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CENTRO DE CIÊNCIAS DA VIDA E DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE E COMPORTAMENTO

Efeito protetor da Curcumina na resposta tecidual a placas de silicone em ratas.

Protective effects of curcumin in tissular response around silicone disks in rats.

Projeto de pesquisa elaborado para o Mestrado em Saúde e Comportamento da UCPEL, sob a orientação da Profª Gabriele Ghisleni e Co-orientação da Prof Dra Elizandra Braganhol

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1. Introdução

A utilização do implante de silicone teve inicio no final da década de 60. Desde então, as cirurgias de implante de silicone mamário tornaram-se cada vez mais frequentes e populares, com as modernas próteses de silicone. Com a crescente utilização destes materiais nas cirurgias reparadoras e estéticas das mamas e em outras partes do corpo, o interesse pelas reações teciduais destes materiais nos tecidos tem sido motivo de estudo para entender as suas principais complicações: a contratura capsular e a perda da integridade do implante[15]. A contratura capsular é uma complicação cirúrgica importante, resultado de uma resposta fibrótica tecidual no processo inflamatório local, ocorrendo em 4 a 17% dos casos quando encontra-se em contato com o tecido mamário[8,625].

O implantes de silicone são materiais aloplásticos, com importante inércia biológica e propriedades hidrofóbicas, o que impede que agentes químicos e enzimáticos do organismo provoquem a sua degradação[15]. O envoltório que compõe estes implantes pode ser de superfície lisa, de superfície texturizada (rugosa) e de superfície de poliuretano, sendo as de superfícies texturizada (rugosa) e de poliuretano as mais utilizadas atualmente[9,13,15]. Dentro das superfícies texturizadas existem os implantes com espessura fina ou média relacionadas à densidade do silicone e à sua trama.

A resposta do organismo ao implante de silicone produz uma cápsula fibrótica, que pode estar relacionada ou não ao tipo de superfície que envolve o implante[8-10,13-15]. A cápsula formada pode se tornar espessada e endurecida como efeito da

prolongada resposta inflamatória ao implante, o que pode levar de 6 meses a 2 anos, quando ocorre [29]. Esta resposta, com conhecida ação dos leucotrienos e das citocininas que modulam a capsulogênese e a reação fibrótica, tem sido alvo de estudo na geração de novas drogas com atividade anti-inflamatória específica [29].

O uso do implante de silicone com superfície texturizada tem mostrado uma redução na incidência de contratura capsular, ao contrário da prótese com superfície lisa. Os mecanismos que envolvem a contratura da cápsula dizem respeito ao alinhamento das fibras de colágeno que fazem parte do tecido capsular, em que nas próteses de superfície texturizada originam forças contráteis multidirecionais que neutralizam a ação destas mesmas forças, reduzindo o risco da contratura [9,14,15], quando comparada à prótese de superfície lisa. Porém, quando comparada à prótese com superfície de poliuretano, a superfície texturizada não apresenta a biodegradação e a formação de microcápsulas, que a prótese de poliuretano tem ao mostrar uma resposta mais exacerbada[6].

Estudos experimentais envolvendo ratos e coelhos tem sido realizados para melhor entender a resposta tecidual à superfície do implante [7,12-15,23,24,29,]. Estudos em humanos tem demonstrado que a escolha do tipo de prótese, a técnica operatória, o local de posicionamento do implante, a presença de hematoma e de infecções influenciam nas respostas inflamatórias exacerbadas aos implantes de silicone[7,12-15,23,24,29]. O uso de fármacos tais como Vitamina E, Prednisona e os inibidores dos leucotrienos tem sido estudado ao longo dos anos como tendo provável efeito inibidor da resposta fibrótica ao implante mamário[5,7,16,24,28,29].

A Curcumina (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, um componente polifenólico, natural, isolado do rizoma Curcuma longa

Linn, tem demonstrado potencial propriedade antioxidante, antiinflamatória e anticancerígena, vem sendo amplamente estudada nos processos inflamatórios em geral e também nos fibróticos [2,18,20,27]. A sua principal ação está relacionada à suas propriedades antioxidadnte e antinflamatória in vitro e in vivo. Esta substância é capaz de modular os processos inflamatórios ao suprimir fatores inflamatórios específicos, ao inibir a cicloxigenase 2 (cox2), o efeito do AP-1 e dos fatores de transcrição do NF-kB e na proteínaquinase [2,20,27].

Ao considerar o potencial efeito inibidor da curcumina no processo inflamatório, na fibrose e na produção da matriz extracelular, se torna de fundamental interesse a investigação desta substância na resposta tecidual que envolve a inclusão dasplacas de silicone.

O presente projeto tem por objetivo investigar o efeito da curcumina no processo inflamatório local envolvido na resposta à inclusão de placas de silicone em ratas; como também, comparar a resposta inflamatória tecidual entre as placas com texturas diferentes.

2. Fundamentação Teórica

2.1 Implantes Mamários

As próteses de silicone são materiais aloplásticos, que têm por característica uma inércia biológica devido às suas propriedades hidrofóbicas, sendo envolvidas por um elastômero de silicone e preenchidas por gel de silicone e/ou soro fisiológico [15]. A superfície que envolve a prótese pode ser lisa, apresentar rugosidades mais finas: como poros, depressões, nódulos ou

[1]pilares sendo então denominada texturizada e apresentar rugosidades mais profundas com uma cobertura esponjosa e microporosa de poliuretano [15].

Os primeiros implantes mamários possuíam uma superfície lisa, mas atualmente tem se utilizado implantes texturizados e de poliuretano, os quais contêm características diferentes em suas superfícies [15]. As superfícies texturizada e de poliuretano podem estar relacionadas a uma menor incidência de contratura capsular, ao considerar que o crescimento tecidual na estrutura microporosa origina forças contráteis multidirecionais, com tendência à neutralização entre elas quando o seu efeito se soma ao do implante, desta forma impedindo a retração da cápsula que envolve o implante [15]. Na superfície lisa as fibras de colágeno estariam dispostas paralelamente e circularmente ao redor da prótese, promovendo o envolvimento de forças contráteis concêntricas que levariam à contratura [15]. Estudos têm demonstrado que superfícies texturizadas retardam ou diminuem a incidência de contratura capsular pela ação destas forças [12,23,24].

A cavidade tecidual que aloja o implante se comporta como uma ferida cirúrgica, desenvolvendo um processo reparador local, com a formação de uma cápsula fibrosa avascular ao redor do implante [15]. A reação local inicial ao implante é o principal fator que leva à contratura da cápsula, caracterizada por deposição aumentada de matriz extracelular (ECM), esta deposição e proliferação é induzida pela ação das citocinas e de seus receptores, e pode desenvolver um tecido conjuntivo com propriedades fibrogênicas [15].

2.2 Resposta tecidual aos implantes mamários

É comum observar à volta do implante mamário um tecido conjuntivo denso de fibrina e de colágeno ou um tecido conjuntivo fibrovascular contendo granulomas ou

células gigantes do tipo corpo-estranho, macrófagos e linfócitos que são compatíveis à resposta inflamatória, como uma resposta imune local ao implante mamário [28]. Além destes tipos celulares se tem identificado outros, tais como células plasmáticas, células de tipo sinovial, fibroblastos e miofibroblastos [9,13,15,,24,26,28].

As primeiras células identificadas no processo inflamatório agudo após inclusão do implante, entre 24-48 horas, são os neutrófilos, os macrófagos e os monócitos que se transformam em células gigantes multinucleadas quando se tem material de corpo estranho no processo, de tamanho grande e não degradável. As próteses mamárias de superfície microporosa, como as texturizadas, provocam uma reação tecidual crônica envolvendo as partículas de silicone [15].

No tecido capsular, a fibrose exacerbada pode se desenvolver durante os primeiros meses de pós-operatório, ou ainda um ou dois anos após a cirurgia [24]. O tecido fibroso formado ao redor da prótese promove assim sua compressão, podendo comprometer a estética mamária (com distorção do formato, assimetrias e endurecimento da mama) e desencadear sintomas dolorosos [1,11,13] , em uma ou em ambas as mamas [22]. A etiologia que desencadeia a contratura capsular é multifatorial, envolvendo a difusão do gel de silicone através do envoltório da prótese, eliminação de partículas de silicone do envoltório da prótese, infecção, hematomas e seromas, fatores imunológicos, miofibroblastos, posição anatômica das próteses e superfície das próteses [15].

Um estudo experimental em coelhos mostrou, na avaliação histológica, diminuição na proporção do colágeno do tipo III quando comparado ao do tipo I na cápsula de implantes texturizados, com uma significativa resposta inflamatória ao redor deste implante em relação ao implante liso [9]. Outros estudos, também em coelhos,

revelaram que as cápsulas dos implantes texturizados foram mais espessas do que as cápsulas de implantes lisos [5]. Estudos comparativos entre roedores e humanos revelaram alterações histológicas do tecido à volta da cápsula que envolve o implante, como o espessamento tecidual, aumento do infiltrado celular, aumento no número de fibroblastos e de miofibroblastos[1,6,9,11,12,26,28]. Ao tentar estudar a fisiopatologia e o possível tratamento da contratura capsular, modelos animais têm sido desenvolvidos. Os estudos experimentais que melhor reproduzem em curto período de tempo o desenvolvimento da contratura capsular foram realizados em ratos, como modelo experimental [5-7,11,29]. Avaliações histoquímicas e de pressão intraimplante têm complementado a investigação a respeito das alterações promovidas pelo implante mamário nos modelos animais, demonstrando ações semelhantes às causadas em humanos[7].

2.3 *Curcumina*

O uso de plantas medicinais para o tratamento de doenças está associado ao folclore de diferentes partes do mundo, estas plantas contém inúmeras substâncias com propriedades biológicas importantes[21,22,23,24,25,26]. O interesse nestas plantas visa encontrar novas drogas com propriedades menos tóxicas para os humanos e que sirvam no tratamento de diversas doenças[21,22,23,24,25,26].

A Curcumina é um rizoma originado da C*urcuma longa L,* que deriva da família Zingiberaceae, distribuída nas regiões tropicais e subtropicais, sendo cultivada nos países asiáticos, principalmente na India e na China. A curcumina, recebeu este nome *no século 19*, sendo utilizada na medicina local dos países asiáticos, pelo seu importante

efeito anti-inflamatório. O interesse nesta planta se desenvolveu durante os anos pelos seus conhecidos efeitos farmacológicos: ação antiinflamatória, antioxidante e anticancerígena **[26].**

A importante ação anti-inflamatória dos componentes da Curcuma longa L foram examinados por Srimal e Dhawan (1973)**[26]**, estes autores relataram que a curcumina foi efetiva nos modelos agudos e crônicos de inflamação, sendo mais potente nos processos agudos e menos tóxica quando comparada à phenilbutazona**[26]**. Ammon et al demonstraram a ação da curcumina como inibidor da formação dos leucotrienos**[26]**, inibição da ciclooxigenase 2 (COX2) e efeitos no fator de transcripção do NFkB (fator de necrose tumoral**)[24,25,26]**. Em um estudo sobre a ação da curcumina no quelóide se demonstrou que esta inibe a cascata do fator de necrose tumoral B (TGF-B) e diminui a expressão da matriz extracelular nos fibroblastos**[37]**. Os efeitos inibitórios na proliferação de células mononucleares e músculo liso vascular também foram avaliados na formação dos leucotrienos e dos neutrófilos polimorfonucleares, em ratos **[24-26]**. Estudos experimentais envolvendo ratos utilizaram a curcumina oralmente na dose de 1 a 5mg/Kg não mostrando efeito adverso aparente, sendo excretada nas fezes e na urina em 75%, e ainda rapidamente metabolizada na circulação **[24-28]**.

A inibir a peroxidação dos lipídios, a curcumina exerce um papel importante no processo inflamatório, desta forma mantendo as atividades antioxidantes de enzimas [2]como a superoxidase dismutase, a catalase e a glutathiona peroxidase, em níveis elevados e reduzindo o dano tecidual**[25,26,27,28]**.[3]

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3. Justificativa

A resposta inflamatória ao implante mamário pode ser uma resposta intensa, capaz de levar a complicações, como a fibrose sintomática da cápsula que envolve o implante. Atualmente, o tratamento definitivo para esta fibrose é cirúrgico[18]. Tratamentos farmacológicos têm sido utilizados na forma de prevenir e tratar o processo fibrótico, porém, as medicações atualmente disponíveis são escassas ou encontram-se em teste.

4. Objetivos

O objetivo deste estudo é avaliar as modificações estruturais e a resposta inflamatória em um modelo animal submetido ao implante de placas de silicone, como também, verificar o efeito da Curcumina como potencial droga antinflamatória e antioxidante protetora da fibrose capsular.

Objetivos específicos:

- Comparar as modificações estruturais e a resposta inflamatória em dois tipos de placas de silicone, a texturizada fina e a texturizada média, através de análise histológica e imunohistoquímica;

- Verificar o efeito da curcumina como potencial droga nas modificações estruturais e na resposta inflamatória.

- Avaliar o perfil pró-inflamatório com as dosagens séricas de IL-1 e TNF-α entre os grupos controle e tratados com a curcumina.

5. Hipóteses

- As próteses de textura média estão relacionadas a um aumento da espessura e do infiltrado inflamatório celular da cápsula que envolve o implante quando comparadas às próteses de textura fina.

- O uso da curcumina irá modificar o infiltrado inflamatório, promover menor número de células inflamatórias, menor reação tecidual, e assim formar uma cápsula menos densa. O uso da curcumina se mostra eficaz na prevenção da formação da fibrose capsular, apresentando maior potencial terapêutico.

- Animais com implante mamário tratados com curcumina apresentarão uma resposta inflamatória menos intensa e menor estresse oxidativo.

6. Material e métodos

6.1. Animais

Utilizaremos ratos Wistar fêmeas adultas , pesando entre 200-250g, de 60 dias, provenientes do biotério da Universidade Federal de Pelotas -UFPEL. Todos os procedimentos com animais serão realizados nos laboratórios do biotério da UFPEL. Os animais serão manipulados de acordo com o protocolo de animais e guia para cuidados

e uso de animais de laboratório (Institute for Laboratory Animal Research, 1996) e de acordo com os princípios éticos do colégio brasileiro de animais para experimentação. Não existe associação comercial por parte dos autores que possa criar qualquer conflito de interesse com as informações aqui colocadas **[15]**.[4]

O cálculo da amostra para experimentos em animais será realizado conforme protocolo previamente descrito pelo laboratório de experimentação animal da USP [7], para obtenção de resultados estatisticamente significativos ($p<0,05$) entre o grupo controle e o tratado, com coeficiente de variação (CV) 15% ou 20%. Os animais serão mantidos em um ciclo claro-escuro (12/12h), com livre acesso a comida e a água. Todos os animais deverão ser anestesiados para os procedimentos via intraperitoneal (i.p) com ketamina 50mg/Kg e Xilazina 7,5mg/Kg - uso veterinário VIRBAC, São Paulo, Brasil. Estes serão medicados e observados por um período de 30 dias. Após este período, serão anestesiados e decapitados.

Este estudo será realizado sob as normas de ética para pesquisa em modelos animais conforme o preconizado pela Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL/COBEA) cumprimentando a Lei Nº 11.794, de 08 de outubro de 2008 e a Constituição do Estado Lei nº 11.915, artigo 82, inciso IV de 21 de maio de 2003 1-4, com cuidados especiais para a utilização do menor número de animais e para manejo da dor e sofrimento durante os procedimentos e eutanásia. O projeto foi aprovado pelo Comitê de Ética em Pesquisa Animal da UFPEL.

6.2. Procedimento cirúrgico

Para as cirurgias utilizaremos placas de silicone do tipo textura fina e textura média com 3,0 cm de diâmetro, produzidos e doados pela Lifesil do Brasil (Paraná). Durante o procedimento cirúrgico faremos uma incisão de 2,0cm na região dorsal perpendicular à linha média, sob o panículo adiposo, abrindo um túnel. Do lado direito será introduzida meia placa de textura fina e do lado esquerdo meia placa de textura média. Os animais serão suturados, com pontos continuos de mononylon 4.0 e mantidos sob cuidados durante o período pós-operatório e separados em grupos.

6.3. Tratamentos

Os animais serão divididos aleatoriamente após a cirurgia em três grupos experimentais: 1) grupo com placas de silicone e com o DMSO (veículo); 2) grupo com placas de silicone, tratados com curcumina 25mg/Kg de peso diluído com DMSO; 3) grupo com placas de silicone, tratados com curcumina 50mg/Kg de peso diluído com DMSO. Todos os tratamentos administrados por gavagem das substâncias e do veículo, 7 dias na semana durante um período de 30dias[32]. Após 30 dias os animais serão anestesiados e decapitados. As cápsulas ao redor dos implantes retiradas com as placas de silicone em seu interior e amostras de tecido hepático coletadas.

6.4. Avaliação bioquímica

O sangue periférico será coletado por punção cardíaca e o perfil pró-inflamatório (IL-1;TNF-a) analisado no soro de todos os grupos animais pelo método ELISA usando kits comerciais (DuoSet ELISA Development, R&D Systems, Inc., USA), em um leitor de microplacas (Molecular Devices, Espectra Max 190).

6.5. Avaliação histológica

Após os tratamentos por 30 dias, os animais serão sacrificados e as cápsulas dissecadas, mantendo as placas de silicone no interior capsular[22]. Cada cápsula deverá ser colocada em um frasco contendo formol e este identificado pela posição e conforme o grupo a que corresponde: sendo a da direita a placa de textura fina e a da esquerda a placa de textura média. As análises histológicas serão realizadas por técnico. No preparo histológico, o tecido deverá ser inicialmente fixado com formalina, após desidratado e infiltrado com parafina. Os blocos de parafina serão cortados no micrótomo em fatias de 5 μm, e as fatias colocadas em lâminas aderidas com albumina e tratadas com xilol pra remoção da parafina. De um simples bloco de parafina obteremos de 10 a 20 seções consecutivas com um intervalo de 200 μm.

O tecido será reidratado com álcool e água, a após corado com Hematoxilina e Eosina (HE) para as análises. Serão avaliadas nas marcações com HE: a espessura da cápsula, medida em milímetross, a intensidade do processo inflamatório local pela presença celular de células gigantes, número de vasos e fibroblastos, bem como a espessura da camada que compõe a cápsula ao redor do implante **[11,13,14,16-19]**. A análise será realizada pela identificação e quantificação em microscópio eletrônico, com o uso de contador manual e do programa de imagem ImageJ.

6.6. Análise estatística

Os dados serão digitados e analisados no programa GraphPad Prism 5.0. Para a comparação dos parâmetros avaliados entre os grupos será realizado o teste de ANOVA seguido pelo *post-hoc* de *Tukey*. Todos os resultados deverão ser apresentados como média± . Valores de $p \leq 0,05$ foram considerados estatisticamente significativos.

7. Cronograma

	2012		2013	
Atividades	1º semestre	2º semestre	1º semestre	2º semestre
Revisão bibliográfica				

Submissão ao Comitê de ética				
Qualificação do Projeto				
Cirurgias				
Tratamento				
Análise histológica, imunohistoquímica, macroscópica				
Análise de resultados				
Elaboração de artigo e Defesa de dissertação				

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9**. Artigo**

Protective effects of curcumin in tissular response around silicone disks implants in rats.

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Introduction

The first silicone implants were used in the early 60. Nowadays, breast augmentation surgery ranks second in plastic procedures, and its use is of high relevance in mammary reconstruction after mastectomy. However, breast implants exhibit particular clinical complications related to inflammatory response with subsequent formation of fibrous capsule surrounding the implant [1,2]. The development of an avascular fibrous capsule constitutes a reaction to the foreign body as a part of local reparative process in which the dissected tissue cavity behaves as a surgical wound [3].

The chronic inflammatory process involved in fibrosis of the capsule can modify the consistency and shape of the breast leading to capsular contracture. This pathological process is triggered by individual reactivity to silicone and its envelope [4,5], which can be a consequence of the implant anatomic position [3,4] or be related to perioperative complications [4,6-8]. Moreover, in regard to the reactivity to silicone envelopes, studies have shown that the incidence of the inflammatory process seems to be higher in thicker texture capsules [3,4,6-8].

The participation of leukotriene and cytokines modulating the fibrotic reaction has been subject of pre-clinical and clinical studies investigating new pharmacological approaches with anti-inflammatory action [2,7-11]. Indeed, pre-clinical studies have shown promising results in reducing the inflammatory process involved in the foreign body reaction [4,10]. Although the oxidative stress represents an important biological marker for a series of disorders where the inflammatory process occur, no evidence was mentioned until now for oxidative stress parameters in patients recipients of breast silicone implants.

In this context, curcumin (1*E*,6*E*)-1,7-*bis*(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, a polyphenolic compound isolated from the rhizomes of *Curcuma longa* Linn, has been shown to exhibit a wide range of pharmacological activities including anti-inflammatory, anti-cancer, anti-oxidant, anti-atherosclerotic, anti-microbial, and wound healing effects [12-14]. The biological activities of curcumin are based on its chemical features, as well as its ability to interact with multiple signaling molecules [15]. The main actions of curcumin could be linked to its antioxidant and anti-inflammatory properties in *in vivo* and *in vitro* models [16]. The anti-inflammatory potential of curcumin is related to the inhibition of nuclear factor kappa B (NFkB) signaling and reduction of pro-inflammatory cytokine production such as IL-1β, IL-6 and TNF-α [14,16]. Curcumin is also a potent inhibitor of reactive-oxygen-generating enzymes such as lipoxygenase/cyclooxygenase, xanthine dehydrogenase/oxidase, and inducible nitric oxide synthase [14,16]. Moreover, the safety, tolerability, and non-toxic doses of curcumin have been well established in many clinical trials [14,16].

Considering the effects of curcumin in inflammatory and oxidative processes, it becomes relevant investigate the action of this compound in the chronic inflammatory process induced by the inclusion of the breast implant. Therefore, the aim of this study was to compare the capsular reaction to two different coverings of silicone disks through the morphological, biochemical and microscopic aspects of the inflammatory reaction. Moreover, we aimed to evaluate the protective effect of curcumin on the local inflammatory and oxidative process as a promising new therapeutic target.

Methods

Chemicals

The chemicals used included curcumin (Sigma Chemical Co., USA) dissolved in peanut oil and administered by oral route (*p.o.*), the anesthetics ketamine and xylazine (VIRBAC, Brazil), dissolved in saline solution (NaCl 0,9%, w/v) and administrated by intraperitoneal route (*i.p.*). Appropriated vehicle groups were also assessed simultaneously. The doses of curcumin, ketamine and xylazine used in the present study were chosen according to the literature [17].

Silicon disks

Circular disks of 3.0 cm in diameter of fine and medium texture were produced and donated by Lifesil, Paraná, Brazil. Ethylene oxide sterilization was done according to the manufacturer's instructions before implantation to avoid contamination or infections.

Animals

Female adult Wistar rats 16, aged 11-12 weeks (250 – 300 g) were obtained from the Central Animal House of the Federal University of Pelotas, Pelotas, RS, Brazil. Animals were maintained under controlled environment (23 ± 2°C, 12h-light/dark cycle, free access to food and water) and handled according to the Federation of Brazilian Societies for Experimental Biology guidelines upon approval by the Ethics Committee of the Federal University of Pelotas, Brazil (protocol 1276).

Experimental surgery protocol

For the surgery procedures, animals were anesthetized with intraperitoneal (i.p.) ketamine 50 mg/kg and xylazine 7.5 mg/kg. Their dorsal hair was clipped with electric shears and then completely removed with a depilatory apparatus. The rats were individually draped and positioned for surgery, and the methodology used was

performed according to previously described [3]. Using strict aseptic techniques, a 2.0-cm midline longitudinal wound was incised over the spine at the scapular level. The incision was deepened to the panniculus carnosus muscle, which allowed the creation of a subcutaneous pocket. By blunt dissection, a 2-cm-wide bloodless pocket was exposed laterally on the right and left side of the incision. The disks were then inserted in the same way, with the textured surface upside covered by the panniculus carnosus. The fine and medium textures disks were placed at right and left side of the incisions, respectively, and the wound was closed by interrupted stitches of monylon 4.0. Animals were housed 4 *per* cage and exposed to 24ºC heating lamps until anesthesia recovery.

Animals groups and treatment

The animal groups were divided as follow and treatment was administered by oral route (p.o.): 1) Peanut oil treated rats (vehicle control); 2) 20 mg/kg curcumin treated rats; 3) 50mg/kg curcumin treated rats. Curcumin administration schedule started on the second postoperative day once a day. Control group received the same volume of peanut oil in absence of curcumin. All experiments were carried out between 9:00 and 16:00 hours, with each animal used only once. Animals were observed and treated for 30 days postoperative. The treatment was well tolerated and there was no loss of weight or animal number.

Histological analysis

First, the two types of silicon disk were metalized and examined with a scanning electron microscopy in order to document their surface.

The animals were anesthetized with ketamine 50 mg/kg and xylazine 7.5 mg/kg i.p. and then were beheaded. After the necropsia, the tissue surrounding the implant (capsule) and the hepatic tissue were collected to evaluate histological differences

between the textures, as well as the effect and toxicity of curcumin treatment. The block of hepatic tissue and the capsule was immersed separately in 10% buffered formalin. After 24 h, the capsule around the implant was embedded in paraffin, and prepared for histological sections (5 µm) [11,18]. Slides were stained with hematoxylin-eosin (HE) and histological evaluation was determined by a pathologist in a blinded manner.

The total capsule thickness was measured in mm with light microscopy at magnification of 40x from selected skull caudal blade. The cell count was also performed in five fields of 40 x magnification and the following parameters were considered for analysis: number of nucleated fibroblasts, inflammatory cells, and number of vessels at the center of each capsule sample on each slide [11].

Oxidative stress analysis

A small portion of tissue extracted from implant periphery was reserved and maintained at -80ºC until the oxidative stress analysis be performed. The tissues were manually dissected and homogenized in 10 volumes (1:10 w/v) of 20 mM sodium phosphate buffer, pH 7.4 containing 140 mM KCl. Homogenates were centrifuged at 750 x *g* for 10 min at 4°C. The pellet was discarded and the supernatant was immediately separated and used for stress oxidative measurements. The protein content was quantified by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Thiobarbituric acid reactive species formation (TBARS)

The measure of lipid peroxidation was determined by TBARS in according to protocol described by Esterbauer and Cheeseman (1990). Briefly, homogenates were mixed with trichloroacetic acid 10% and thiobarbituric acid 0.67% and heated in a

boiling water bath for 25 min. TBARS was determined by the absorbance at 535 nm. Results were reported as nmol of TBARS per mg of protein.

Nitric Oxide assay (NO)

Concentration of NO was measured in terms of its products nitrite (NO^{2-}) and nitrate (NO^{3-}). The test is based on the reduction of nitrate to nitrite using the granulated cadmium and conversion, using Greiss reagent, of nitrite into a purple compound – chromophore, which is measured colometrically at 540 nm. NO products were expressed as μM/L [2].

Statistical analysis

All experimental results are given as the mean ± S.E.M. Statistical analysis was performed by One way ANOVA. In all cases, Tukey test was applied for post-hoc comparison when appropriate. A value of $p \leq 0.05$ was considered significant. Analysis was made in the GraphPad Prism 5.0.

Results

Histological Evaluation

Morphological changes in tissue surrounding the two texture of silicon disk implanted were analyzed by both HE histological for all groups of animals. Examining the sections from implanted medium texture disk (Fig. 1), the tissue inflammatory response invaded the rough surface of the disk (Fig. 1A) which was composed by giant cells, blood vessels and fibroblasts (Fig. 1B). In according, the ultrastructural findings analyzed by scanning electron microscopy showed a very rough and thicker surface in the medium texture (Fig. 1C and D). In opposite, the implanted fine texture disk had a less intense tissular response (Fig. 2A), but it also exhibited the presence of blood

vessels, giant cells and fibroblasts (Fig. 2B). The ultrastructure of fine texture disk showed a more regular (Fig. 2C) and thinner surface (Fig. 2D). Although the different intensity in the response in the rough surface, the cell count in 4 different fields in the fine and medium texture reported no differences for the number of giant cells (p=0.868), blood vessels (p=0.568), number of fibroblasts (p=0.178) and total thickness of sections (p=0.576) as shown in Table 1.

The Figure 3 exhibits the effects of curcumin treatment at doses of 20 and 50 mg/kg in the histological parameters for fine and medium textured implants. Figure 3A shows the analysis of fine textured, where curcumin treatment at 20 (2.1±1.6) and 50 mg/Kg (3.2±1.2) decreased the number of giant cells compared to control (5.1±1.7) ($p\leq0.001$). In Fig 3B representing the medium textured, we also observed a decrease in the number of giant cells in animals treated with curcumin 20 (2.8±2.1) and 50 mg/kg (2.7±0.8) compared to control (4.7±1.6) ($p\leq0.05$).

We demonstrated, for fine and medium texture implanted, a decrease in blood vessel number in animals treated with curcumin 20 (7.9±2.7 and 7.1±5.3; respectively) and 50 mg/kg (5.9±3.5 and 5.3±2.2; respectively) compared to control (11±2.0 and 13.5±5.7; respectively) ($p\leq0.01$) (Fig. 3C and 3D; respectively). The Figure 3E represents the number of fibroblasts in the fine textured implants, showing that the treatment with curcurmin 20 (82.6±33.4) and 50 mg/kg (81.6±19.2) increased the number of fibroblasts when compared to control (51.1±18.6) ($p\leq0.05$). In Figure 3F, representing the medium textured, we also observed an increase in the number of fibroblasts after curcumin treatment at 20 (97.1±30.8) and 50 mg/kg (95.4±32.5) in comparison to control (51.3±25.5) ($p\leq0.01$). Possible toxicological effects of curcumin treatment were evaluated in liver tissue and no morphological changes were observed in the histological analysis (data not shown).

Microscope and Morphometric Examination

On light of microscopic examination, comparing the fine and medium textured implants, no differences were observed in the total thickness (Table 1). Figure 4 represents the effects of curcumin treatment at 20 and 50mg/kg in the total thickness of fine texture and medium texture (Fig. 4A and B). Total thickness of rats implanted with fine texture was reduced in animals treated with curcumin 50 mg/kg (0.17±0.04) in comparison to control group (0.25±0.05; $p \leq 0.05$). Animals with medium textured implant also showed reduced total thickness for curcumin 20 (0.15±0.05) and 50 mg/kg (0.15±0.03) related to control (0.2±0.04; $p \leq 0.05$).

Measurement of Oxidative Stress Parameters

We firstly performed a comparative analysis of oxidative stress parameters evaluated by TBARS and NO levels of fine and medium textured implants in order to determine the response to different surfaces. In Table 1 we demonstrated that NO levels are higher in medium texture comparing to fine ones ($p \leq 0.0001$). However, the measure of oxidative damage evaluated by the lipid peroxidation (TBARS) did not differ between fine and medium textured implants (p=0.712) (Table 1).

The antioxidant profile of curcumin was evaluated for both fine and medium textured implants. Figure 5A shows the NO levels in fine textured implant of rats treated with curcumin 20 and 50 mg/kg and no effects were observed (24.4±8.3 and 30.5±8.7; respectively) in comparison to control (33.6±3.1; p=0.235). However, in figure 5B for medium textured implant group, a significant decrease in NO levels was observed in curcumin-treated rats with 20 and 50mg/kg (30.4±6.9 and 71.1±18.1; respectively) when compared to control (96.3±3.31; $p \leq 0.0001$).

The measures of TBARS in fine textured implant reveals decreased levels in curcumin-treated rats with 20 and 50 mg/kg (0.07±0.02 and 0.07±0.02; respectively) related to control (0.19±0.05; p≤0.002) (Fig. 5C). Similarly, in Figure 5D we demonstrated in medium textured implant that curcumin 20 and 50mg/Kg decreased the levels of TBARS (0.077±0.03 and 0.08±0.006; respectively) when compared to control (0.137±0.006; p≤0.0006).

Discussion

In this study, we evaluated the capsular reaction to two types of prosthesis surfaces and investigated the protective potential of curcumin in the tissular response assessing the inflammatory and oxidative stress reaction. Although no differences were observed between the surfaces of the implants, the inflammatory reaction implant-induced known as foreign body reaction, was prevented by the treatment with curcumin. Here, we showed for the first time the role of curcumin in prevent morphological, biochemical and morphometrics alterations evidenced in the response to fine and medium textured implants.

The normal tissue response to silicone implants involves inflammatory infiltration, increased vasculogenesis and fibroblast proliferation, which leads to a collagenous capsule surrounding the foreign material [8]. In this study, we firstly evaluate the differences in tissular response between fine and medium textured of silicone particles implanted in rats. Our results are in agreement with previous studies concerning the fibrotic process using two smooth and texturized implants, where evidences of eight weeks treatment showed no differences in histological analysis [4]. Indeed, in a clinical study comparing the capsular contracture between the smooth and texturized implants, no significant changes in tissular response was observed [8].

However, in a different way, Escudero et al. (2005) [3], affirmed that there is a more intense chronic inflammatory reaction with macrophages and giant cells in textured prostheses due to tissue growth inside the coating. Similarly, new findings evidenced larger number of giant cells and granulomes in sub-groups of silicone foam which presented greater tissue growth inside the coating [17]. However, it is important emphasize that the absent of differences between the surfaces in our study could be explained by the fact that both types of implants analyzed exhibit texturized surfaces, being distinguished by the plot of silicone particles and higher density layer in medium than in fine implant.

It is well known that the capsule-inducing process is a multifactorial event cascade that includes inflammation, cell proliferation, matrix deposition and remodeling phases, often associated with oxidative stress and consequent prolonged inflammation that results in impaired healing. Thus, several drugs are being used to diminish post-surgery complications in breast implants to improve the healing process and avoid the formation of capsular contracture [8]. In this context, curcumin, that has stronger anti-inflammatory and antioxidant properties than vitamins C and E clinically used in the post-operatory, was described to inhibits the oxidative stress by quenching free radicals and reducing inflammation through the inhibition of nuclear factor-kappa B (NFK-β) [14,18,19]. In respect to inflammatory process, the anti-inflammatory potential of curcumin has been demonstrated in a series of pathological states [14]. Moreover, the transforming growth factor beta 1 (TGF-B1), known by stimulate the synthesis of matrix proteins, was documented by increased expression in fibrotic cells and keloid process. In this context, curcuminoids were described by inhibit the TGF-B1 signal cascade and decrease the expression of extracellular matrix in the fibroblasts of keloid process [20]. Although we did not evaluate the mechanisms for curcumin actions, our results showed

that this compound was able to decrease the number of giant cells and blood vessels in the inflammatory infiltrate for both silicone textures analyzed, reducing in this way the inflammatory process induced by the implants.

The event of oxidative stress is recognized to play a role in the healing process, since reveals a positive impact in the proliferation and mobilization of cells by acting in a series of cell signaling events[14]. The curcumin is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals, which are important to the initiation of lipid peroxidation. Here, we noticed that curcumin alters parameters of oxidative stress by decreasing NO levels and lipid peroxidation in tissue that surround both fine and medium textured implants. In contrast to our study, curcumin was described to increase the inducible nitric oxide synthase (iNOS) by regulation of the transforming growth factor-beta (TGF-β), consequently increasing the NO levels, important in the normal and impaired healing wounds process (Mani et al., 2002).

Important to note that higher number of fibroblasts concomitant to the increased inflammatory process and oxidative stress, takes part of the initial process of healing. In this study we provide evidences of the curcumin efficacy in the prevention of chronic inflammatory process involved in the silicone implants. Moreover, we observed a stimulatory effect in fibroblast proliferation. In this way, we can suggest that curcumin could be acting to benefit the healing process preventing the formation of a pathological fibrotic process. In line with our study, the regenerative potential of curcumin has been evaluated for future application in skin regeneration and wound healing [19]. Similar to our findings, studies have been shown the potential of curcumin in facilitate the healing process by affects signaling pathways as transforming growth factor-β (TGF-β) and mitogen-activated protein kinase pathway (MAPK), increasing the cells proliferation [19,20] . However, TGF-B is described to simulate the synthesis of matrix proteins in the

fibrotic and keloid process, and curcumin was able to inhibit this signaling cascade decreasing the expression of extracellular matrix (ECM) in the keloid fibroblasts [20].

Notwithstanding the complexity of fibrogenesis around the implants, our data support the hypothesis of a reduced inflammatory cell infiltration and oxidative damage, as well as an increase in fibroblast response in the pericapsular space surrounding the implants, as a way to reduce the formation of fibrotic process improving the healing one. Furthermore, we observed that total thickness surrounding the implants is reduced after curcumin treatment in both fine and medium textured implants, showing the general effect of curcumin in the inflammatory infiltration. Right now we have achieved reasonable experimental evidence that curcumin is a good candicate to modulate the tissular reaction, showing for the first time the preventive effects of curcumin and corroborating with the efficacy in enhances the healing process. However, further studies would be conducted to better define the signaling ways leading to the preventive effects of curcumin as well as for investigation of other parameters not evaluated in this study.

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Legend to Figures:

Figure 1. Light microscopy of medium texture implanted disks in control group. (A) Inflammatory infiltrate which was formed in the rough surface of medium texture (HE; magnification x 100). (B) Detail of the inflammatory infiltrate with lymphocytes, fibroblasts, giant cells and new blood vessels (HE; magnification x400). (C) Scanning electron microscopy showing important irregular surface (magnification x70). (D) Lateral view of the disk showing the rough surface (arrow), in which the inflammatory inflitrate takes place (magnification x70)

Figura 2. Light microscopy of fine texture implanted disks in control group (A) inflammatory infiltrate which was formed in the rough surface of fine texture (HE; magnification x100). (B) Detail of the inflammatory infiltrate with fibroblasts and giant cells (HE; magnification x400). (C) Scanning electron microscopy exhibiting surface with a more regular structure when compared to Figure 1C (magnification x70). (D) Lateral view of the disk showing the rough surface (arrow) (magnification x150).

Figure 3. Represent the effects of curcumin at 20 and 50 mg/kg on histological analysis for fine (left panel) and medium textures (right panel). Number of giant cells in fine (A), and medium texture implants (B). Number of vessels in fine (C), and medium texture implants (D). Number of fibroblasts in fine (E), and medium texture implants (F). Data

was expressed as mean ± S.E.M. (*) $p<0.05$ and (**) $p<0.01$ when compared to control (vehicle group).

Figure 4. Represent the effects of curcumin treatment at 20 and 50mg/kg in the total thickness of fine texture (A), and medium texture (B). Data was expressed as mean ± S.E.M. (*) $p<0.05$ when compared to control (vehicle group).

Figure 5. Represent the effects of curcumin treatment at 20 and 50 mg/kg in the oxidative stress parameters. NO levels in fine (A), and medium texture implants (B); TBARS in fine (C), and medium texture implants (D). Data was expressed as mean ± S.E.M. (*) $p<0.01$ and (**) $p<0.001$ when compared to control (vehicle group).

Figure 1.

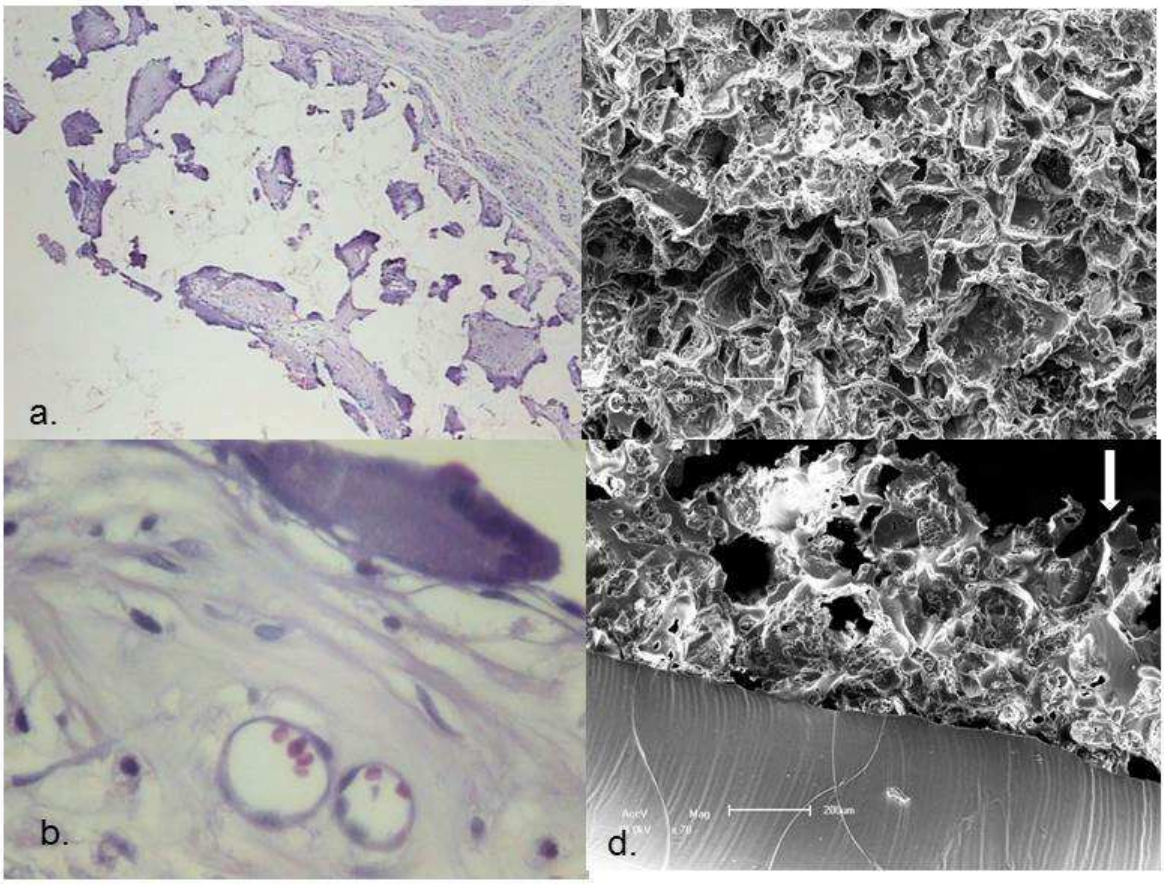


Figure 2.

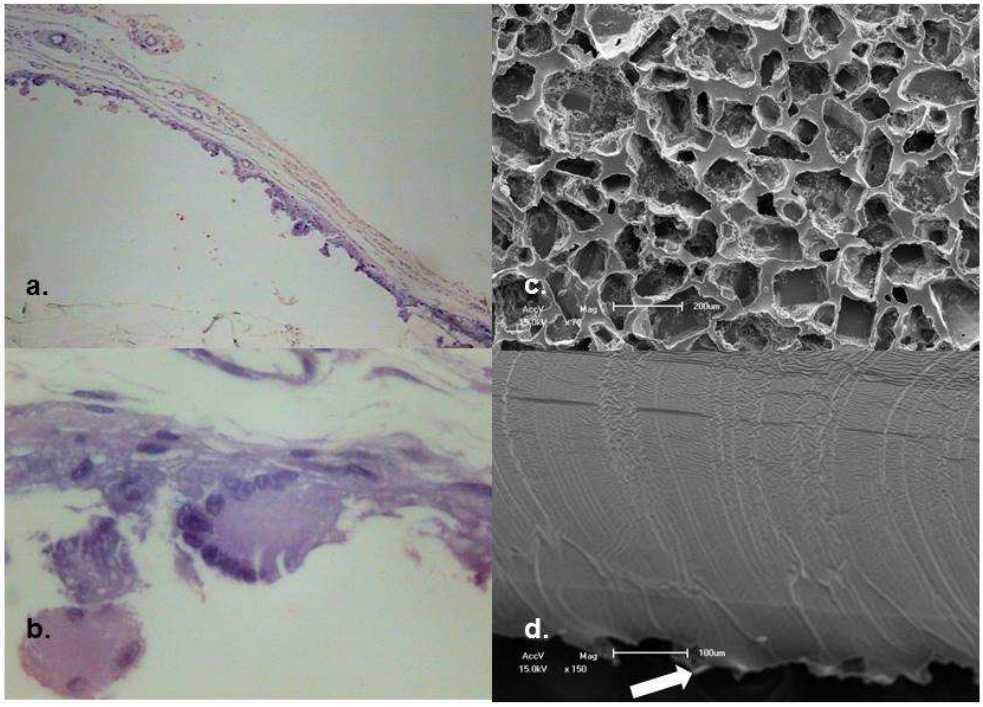
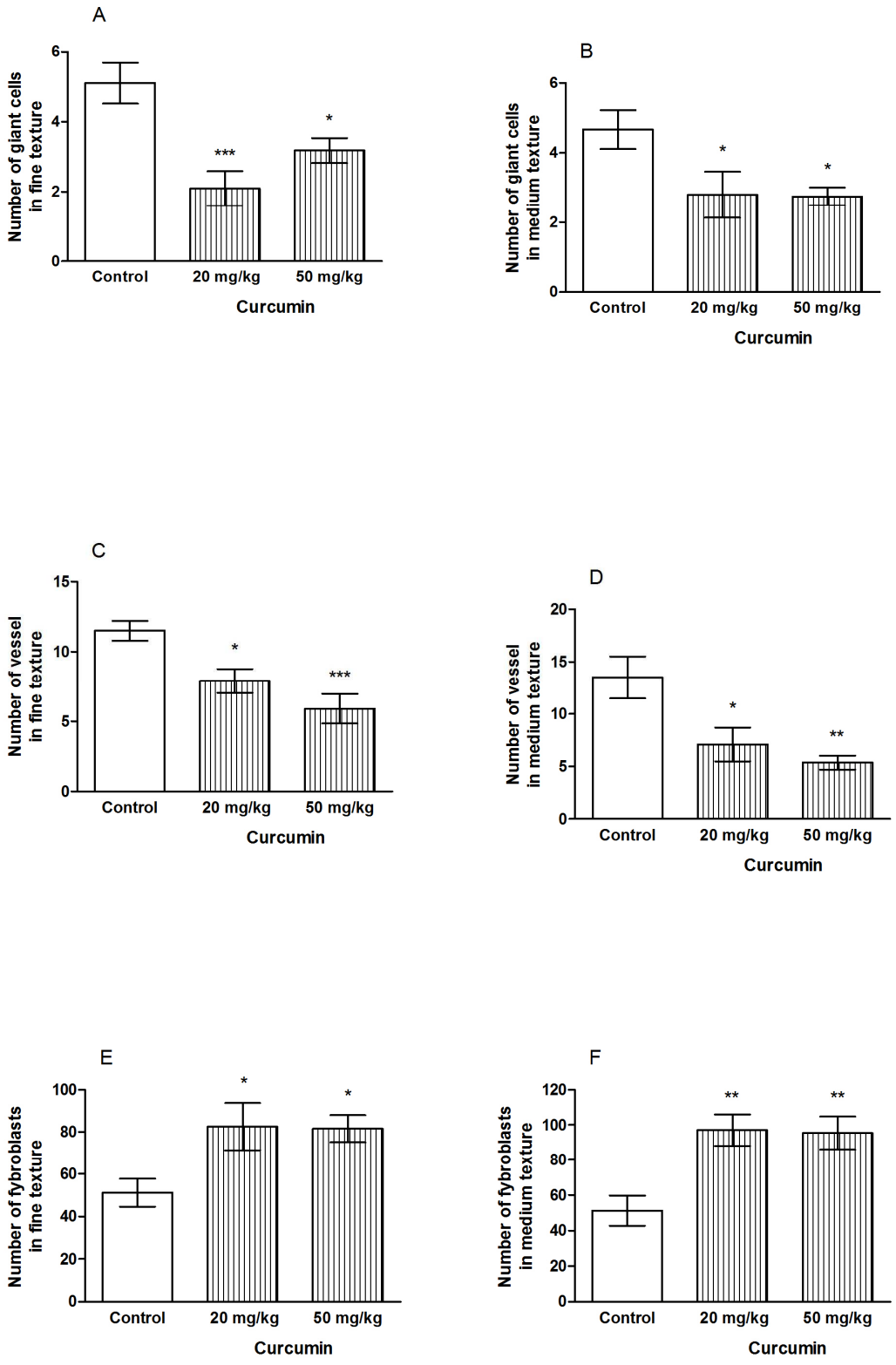


Figure 3.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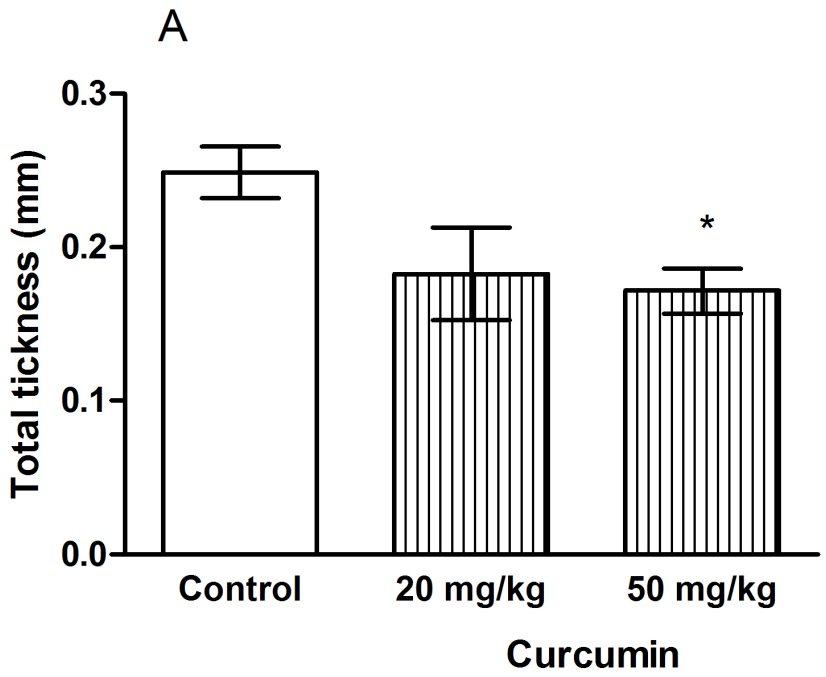
Curcumin
E
Number of fybroblasts in fine texture
100
80
60
40
20
0
*
*
Control
20 mg/kg
50 mg/kg
Curcumin
F
Number of fybroblasts in medium texture
120
100
80
60
40
20
0
**
**
Control
20 mg/kg
50 mg/kg
Curcumin

Figure 4.


A
Total tickness (mm)
0.3
0.2
0.1
0.0
*
Control
20 mg/kg
50 mg/kg
Curcumin

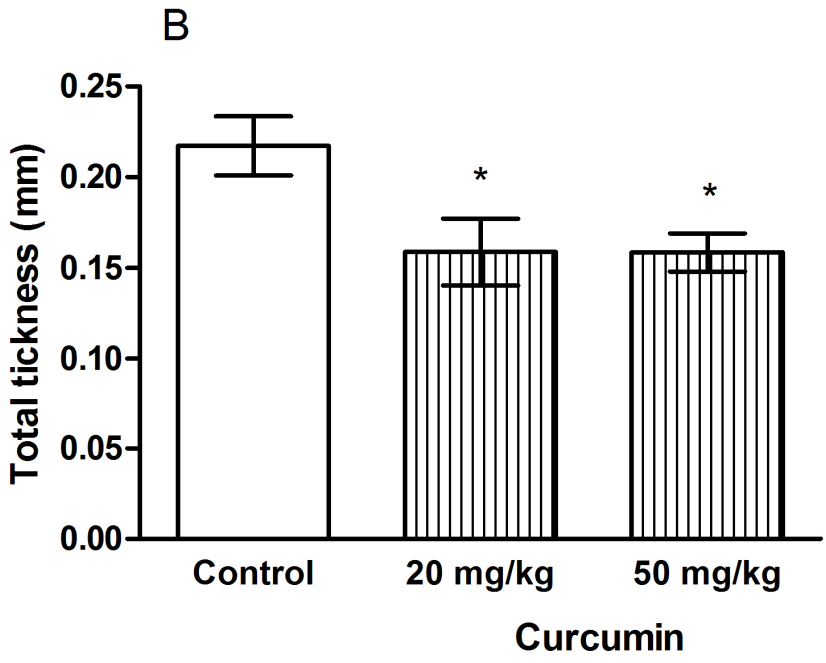
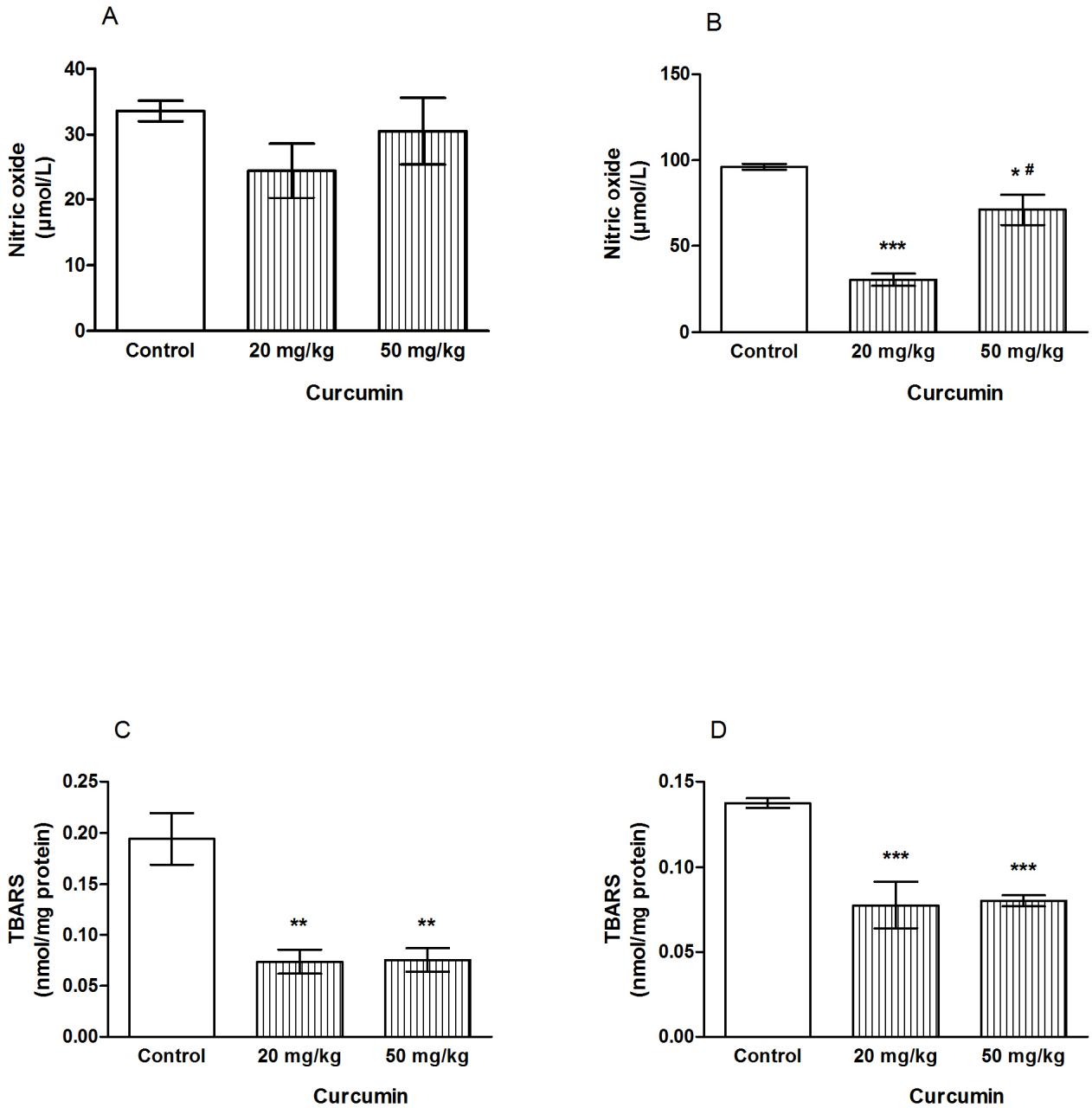

B
Total tickness (mm)
0.25
0.20
0.15
0.10
0.05
0.00
*
*
Control
20 mg/kg
50 mg/kg
Curcumin

Figure 5.


A
Nitric oxide (µmol/L)
40
30
20
10
0
Control
20 mg/kg
50 mg/kg
Curcumin
B
Nitric oxide (µmol/L)
150
100
50
0
***
* #
Control
20 mg/kg
50 mg/kg
Curcumin
C
TBARS (nmol/mg protein)
0.25
0.20
0.15
0.10
0.05
0.00
**
**
Control
20 mg/kg
50 mg/kg
Curcumin
D
TBARS (nmol/mg protein)
0.15
0.10
0.05
0.00
***
***
Control
20 mg/kg
50 mg/kg
Curcumin