osteocytes, and the hematopoietic elements of bone marrow. Osteoblasts are mature, metabolically active, bone forming cells. Osteocytes are mature osteoblasts trapped within the bone matrix. Osteoclasts are multinucleated, bone resorbing cells controlled by hormonal and cellular mechanisms. There are three primary types of bone; woven bone, cortical bone, and cancellous bone. Osteons consists of cylindrical shaped lamellar bone that surrounds longitudinally oriented vascular channels called haversian canals. [(3,4)]

Bone Healing Process:

The bone healing can either directly as primary bone healing or secondarily (in direct) demonstrating an intermediate cartilaginous phase. Phases of bone healing 1) Inflammatory phase 2) Reparative phase 3) Remodeling phase.[(5)]

Non steroidal anti-inflammatory drugs: are drugs with analgesic and anti-

pyretic (fever-reducing) effects and which have, in higher doses, anti-inflammatory effects.[6] *Diclofenac Na.*: marketed as Voltaren is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury. It can also be used to reduce menstrual pain, dysmenorrhea . The name is derived from its chemical name: 2-(2,6-dichloranilino) phenyl acetic acid. Formula: C14 H11CL 2NO2. [7]

Mechanism of action: The exact mechanism of action is not entirely known, but it is thought that the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX) and it appears to inhibit DNA synthesis. [8]

Indications: Diclofenac is used for musculoskeletal complaints, especially arthritis, rheumatoid arthritis, polymyositis, dermatomyositis, osteoarthritis, dental pain, TMJdysfunction, spondyl arthritis , ankylosing spondylitis, gout attacks, and pain management in cases of kidney stones and gallstones An additional indication is the treatment of acute migraines. Diclofenac is used commonly to treat mild to moderate post-operative or post-traumatic pain, particularly when inflammation is also present, and is effective against menstrual pain and endometriosis.[9]

MATERIALS AND METHODS

Twelve rabbits average weight (500 - 1000) gram were anesthetized with an intra muscular injection of ketamine (Hcl) (10mg/kg body weight), and xylazine(0.15ml /kg body weight). [10]The right mandibular incisors were extracted with forceps after disconnection of the surrounding gingiva and luxation with an enamel hatchet. Immediately after extraction , a single intramuscular dose of antibiotic oxytetracycline 0.2 ml/rabbit; was given. [11] The sample included the treated group (six rabbits), received intramuscular injections of diclofenac sodium (10 mg/kg/day) one day before extraction, at day of extraction and 4 days after extraction of the right mandibular incisors and six rabbits received the same volume of normal saline by intramuscular injections. The animals were sacrificed in each group at 7, 14 and 21 days (n=2per group) after tooth extraction, and mandibles were immersed in 10% formalin for 48 hours. After fixation, the mandibule was dissected and divided along the median sagittal plane and decalcified, and processed for paraffin embedding. Longitudinal semi serial 6-µm thick sections were cut at intervals of 60-µm and were stained with hematoxylin and eosin. The microscopical finding included granulation tissue and area of old and new bone during alveolar socket healing. Measuring area of granulation tissue by image J soft ware used for the digital assessment of the healing area. All sections were captured digitally using a generic digital camera mounted on alight microscope magnification at 10x,40x. Measuring of area of bone formed using the measuring rule of the visopan projection microscope (Reichert A G) at 50x, magnification at 4/0.10 ,manufacture in Austria.[12]

RESULTS

The clinical manifestations:

The results showed that all animals remained clinically healthy, non of the rabbits died or loss weight with normal healing of the extraction site.

Histopathological examination:

The histopathologic examination was performed and the area of socket of the control and experimental specimens were examined. The different phases of alveolar wound healing observed using histological examination 1 to 3 weeks after tooth extraction.

*At the end of the first week***:**

Control group (C1): The alveolus of control animals was filled with many dilated blood vessels and granulation tissue which contain blood vessels, fibroblasts, and chronic inflammatory cells with predominate osteoblastic activity and newly formed trabecular bone was observed mainly on the internal surfaces of the alveolar socket and remnant of the blood clot observed in the apical third (Figures 1A and 3).

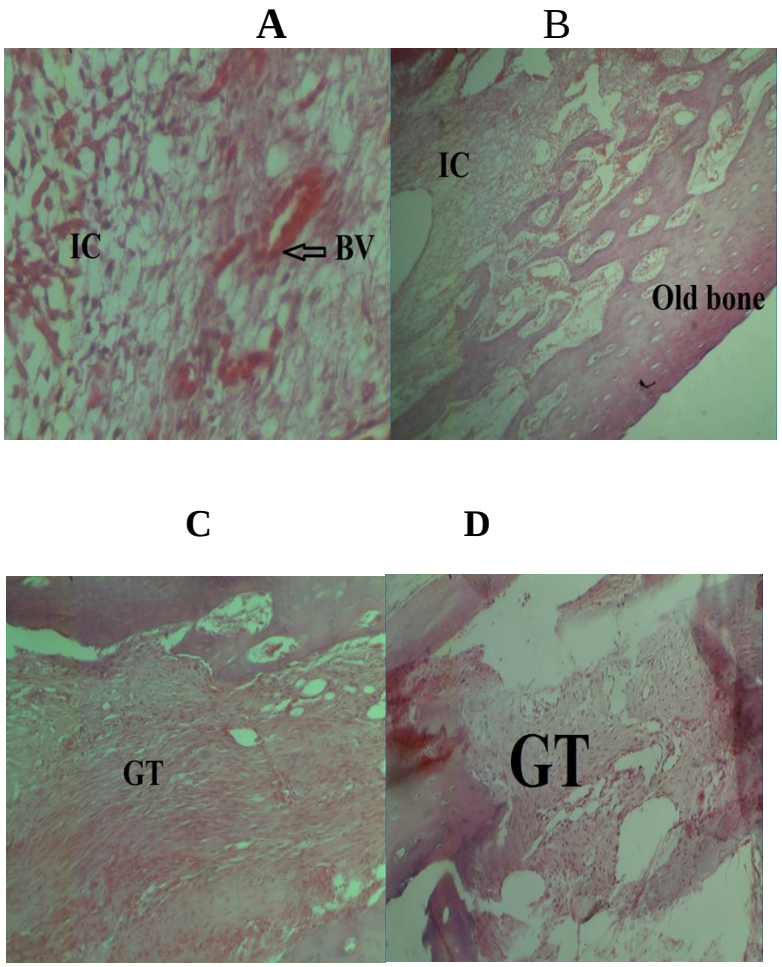


Figure (1): Microphotograph for histological section of alveolar wound healing of control group (A) at the end of the first week, control group (C) at the end of the second week (10X). (B) experimental group at the end of the first week, (D) experimental group at the end of the second week (10X) . IC= inflammatory cells, BV = blood vessels, GT= granulation tissue

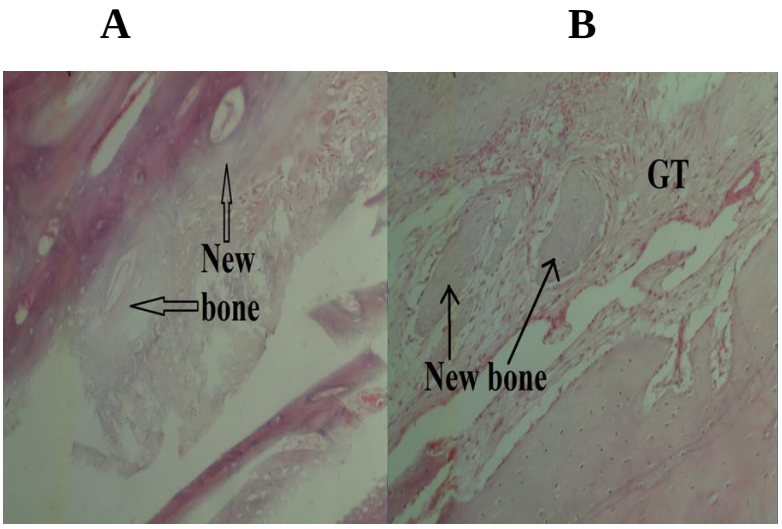


Figure (2): Microphotograph for histological section of alveolar wound healing of control group (A) at the end of the third week, (B) experimental group at the end of the third week, (10X) , GT= granulation tissue.

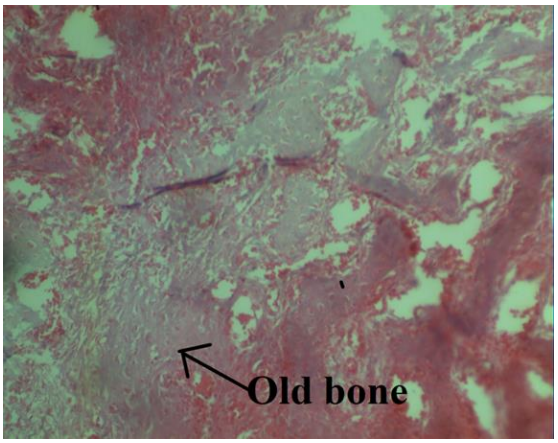


Figure (3): Microphotograph for histological section of alveolar wound healing of control group at the end of the first week (40X) .

Experimental group (E1): More blood clot formation and more amount of granulation tissue and small amount of new bone formation (Figures 1B and 6).

At the end of the second week:

Control group (C2): There is fibrous tissue formation and moderate amount of granulation tissue, but the bone trabecule were more than that observed in E2 (Figures 1C and 4).

Experimental group (E2): More fibrous tissue formation and hemorrhage and more amount of granulation tissue than C2 but bone formation is less than that in C2 (Figures 1D and 7).

At the end of the third week:

Control group (C3): Few fibrous tissue and the area filled with new bone (Figures 2A and 5).

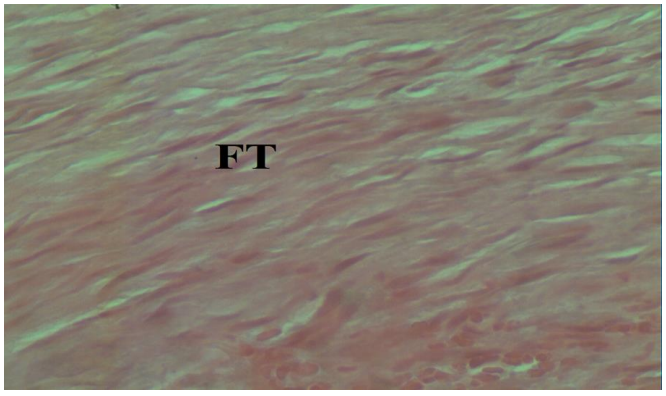


Figure (4): Microphotograph for histological section of alveolar wound healing of control group at the end of the second week (40X) .FT=fibrous tissue.

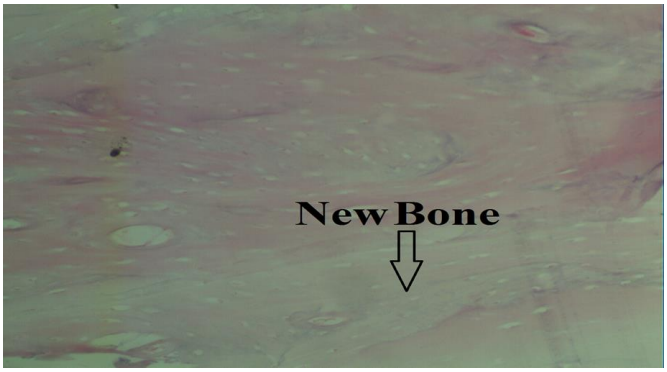
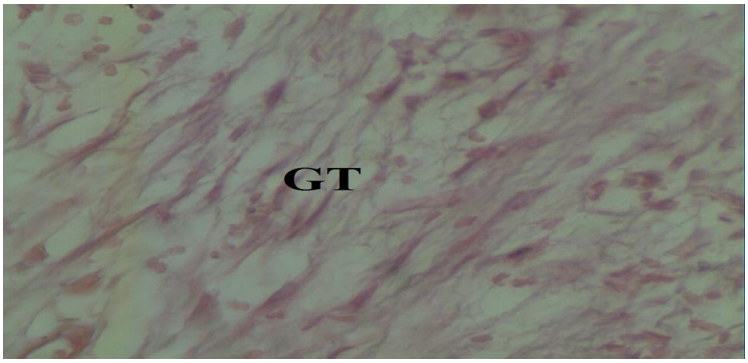
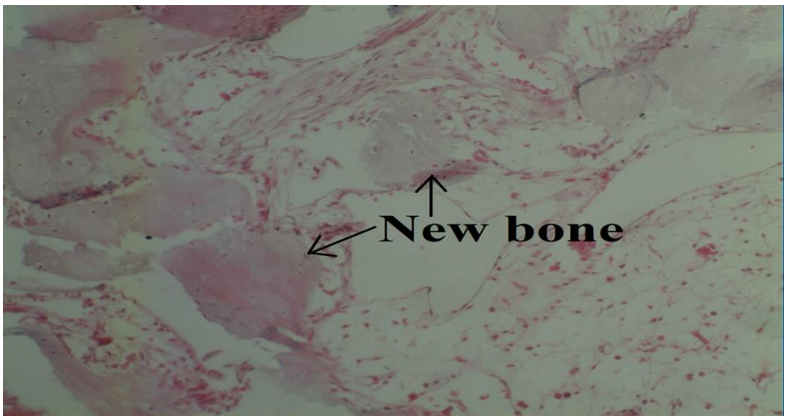


Figure (5): Microphotograph for histological section of alveolar wound healing of control group at the end of the third week (40X).



Figure(6): Microphotograph for histological section of alveolar wound healing of experimental group at the end of the first week (40 X) . GT = granulation tissue



Figure(7): Microphotograph for histological section of alveolar wound healing of experimental group at the end of the second week (40 X).

Experimental group (E3): high amount of fibrous tissue with a number of dilated blood vessles and granulation tissue started to accumulate more than what was seen in C3 which may mean slow down the process of bone healing in E3 (Figures 2B and 8).Histological study of therapy with Diclofenac treatment after tooth extraction caused non significant decrease in bone formation of socket which was more prounced at the end of third week.

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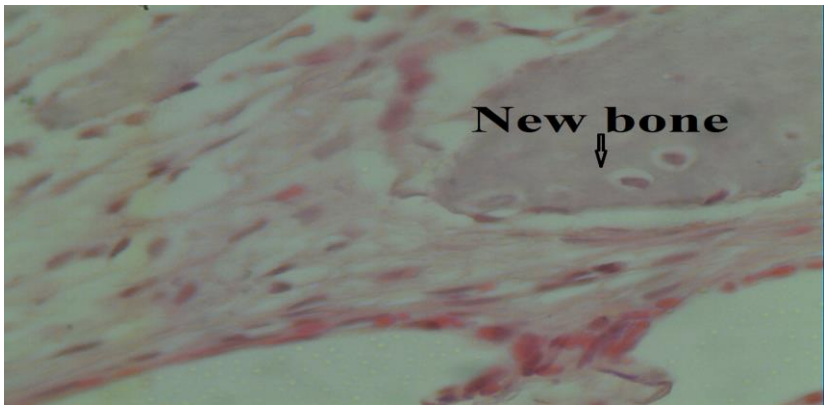

Figure (8): Microphotograph for histological section of alveolar wound healing of experimental group at the end of the third week (40 X).

Statistical analysis for histopathological findings:

The mean bone trabacule of experimental groups was less than control groups. In all three time intervals when bone area was measured by measuring rule of the visopan projection microscope (Reichert A G) at 50x magnification but this difference was non significant in the first and significant in the second weeks using student،s T test, to determine whether difference was significant or not.

After three weeks the results showed non significant(P value > 0.05)decrease in bone formation in the experimental group compared with the control group using student،s T- test (Tables 1 and 2).

Table (1): means, std. deviation of the healing bone area of the different groups at 1, 2, 3 weeks.

Groups number	No.	Mean of bone area inmicron	Std. deviation
E1	2	416.65000	23.546656
C1	2	716.65000	164.968012
E2	2	466.60000	0.000000
C2	2	783.30000	70.710678
E3	2	983.30000	117.803990
C3	2	1266.6500	94.257334

E= experimental group, C=control group, No.= Number of the samples

Table (2): Compares the means of bone healing area between experimental groups and control group1, 2and 3weeks after tooth extraction.

Groups number	Groups name	t	DF	p-value
1	E1αC1	-2.546	2	0.126 NS
2	E2αC2	-6.334	2	0.024 S
3	E3αC3	-2.656	2	0.117 NS

DF= degree of freedom, T= t test, Statistic value , NS= non significant, S = significant.

The mean of granulation tissue of experimental groups more than control groups but this difference is non-significant in the first and significant in the second weeks. by using student،s T- test.

After three weeks the results showed non-significant(P value > 0.05) increase area of granulation tissue in experimental group compared with control group by using student،s T test . Which mean slowdown of the process of bone healing which is less than that seen in control group (Tables 3, 4).

Table (3) Means ،std .deviation of the granulation tissue area of the different groups at 1,2,3 weeks.

Groups number	No.	Mean area of granulation tissue	Std. deviation
E1	2	180.86400	2.446589
C1	2	81.25900	9.357592
E2	2	216.97000	0.502046
C2	2	174.08950	5.446844
E3	2	169.26150	7.708171
C3	2	155.47200	39.597980

E= experimental group, C= control group, No.= Number of the

Table (4): Compares the means of granulation tissue area between experimental groups and control group1,2and 3weeks after tooth extraction.

Groups number	Groups name	t	DF	p-value	
1	E1αC1	1.477	2	0.278	NS
2	E2αC2	11.086	2	0.008	S
3	E3αC3	0.483	2	0.677	NS

DF= degree of freedom, T= t test, Statistic value , NS= non significant, S = significant.

DISCUSSION

The present results showed that the effects of early NSAID administration can persist and be measured weeks after treatment has ended. There is also evidence that NSAID induced inhibition is proportional to the duration of treatment, and that bone can heal normally once NSAID administration is discontinued. [13-16]

While NSAID negatively affect bone healing as indomethacin.[16,17] There is insufficient evidence to support with holding NSAID after tooth extraction. Simon and others [18] included in their study a group of 23 rats that were treated with celecoxib for five days before femoral fracture in order to investigate whether NSAID would inhibit bone healing if discontinued at the time of bone trauma . This pretreatment with celecoxib had little to no effect on fracture healing, suggesting that patients chronically taking NSAID, such as patients with osteoarthritis, would not suffer diminished bone healing as a result of the drug.

The present results showed that at the end of the first and second week non significant effect of diclofenac Na in bone formation between experimental and control groups. This result coincided with Akman *et al*. When they found the effect of diclofenac sodium, IM administered at daily doses of 1mg and 2mg, on the repair process of tibia closed fracture in 55 Wistar rats that were sacrificed after 2, 4 and 6 weeks for clinical-radiological and histological analyses, after two weeks, bone callus in control group animals was more stable than that in animals treated with diclofenac sodium. Radiological and histological analyses did not show any difference among groups in the subsequent periods.[19]

Keller showed the indomethacin had no effect on the activated remodeling process in cortical bone neighboring a small drill hole or on remodeling in non traumatized cortical and cancellous bone**,** in the first and second weeks show moderate amount of granulation tissue in experimental groups when compared with control groups diclofenac treatment caused significant decrease in bone formation which more prounced in third weeks and significant increase in the amount of granulation tissue which mean slow down the process of bone healing this concide with result of Burd.[20-21]

The present results showed the short term therapy with diclofenac after tooth extraction caused non significant decrease in bone formation of socket healing this coincided with the results of Krischak *et al*. [22] when taken calluses from rats given diclofenac for seven days after osteotomy were not histologically different than calluses from rats given placebo when evaluated at 21 days, whereas calluses from rats treated for the entire 21 days contained significantly less bone and more cartilage.

Also Dimmen *et al*. when used parecoxib given for seven days only transiently decreased bone mineral density after tibial fractures in rats , bone mineral density returned to normal by three weeks.[23]

Reikeraas& Engebretsen used ketorolac (1 mg/kg/day) given for three days and showed no effect on callus mechanical strength measured six weeks after fracture in rats.[24]

Gerstenfeld *e t al*. used ketorolac given at a higher dose (4 mg/kg/day) and for the entire time course of the experiment and showed reduced mechanical strength and decreased histological scores of fracture calluses compared with untreated rats.[25]

Similarly, the authors of an experiment involving spinal fusion in rabbits concluded that while indomethacin reduced spinal fusion in their model (validating their study as one that can detect spinal fusion inhibition), celecoxib had no significant

effect.[26]

All these studies suggest that the effects of short-term treatment with NSAID were reversible.[19,24,25,27]

Many other local and systemic factors can affect bone healing, making it difficult to isolate the independent effect of NSAID in a clinical setting. Local factors include type of fracture, fracture gap, the presence of infection or debris, and the degree of vascularisation. Systemic factors include age and gender, metabolic and nutritional status, concurrent disease (endocrinopathies, neoplasia, immunodeficiency, chronic inflammatory disease, primary musculoskeletal disease), drug administration (corticosteroids, chemotherapeutic agents, antibiotics, anticoagulants, bisphosphanates, smoking), and others. [28]

CONCLUSION

Short term therapy with diclofenac sodium after tooth extraction in rabbits caused non significant decrease in bone formation of socket healing when examined histologically.

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