

NOM DU PRODUIT : SVELTYSS

CLASSE : Multiples Ingrédients  
Présentation non traditionnelle

RÉCLAME : Utilisé pour aider à éliminer l'eau, les toxines et les ballonnements, faciliter la digestion et contribuer à la perte de poids dans un programme d'amaigrissement

### RAPPORT DE SYNTHÈSE SUR L'INNOCUITÉ

Le produit SVELTYS est une capsule contenant du thé vert, de l'ananas, de la papaïne et de la bromélaïne. Le thé vert est un antioxydant qui a aussi des propriétés diurétiques, la papaïne et la bromélaïne sont utilisées pour améliorer la digestion et l'absorption de certains suppléments.

#### A. MENTIONS DE RISQUE

Le produit SVELTYS de par ses composantes botaniques présentent peu de risques pour la santé, selon la documentation retrouvée sur PubMed, le natural database et autres références. Considérant qu'il n'y a pas d'étude d'innocuité à long terme, il est préférable d'éviter ce produit chez les femmes enceintes ou qui allaitent. Pour en atténuer les risques les précautions ou contre-indications seront ajoutées sur l'étiquette.

Précautions/Avertissement :

- Aucune

Contre-indications :

- Ne pas utiliser si vous êtes enceintes ou allaitant (Natural database).
- Ne pas utiliser en cas d'allergies à la bromélaïne, ananas, papaye, papaïne, figue, kiwi (Natural database<sup>10</sup>, Wuthrich,1985<sup>2</sup>, Taylor, 2001, Diez-Domez, 1998, Braun, 2005<sup>5</sup>, Baur, 1979)

## B. FACTEURS D'INNOCUITÉ

1. L'usage de cette drogue nécessite-t-elle des instructions individualisées ou la supervision directe d'un praticien, un traitement auxiliaire nécessitant un médicament d'ordonnance inscrit à l'annexe F ou un contrôle de routine en laboratoire pour que le produit ou l'ingrédient médicinal soit sûr ou efficace?
2. Le produit ou l'ingrédient médicinal est-il utilisé pour le traitement d'une maladie qui n'est pas approprié pour l'autogestion de la santé, p. ex. une maladie grave souvent mal diagnostiquée par le public?
3. L'utilisation du produit ou de l'ingrédient médicinal masque-t-elle d'autres problèmes de santé ou leur développement?
4. Le produit ou l'ingrédient médicinal a-t-il des réactions indésirables connues à des doses thérapeutiques normales ou à la posologie recommandée?
5. La marge de sécurité entre les doses thérapeutiques et toxiques est-elle mince, surtout lorsqu'il s'agit de certains groupes de la population comme les personnes âgées, les enfants et les femmes enceintes ou qui allaitent?
6. Le produit ou l'ingrédient médicinal risque-t-il de créer une dépendance ou de conduire à l'abus, pouvant mener à un usage non médical dangereux?
7. Le produit ou l'ingrédient médicinal présente-t-il un effet thérapeutique fondé sur des concepts pharmacologiques récemment élaborés et dont les conséquences n'ont pas encore été établies?
8. Des données expérimentales démontrent-elles que le produit ou l'ingrédient médicinal est toxique pour les animaux? Dans l'affirmative, a-t-il fait l'objet d'un usage clinique depuis assez longtemps pour qu'un modèle ou que la fréquence des effets à long terme chez l'être humain soit établi?
9. Le produit ou l'ingrédient médicinal a-t-il des interactions indésirables connues avec d'autres produits de santé naturels, des médicaments ou de la nourriture?
10. Le produit ou l'ingrédient médicinal a-t-il une incidence sur les résultats des tests de diagnostic ou de laboratoire standard?
11. Le produit ou l'ingrédient médicinal contribue-t-il, ou risque-t-il de contribuer, au développement de souches résistantes de micro-organismes chez l'être humain?
12. Le produit ou l'ingrédient médicinal présente-t-il un niveau élevé de risque par rapport aux avantages escomptés?

### Réponses aux facteurs d'innocuité

1. Non
2. Non
3. Non
4. Voir commentaires sur les allergies dans les mentions de risque
5. Voir commentaires pour les femmes enceintes dans les mentions de risque
6. Non
7. Non
8. Non
9. Non
10. Non
11. Non
12. Non

Pour mener à bien l'innocuité de ce produit, une recherche sur PubMed a été effectuée le 24 février 2006, sur le site :  
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Search&DB=pubmed>

a) Thé vert (*Camellia sinensis*)

*Camellia sinensis* AND safety : 5 références, 2 retenue (Isbrucker, 2005a, Ullmann, 2004), 4 rejetées car traite de l'action sur la plaque dentaire, de la fermentation des feuilles, de fertilisant pour la plante.

*Camellia sinensis* AND adverse reaction : 0 référence

*Camellia sinensis* AND drug interaction : 15 références, 3 retenues (Ullmann, 2005, Ahmed, 2004, Anderson, 2002), 12 rejetées (car réfère au rôle modulateur, au rôle de certaines composantes du thé noir (2), évaluation odorante, effet sur un ingestion continue, effet positif dans le traitement de la démence sans innocuité, activité antimicrobienne (4), potentiel anti-cancer, action sur les jonctions neuromusculaires).

Green tea AND safety : 29 références, 7 retenues (Bettuzzi, 2006, Isbrucker, 2006, Isbrucker, 2005b, Ullmann, 2004, Chow, 2003, Heck, 2000<sup>10</sup>, Kaegi, 1998), 22 rejetées (généralités des polyphénols et les maladies gastrointestinales, thérapie alternative pour l'ostéarthrite, généralités sur les herbes médicinales et les interactions possibles (2), usage d'un mélange de plantes comme rince-bouche, suppléments pour la perte de poids en général, composition d'extrait d'épinard, conseils aux patients cancéreux au sujet des herbes médicinales (2), effets biologiques utiles pour la maladie de Parkinson, entrevue sur l'usage des suppléments chez les femmes ménopausées, composantes hypocholestérolémiantes, suppléments nutritionnels, plantes et cancer (2), analyses chimiques, mécanisme de transduction, prévention du cancer sans innocuité, généralité sur les traitements non conventionnels du cancer, sur les travailleurs du thé).

Isbrucker et al. (2005a<sup>1</sup>, 2005b<sup>2</sup>, 2006<sup>3</sup>) ont récemment publié 3 études sur l'innocuité d'une préparation d'epigallocatechin gallate (EGCG), qui est le composé polyphénolique principal du thé vert. Ainsi Isbrucker et al. (2005a<sup>1</sup>) a évalué le potentiel génotoxique de cette préparation EGCG sur une souche bactérienne de *Salmonelle* et sur des cellules de lymphome de souris (L5178Y tk(+/-)). Aucune activité mutagénique n'a été détectée sur le système bactérien, cependant une "tendance" clastogénique a été observée sur les cellules de souris. La dose orale administrée de 500, 1000 ou 2000 mg EGCG/kg de souris n'ont pas induit de formation micronucléi dans les cellules de la moelle osseuse. Similairement des doses moins importantes (400, 800 ou 1200 mg EGCG/kg/j) pendant 10 jours, n'ont pas induit la formation de micronucléi dans les cellules de moelle osseuse. L'injection de 10, 25 et 50 mg EGCG/kg/j résultent en de concentrations plasmatiques plus élevées et démontrent une absence de génotoxicité. Les auteurs ont conclu que le EGCG n'est pas génotoxique.

La même équipe (Isbrucker et al., 2005b<sup>2</sup>) ont évalué la toxicité dermique et des doses orales aiguës à court terme de cette même préparation EGCG. Les préparations topiques de EGCG ont causé des irritations mineures chez les rats et cobayes, mais pas chez les lapins. Une dose orale de 2000 mg EGCG/kg fut létale pour les rats, alors qu'une dose de 200 mg/kg n'induit pas de toxicité. L'administration de la préparation EGCG pendant 13 semaines ne fut pas toxique à des doses jusqu'à 500 mg/kg/j. Il n'y a

pas eu de réactions adverses pour 500 mg/kg/j chez les chiens nourris (en doses divisées) non plus. De ces études, la dose de 500 mg EGCG/kg/j a été établie comme étant le niveau où il n'y a pas d'effet adverse observé (NOAEL).

Finalement Isbrucker et al. (2006<sup>3</sup>) ont étudié l'effet tératogène et la toxicité sur le système reproducteur des rates (femelles) des préparations EGCG. Les préparations EGCG (pur à 91%) ont été administrées à des rates enceintes pendant l'organogénèse et le développement des fœtus. Dans une étude préliminaire précédente, utilisant les voies d'administration de gavage et sous-cutanée, il n'y a pas eu d'évidence de toxicité embryo-fœtal, cependant un peu de toxicité maternelle a été observée. Dans l'étude de tératogénicité, les rates enceintes ont été nourries avec une supplémentation de 1400, 4200 ou 14 000 ppm pendant l'organogénèse, ces concentrations ne furent pas toxiques pour les fœtus et les nouveaux nés. Une étude sur 2 générations de rats nourries avec des préparations EGCG de 1200, 3600 ou 12,000ppm n'a eu aucun effet adverse sur la reproduction ou la fertilité. La dose la plus faible fut considérée NOAEL. Les nouveaux nés ont consommé deux fois plus de la préparation pendant la période de lactation. La dose ne causant pas d'effet adverse (NOAEL) était équivalente à 200 mg/kg/j EGCG. Si nous ramenons ce dosage à l'humain, il faudrait consommer 14 000 mg/jour (200 mg x 70 kg) pour avoir la dose sécuritaire. Le produit SVELTYS contribue pour 100 mg de polyphénol de thé vert. Nous sommes bien loin de ce dosage sécuritaire.

Bettuzzi et al. (2006<sup>4</sup>) rapportent que les catéchines (polyphénols) du thé vert sont efficace pour inhiber la croissance de cellules cancéreuses dans plusieurs modèles expérimentaux. Des études récentes ont montré que 30% des hommes avec une néoplasie intra-épithéliale élevée de la prostate, vont développer un cancer de la prostate dans l'année suivant la biopsie. Les auteurs ont donc fait une étude en double aveugle, placebo contrôlé et éthique sur 60 volontaires atteints de néoplasie intra-épithéliale élevée de la prostate (NIEP) pour évaluer l'innocuité et l'efficacité des catechines du thé vert (CTV) comme traitement de prévention dans les cas de cancer de la prostate causé par NIEP. Les patients ont reçu 600 mg CTV/jour (200 x 3 capsules). Après un an, seulement une tumeur a été diagnostiquée parmi les 30 hommes traités à la catéchine de thé vert (incidence 3%), alors que l'incidence était de 30% dans le groupe placebo. Les antigènes spécifiques de la prostate étaient plus faibles dans le groupe expérimental. Il n'y a pas eu d'effet secondaire significatif, ni de réaction adverse documenté. Selon les auteurs, c'est la première étude qui montre que les catechines de thé vert (CTV) sont sécuritaires et efficaces dans le traitement de lésions pré-malignes avant qu'un cancer de prostate se développe. De plus les CTV ont réduit les symptômes reliés au tractus urinaire, suggérant que ces composés pourraient aider dans le traitement des symptômes de l'hyperplasie bénigne de la prostate.

Le thé vert est donc riche en catéchines et plusieurs études épidémiologiques ont démontré que la consommation de thé vert est associée à des bienfaits pour la santé. Ahmed et al. (2004<sup>5</sup>) s'intéresse aux articulations et à l'effet anti-inflammatoire du thé. Bien que notre produit ne vise pas cette propriété, Ahmed et collaborateurs ont démontré que le polyphénol epigallocatechin-3-gallate (EGCG) du thé vert n'est pas toxique pour les chondrocytes humains in vitro.

Ullmann et al. (2004<sup>6</sup>) ont fait une étude aléatoire, en double aveugle, placebo contrôlé pour étudier entre autre l'innocuité et la tolérance de l'epigallocatechin gallate (EGCG)

chez des volontaires en santé. Les volontaires ont reçu selon les groupes une dose unique variant entre 200 à 800 mg par jour, pendant 10 jours. Les auteurs ont conclu que ce polyphénol est sécuritaire à une dose de 800 mg par jour et est bien toléré. Notre produit contient 100 mg de polyphénols totaux, ce qui correspond à seulement 12,5% de la dose sécuritaire.

Chow et collaborateurs (2003<sup>7</sup>) ont aussi fait une étude aléatoire contrôlée avec EGCG du thé vert et du Polyphenon E (un mélange défini de polyphénol provenant de thé vert décaféiné). Cette fois-ci les auteurs ont voulu déterminer l'innocuité et la pharmacocinétique après 4 semaines d'une administration quotidienne de EGCG ou Polyphenon E. De plus, ils ont voulu déterminer l'effet de l'administration chronique des polyphénols sur la réponse érythème à une induction au ultraviolet. Les participants en santé ont un type de peau Fitzpatrick type II ou III et ont reçu selon le traitement 800 mg EGCG une fois par jour, ou 400 mg EGCG deux fois par jour, ou 800 mg EGCG comme Polyphenon E une fois par jour ou 400 mg EGCG comme Polyphenon E deux fois par jour ou un placebo une fois par jour (8 personnes/groupe). Les réactions adverses ont été principalement des désordres gastrointestinaux et ont été classées comme doux. Pour la plupart, les incidents rapportés n'ont pas été plus nombreux que dans le groupe placebo. Aucun changement significatif fut observé dans le compte sanguin et les profils chimiques sanguins. Il y a eu une augmentation de 60% sous la courbe concentration - temps pour EGCG sanguin après 4 semaines au dosage de 800 mg une fois par jour. Aucun changement significatif ne fut observé dans la pharmacocinétique à un dosage de 400 mg deux fois par jour. Quatre semaines de traitement au polyphénol de thé vert aux différents dosages décrits n'ont pas fourni une protection contre érythème induit par les ultraviolets. Les auteurs ont conclu qu'il est sécuritaire pour les personnes en santé de prendre des produits contenant du polyphénol de thé vert en quantité équivalente en contenu EGCG à 8-16 tasses de thé vert une fois par jour ou en dose divisée deux fois par jour pendant 4 semaines.

Anderson et Polansky (2002<sup>8</sup>) rapporte que les bienfaits du thé sont associés aux polyphénols, qui ont des propriétés antioxydantes, antimicrobiennes, anticarcinogènes, antimutagéniques, de plus les polyphénols du thé pourrait augmenter l'activité de l'insuline. L'objectif de l'étude de Anderson et Polansky (2002) était donc de déterminer les propriétés pouvant augmenter l'insuline par le thé et ses composés in vitro. Les résultats ont démontré que l'EGCG serait la principale composante qui influencerait l'activité de l'insuline dans le thé vert. Si nous remettons ces résultats en perspective, une tasse de thé vert contient environ 80 à 107 mg de polyphénols (qui ne sont pas tous des EGCG) (Natural database<sup>9</sup>) et notre produit contient 100 mg polyphénols, nous sommes bien loin d'une dose pouvant influencer l'activité de l'insuline. Il n'est donc pas justifié de faire une mise en garde pour les diabétiques, puisqu'une tasse de thé vert n'est pas contre-indiquée pour ce groupe cible.

Selon Kaegi (1998<sup>10</sup>), aucun effet adverse n'est rapporté en association à un usage médicinal du thé vert. Cependant une tasse de thé contient 10-80 mg de caféine (le thé vert contient 2 - 4% de caféine). Un surdosage de caféine peut entraîner de la nervosité, de l'insomnie et des irrégularités du rythme cardiaque, c'est pourquoi l'Association Médicale Canadienne recommandent aux personnes ayant des problèmes cardiaques de se limiter à 2 tasses par jour (Kaegi, 1998<sup>10</sup>). L'extrait de thé vert contenu dans notre produit contient environ 9% de caféine (certificat d'analyse) soit un apport de 9 mg de caféine par jour, il n'y a donc pas lieu de faire une mise en garde pour les gens ayant des problèmes cardiaques.

## Interactions médicamenteuses avec le thé vert :

Heck et collaborateurs (2000<sup>11</sup>) ont fait une revue de la littérature pour mettre en évidence les interactions potentielles entre les thérapies alternatives et le warfarin. Pour ce qui est du thé vert, les feuilles contiennent une certaine quantité de vitamine K. Ainsi une grande quantité de thé pourrait potentiellement être antagoniste au warfarin. Taylor (1999<sup>12</sup>) rapporte le cas d'une inhibition de l'effet du warfarin par le thé vert. Un homme de 44 ans consommait un demi à un gallon de thé vert par jour entraînant une diminution du ratio normalisé international (INR) de façon significative. Une consommation normale de thé vert (environ 5 g= 1 cuil. thé /tasse) n'occasionne pas une diminution significative de INR (Heck, 2000<sup>11</sup>) et encore moins la faible quantité (100 mg d'extrait/ 2 capsules) contenue dans SVELTYS, il n'y a donc pas nécessaire de faire une mise en garde pour les gens qui prennent du warfarin.

La monographie du natural database<sup>9</sup> rapporte que le thé vert est consommé quotidiennement dans les cultures asiatiques et n'a pas été associé à des effets adverses significatifs. Les effets adverses documentés sont reliés à un surdosage équivalent à 5-6 litres de thé par jour. Les interactions médicamenteuses potentielles sont soit théoriques ou reliées à une forte teneur en caféine, ce qui n'est pas notre cas.

Ullmann et collaborateurs (2005<sup>13</sup>) ont récemment démontré que les catéchines du thé n'inhibe pas l'absorption intestinale du fer. Cette étude aléatoire, en double aveugle placebo contrôlé, avec 3 périodes de croisement examinaient le degré d'inhibition de l'absorption non hémique du fer par l'isolat purifié d'épigallocatechine gallate (EGCG). L'étude a été conçue pour démontrer le maximum d'action inhibitrice de EGCG chez 30 femmes en santé avec une faible réserve en fer. Les traitements étaient de 150 mg, 300 mg EGCG et placebo, chacun pendant 8 jours consécutifs avec une période de « nettoyage » de 14 jours entre les traitements. L'incorporation de fer s'est fait grâce à un apport d'isotope <sup>57</sup> Fe oralement et <sup>58</sup> Fe par intraveineuse. Les résultats ont démontré une réduction relative de l'absorption du fer non hémique de 14% avec 150 mg EGCG et 27% pour 300 mg EGCG comparé au placebo. Les différences furent significatives entre le placebo et le traitement à 300 mg EGCG. Dans cette étude, l'ampleur de l'action inhibitrice de EGCG sur l'absorption du fer non hémique fut beaucoup plus faible que ce qui est rapporté dans la littérature pour le thé noir et les composés similaires. Les auteurs ont conclu que les doses EGCG dans les suppléments, lesquelles sont moindre que celles utilisées dans l'étude, ne devraient pas avoir d'effets pertinents sur l'absorption du fer chez des personnes avec une réserve de fer normal. Le produit SVELTYS ne contient que 100 mg de polyphénols totaux, ce qui n'a aucune mesure avec 300 mg d'un isolat purifié de EGCG. Notre produit n'a donc pas d'effet sur l'absorption du fer. Le natural database<sup>9</sup> rapporte une autre étude cette fois-ci faite sur des personnes âgées ayant une déficience en fer, les résultats ont démontré que l'usage du thé vert n'altérerait pas l'absorption de fer chez cette population.

## b) papaine

Papain safety : 27 références, 3 retenues (Popiela, 2001, Beuth, 2001, Taylor, 2001 ), 24 rejetées car 1 sur une technique pour l'emphysème, 8 sur un test immunohématologie et caractéristiques sérologiques, 1 sur immunoglobuline rhésus, 3 sur la production et caractérisation de bactériocine, 1 sur un système chirurgical chez le chien, 1 sur la préparation d'antivenin, 1 sur un rapport WHO sur les antivenins, 1 sur méthode de détection de papaye modifiée génétiquement, 1 trop général, 2 sur activité antiparasitaire de la papaine, 1 sur phytobezoars, 1 sur mesure immunochimique et distribution de particules de papaine dans une entreprise alimentaire, 2 sur l'usage du glucagon pour l'obstruction de l'œsophage par des aliments.

Papain adverse reaction : 17 références, 10 retenues (Gujral, 2001, Diez-Gomez, 1998, Iliiev, 1997, Wuthrich, 1985, Bernstein, 1984, Mansfield, 1983, Baur, 1979a, Baur, 1979b, Flindt, 1978, Milne, 1975), 7 rejetées parce que 2 sur des caractéristiques sérologiques, 1 sur un vaccin antiviral, 2 sur une nouvelle drogue : chomopapaine, 1 sur allergie au kiwi, 1 sur methyl dopa.

Papain drug interaction : 18 références, 4 retenues (Gunda, 2002, Heck, 2000, Rodeheaver, 1975, Brisou, 1975), 14 rejetées dont 1 sur l'oxydation de la papaine, 1 sur l'étude structurale de la reconstruction pulmonaire induit par l'administration de cyclophosphamide et de papaine, 1 sur la production de cytokine induit par des enzymes protéolytiques (bromélaïne, papaine) in vitro, 2 sur propriétés de bactériocine, 1 sur l'effet de protéase sur les glycoprotéines in vitro, 1 sur l'effet de la prostaglandine E1 sur l'histamine et la papaine, 1 hors sujet, 1 sur l'activité de la papaine sur le N-ethylmaleimide (sensitive fusion protein), 2 sur réactions pulmonaires déjà mentionnées, 2 anciennes études (1967, 1970) qui n'ont pas été reprises plus tard : une sur le potentiel d'utilisation de la papaine et un anti-inflammatoire dans un onguent et l'autre : l'utilisation d'enzymes dans le traitement otorhinolaryngologique.

Selon le FDA, la papaine est généralement reconnue comme sécuritaire (GRAS), et sécuritaire lorsque utilisée oralement pour des raisons médicinales (papain-natural database<sup>14</sup>).

Une sensibilité croisée à la papaine peut survenir chez les personnes sensibles aux figes et kiwi (papain-natural database<sup>14</sup>).

Une préparation d'enzyme contenant de la papaine a été donnée complémentirement à une thérapie antinéoplastique chez des patients souffrant de cancer colorectal (Popiela, 2001<sup>15</sup>). Dans le cadre de cette étude clinique contrôlée 616 personnes sur 1242 ont reçu cette préparation d'enzymes, pendant 9.2 mois. Une réduction significative des signes et symptômes associés à la maladie a été observé chez les patients recevant les enzymes seules, c'est-à-dire sans recevoir un autre traitement additionnel. Les réactions adverses dues à la chimio- et radiothérapie ont diminué chez tous les patients recevant les enzymes. Popiela et collaborateurs (2001<sup>15</sup>) ont conclu que les enzymes ont amélioré la qualité de vie de ces patients en réduisant les signes et les symptômes de la maladie et les réactions adverses associées aux thérapies antinéoplastiques. Ces enzymes furent généralement bien tolérées. Considérant que ces patients ont un intestin fragile mais ont bien toléré les enzymes, nous pouvons

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Une préparation d'enzyme contenant de la papaine a été donnée complémentirement à une thérapie antinéoplastique chez des patients souffrant de cancer colorectal (Popiela, 2001<sup>15</sup>). Dans le cadre de cette étude clinique contrôlée 616 personnes sur 1242 ont reçu cette préparation d'enzymes, pendant 9.2 mois. Une réduction significative des signes et symptômes associés à la maladie a été observé chez les patients recevant les enzymes seules, c'est-à-dire sans recevoir un autre traitement additionnel. Les réactions adverses dues à la chimio- et radiothérapie ont diminué chez tous les patients recevant les enzymes. Popiela et collaborateurs (2001<sup>15</sup>) ont conclu que les enzymes ont amélioré la qualité de vie de ces patients en réduisant les signes et les symptômes de la maladie et les réactions adverses associées aux thérapies antinéoplastiques. Ces enzymes furent généralement bien tolérées. Considérant que ces patients ont un intestin fragile mais ont bien toléré les enzymes, nous pouvons

supposer que les enzymes dans la papaine seront bien tolérées chez des individus en santé.

Une étude similaire (Beuth, 2001<sup>16</sup>) a été effectuée sur 2339 femmes atteintes d'un cancer du sein, dont 1283 ont reçu des enzymes en complément au traitement de chimio- ou radio-thérapie. Là encore, les patientes prenant des enzymes ont vu leur qualité de vie s'améliorer en ce que leurs signes et symptômes post-thérapie ont diminué significativement. Ces symptômes sont des malaises gastrointestinaux, symptômes mentaux, maux de tête, cachexie, désordres cutanés et effets secondaires associés à la thérapie antinéoplasique. Les enzymes furent bien tolérées. Gujral et collaborateurs (2001<sup>17</sup>) rapportent les mêmes bienfaits chez des patients souffrant de cancers de la tête et du cou, ayant pris des enzymes protéolytiques 3 jours et jusqu'à 5 jours après un traitement de radiothérapie.

Réactions adverses de la papaine :

En règle générale, les enzymes protéolytiques ont un potentiel allergénique. Le risque de sensibilisation aux enzymes par inhalation est présent. La papaine et la bromélaïne auraient aussi le potentiel d'être des allergènes alimentaires (Wuthrich, 1985<sup>18</sup>).

Taylor et Hefle (2001<sup>19</sup>) dans une revue de littérature, rapportent une série d'aliments allergènes et d'autres moins. Ainsi la papaine serait considérée comme ayant la capacité de déclencher une sensibilisation allergique, mais cet ingrédient serait classifié comme rarement allergénique. Puisque la papaine a la capacité de déclencher une sensibilisation allergique, il est préférable de faire mention sur l'étiquette de s'abstenir de prendre ce produit si la personne est allergique.

Diez-Gomez et collaborateurs (1998<sup>20</sup>) ont montré qu'une allergie aux figes (*Ficus benjamina*) ou aux kiwis pouvait aussi entraîner une réactivité croisée avec la papaine. Les structures allergènes pourraient expliquer l'association allergique de la fige et de la papaine. Il est préférable d'en faire mention sur l'étiquette à l'effet d'éviter de prendre ce produit si une personne est allergique aux figes ou aux kiwis.

Kiev (1997<sup>21</sup>) signale le cas d'une femme de 55 ans ayant eu des démangeaisons deux jours après avoir ingéré une pastille contenant du jus de papaye. C'est le premier cas, de dermatite de contact à la papaye sans hypersensibilité à la papaine. Bien que cela soit un cas isolé, la mention d'allergie à la papaye sera mentionnée sur l'étiquette.

Un autre cas isolé, de conjonctivite due à une solution de nettoyage contenant de la papaine (Bernstein, 1984<sup>22</sup>).

Un patient a eu une réaction allergique systémique après avoir ingéré de l'attendrisseur de viande. Les produits contenant de la papaine sont communs dans notre société et l'hypersensibilité à la papaine peut représenter une cause non reconnue de symptômes allergiques (Mansfield, 1983<sup>23</sup>).

Baur (1979a ) rapporte que 7 travailleurs sur 11, ayant été exposés à de la poudre de papaïne en suspension, ont développé des réactions immédiates d'hypersensibilité, en prédominance de l'asthme et des rhinites. Flindt (1978<sup>25</sup>) et Milne (1975<sup>26</sup>) avait fait la même observation chez des travailleurs ayant eu des attaques d'asthme suite à l'exposition à la poussière atmosphérique de papaïne. Une mention d'allergie à la papaïne sera aussi inscrite sur l'étiquette.

Baur (1979b<sup>27</sup>) a aussi rapporté le cas d'une dame de 58 ans, qui a travaillé 10 ans dans une compagnie pharmaceutique, a développé de l'asthme et une rhinite au contact de la bromélaïne. Certains travailleurs sensibles à la papaïne ont aussi développé des allergies de contact et/ des réactions asthmatiques à la bromélaïne. Il faut se rappeler que le SVELTYS n'implique pas une exposition à la papaïne au même titre que les travailleurs qui manipule et inhale la matière première, malgré tout, une mention d'allergie à la bromélaïne et la papaïne sera inscrit sur l'étiquette.

Interactions médicamenteuses de la papaïne :

Le composé beta-lactam inhiberait l'activité d'une variété d'enzymes (cystéine ou serine protéase) dont celle de la papaïne (Gunda, 2002<sup>28</sup>). Des études supplémentaires devront être mis de l'avant pour déterminer l'effet in vivo.

Les enzymes protéolytiques pourraient hydrolyser le coagulum protéique et augmenter l'activité d'antibiotique sur des blessures expérimentales (Rodeheaver, 1975<sup>29</sup>, Brisou, 1975<sup>30</sup>).

Le natural database sur la papaïne<sup>14</sup>, la Commission E<sup>31</sup>, le PDR<sup>32</sup> et Heck (2000<sup>11</sup>) soulèvent la possibilité que la papaïne augmenterait le risque de saignement, mais ces 4 références demeurent prudents puisqu'il n'y a pas d'étude pour démontrer l'augmentation du temps de coagulation. L'interaction avec des médicaments anti-coagulants reste théorique jusqu'à preuve du contraire. Il est alarmiste d'ajouter une remarque sur une interaction potentielle sans étude à l'appui.

### C) Bromélaïne

Bromelain and safety : 11 références, 4 retenues, 7 rejetées car 2 en allemands, 1 sur un usage topique pour les brûlures, 1 trop général, 1 sur immunosérologie, 1 sur l'efficacité sans innocuité.

Bromelain and adverse reaction : 7 références, 3 retenues, 4 rejetées car 2 en langues étrangères, 1 sans résumé, 1 sur la papaïne et non la bromélaïne.

Bromelain and drug interaction : 17 références, 3 retenues (dont 1 référence déjà citée précédemment), 14 rejetées car 8 en langues étrangères, 4 sans résumé et ancien (avant 1975), 1 trop général sur les plantes pouvant interagir avec les drogues dentaires, 1 sur mécanisme d'action in vitro.

Comme déjà mentionné pour la papaïne, les enzymes protéolytiques ont un potentiel allergénique. Le risque de sensibilisation aux enzymes par inhalation est présent. La

papaïne et la bromélaïne auraient aussi le potentiel d'être des allergènes alimentaires (Wuthrich,1985<sup>18</sup>).

Baur (1979b<sup>27</sup>) rapporte le cas d'une dame de 58 ans, qui a travaillé 10 ans dans une compagnie pharmaceutique, a développé de l'asthme et une rhinite au contact de la bromélaïne (déjà rapporté dans l'innocuité de la papaïne, voir plus haut).

Brien et al. (2004<sup>33</sup>) ont fait une revue de littérature d'études cliniques sur la bromélaïne dans le traitement de l'ostéoarthrite (genoux et épaules). Aucune réaction adverse sérieuse n'a été rapportée en relation avec la consommation de bromélaïne ou ananas dans les études (16) analysées. Les réactions adverses répertoriées sont principalement des malaises gastrointestinaux, de même que des maux de tête, fatigue, bouche sèche et réactions allergiques non spécifiées. Ces études utilisaient des doses variant de 540 à 1890 mg bromélaïne par jour. L'innocuité et la tolérance à la bromélaïne à faible dose (540 mg/j) apparaissent bonnes et le profil d'innocuité est similaire sinon meilleur au traitement conventionnel (anti-inflammatoire NSAID). À des dosages supérieurs (945 mg - 1890 mg/j), les réactions adverses mentionnées plus haut sont plus fréquentes. Notre produit SVELTYS ne fournit que 400 mg de bromélaïne par jour et est donc inférieure à la dose jugée sécuritaire (540 mg/j).

Braun et al. (2005<sup>34</sup>) ont évalué dans une étude multicentrique, l'efficacité et l'innocuité de la bromélaïne (d'ananas) chez des enfants de moins de 11 ans ayant une sinusite aigüe. Ainsi 116 enfants de 19 centres ont reçu soit de la bromélaïne seule (62 personnes) ou en combinaison avec la thérapie conventionnelle (34 personnes) ou la thérapie conventionnelle seule (20 personnes). Les patients utilisant la bromélaïne seule ont vu leur rétablissement (pas de symptômes) plus rapidement et de façon significative que dans les autres groupes. Il n'y a eu qu'un enfant de 10 ans, avec une allergie connue aux ananas, qui a eu une réaction allergique légère. Aucun autre effet secondaire n'a été rapporté.

Akhtar et al. (2004 ) ont comparé l'efficacité et l'innocuité d'enzymes de bromélaïne et trypsine avec le diclofenac chez des patients ayant l'ostéoarthrite du genou. Cent trois personnes ont été traitées pour leurs douleurs ostéoarthrites au genou dans le cadre d'une étude clinique aléatoire, double aveugle et en parallèle dans 2 centres d'études. Les personnes ont été divisées en 2 groupes, 52 personnes traités à la bromélaïne et trypsine et 51 personnes ont reçu le diclofenac. Une amélioration considérable fut observée dans les 2 groupes. La tolérance au traitement fut jugée bonne à très bonne dans les 2 groupes. Les auteurs ont conclu que le traitement à la bromélaïne - trypsine peut être considéré comme efficace et une alternative sécuritaire au anti-inflammatoire non stéroïdien (NSAID) comme le diclofenac dans le traitement des épisodes de douleur causées par l'ostéoarthrite du genou. Une fois encore la bromélaïne s'est avérée sécuritaire.

Kerkhoffs et al. (2004<sup>36</sup>) ont voulu comparer l'efficacité et l'innocuité des enzymes rutoside, bromélaïne et trypsine en combinaison (Phlogenzym) ou ingrédient seul ou un placebo dans le traitement d'entorse latérale de la cheville. C'est une étude multicentrique, double aveugle, aléatoire, placebo contrôlé. Huit groupes en parallèle ont été étudiés, dont un groupe a reçu 270 mg bromélaïne par jour. Les résultats des groupes traités n'ont pas été plus concluants que le groupe témoin. Les réactions adverses ont été plus nombreuses dans le groupe avec la combinaison des 3

ingrédients, mais il n'y a pas eu de différence substantielle dans l'intensité des réactions adverses. Parmi les 692 patients, seulement 13 (1,9%) ont interrompu le traitement à cause de réactions adverses. L'étude visait surtout à étudier un produit (Phlogenzym) qui est une combinaison de rutoside, bromélaïne et de trypsine, les réactions adverses ne sont pas énumérées dans l'étude et il n'y a pas non plus de précision à l'égard du groupe à ingrédient unique (bromélaïne seule). Il en demeure pas moins que 270 mg de bromélaïne par jour fut bien toléré.

Selon Gailhofer et al. (1988 ), il est rapporté que la bromélaïne peut occasionner des allergies chez les travailleurs qui la manipulent. Les auteurs ont voulu connaître le taux de sensibilisation à la bromélaïne en particulier chez le personnel de laboratoire qui est en contact avec la bromélaïne. Ces résultats ont ensuite été comparés avec des personnes en santé, sélectionnées de façon aléatoire et qui ne sont pas exposées à la bromélaïne de manière évidente. Leurs conclusions indiquent que :

- La bromélaïne est un fort sensibilisant
- La sensibilisation apparaît habituellement par inhalation et non par ingestion
- L'allergie à la bromélaïne est acquise dans les cas reliés au travail et des mesures de précautions dans les laboratoires sont nécessaires.
- Les tests de sensibilité cutanée avec des allergènes relativement purs, comme la bromélaïne (protéase isolée) peut induire des réactions systémiques, même à de hautes dilutions.

En ce qui concerne notre produit la bromélaïne n'est pas inhalée, mais bien ingérée. Le produit ne sert pas à des tests de sensibilité cutanée et la bromélaïne est dans une capsule, donc ne vient pas en contact avec la peau.

Kelly (1996<sup>38</sup>) a fait une revue de la littérature sur l'usage thérapeutique de la bromélaïne. En plus de préciser ces bienfaits, il mentionne qu'elle est considérée non toxique et sans effets secondaires, c'est pourquoi la bromélaïne peut-être utilisée à des doses variant entre 200 et 2000 mg en toute sécurité et cela pendant une période de temps prolongée.

Le Natural database<sup>39</sup> et Heck (2000<sup>11</sup>) soulève la possibilité que la bromélaïne augmente le risque de saignement, mais ces 2 références demeurent prudents puisqu'il n'y a pas d'étude pour démontrer l'augmentation du temps de coagulation. L'interaction avec des médicaments anti-coagulants reste théorique jusqu'à preuve du contraire. Il est alarmiste d'ajouter sur l'étiquette une remarque sur une interaction potentielle sans étude à l'appui.

En conclusion, après avoir consulté PubMed et autres références, il s'avère que le produit SVELTYS est sécuritaire, car ces composantes présentent peu de risques pour la population et les bienfaits du produit dépassent largement les inconvénients que celui-ci pourrait entraîner. De plus ce produit est vendu depuis plusieurs années et cela sans danger pour les consommateurs. La mise en garde dans les cas d'allergie sera inscrite sur l'étiquette.

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## Safety studies on epigallocatechin gallate (EGCG) preparations. Part 1: Genotoxicity.

**Isbrucker RA. Bausch J. Edwards JA, Wolz E.**

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Public interest in green tea has grown recently due to the potential health benefits from its consumption. Epigallocatechin gallate (EGCG), a principal polyphenolic component of green tea, is considered key to these healthful qualities. Although numerous studies have evaluated the anti-cancer effects of green tea and EGCG, few have examined the safety of EGCG consumption. The genotoxic potential of a concentrated EGCG preparation was tested in Salmonella and L5178Y tk(+/-) mouse lymphoma cell assays to further define the safety of Teavigotrade mark, a high-concentration EGCG extract of Camellia sinensis leaves produced by the same novel method. No mutagenic activity was detected in the bacterial system; however, a clastogenic 'trend' from the formation of hydrogen peroxide was noted in the murine cells. The oral administration of 500, 1000, or 2000mg EGCG/kg to mice did not induce micronuclei formation in bone marrow cells. Similarly, administering 400, 800, or 1200mg EGCG/kg/day in their diet for 10 days did not induce bone marrow cell micronuclei and produced plasma EGCG concentrations comparable to those reported in human studies. The intravenous injection of 10, 25 and 50mg EGCG/kg/day to rats resulted in much higher plasma concentrations and demonstrated an absence of genotoxic effects. From these studies, it is concluded that Teavigotrade mark (EGCG) is not genotoxic.

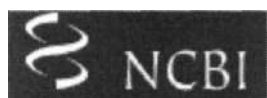
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### **Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: Dermal, acute and short-term toxicity studies.**

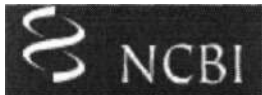
**Isbrucker RA, Edwards JA, Woiz E, Davidovich A, Bausch J.**

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Green tea extract and its principal active ingredient, epigallocatechin gallate (EGCG), are gaining attention and increased usage due to their healthful properties. Despite the increasing demand for these products, few studies have examined their safety. The toxicity of purified green tea extracts containing high concentrations of EGCG have been evaluated in a series of studies in order to define the safety of Teavigotrade mark, a high-concentration EGCG extract produced by the same novel method. Topical EGCG preparations caused minor dermal irritation in rats and guinea pigs, but not rabbits, and was a moderate dermal sensitizing agent in the guinea pig maximization test. A rabbit eye irritation test produced a strong enough response to not warrant any further testing in this assay. An oral dose delivering 2000mg EGCG preparation/kg was lethal to rats; whereas, a dose of 200mg EGCG/kg induced no toxicity. The dietary administration of EGCG preparation to rats for 13 weeks was not toxic at doses up to 500mg/kg/day. Similarly, no adverse effects were noted when 500mg EGCG preparation/kg/day was administered to pre-fed dogs in divided doses. This dose caused morbidity when administered to fasted dogs as a single bolus dose, although this model was considered an unrealistic comparison to the human condition. From these studies a no-observed adverse effect level of 500mg EGCG preparation/kg/day was established.

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### **Safety studies on epigallocatechin gallate (EGCG) preparations. Part 3: Teratogenicity and reproductive toxicity studies in rats.**

**Isbrucker RA, Edwards JA, WojzJE, Davidovich A, Bausch J.**

Burdock Group, 888 17th Street, N.W., Suite 810, Washington, DC 20006, United States.

Green tea and its principal active ingredient, epigallocatechin gallate (EGCG), have been demonstrated to have anticancer properties through interactions with multiple biochemical processes. Since these processes are often crucial in normal fetal development it is important to evaluate the potential effects of EGCG on the fetus. EGCG preparations of >91% purity were administered to pregnant rats during organogenesis and development in order to define the safety of Teavigotrade mark, a high-concentration EGCG extract produced by the same novel method. In an initial preliminary study using subcutaneous and gavage routes, there was no evidence of any direct embryo-fetal toxicity, although some maternal toxicity was seen. In the main teratogenicity study, feeding pregnant rats diets supplemented at 1400, 4200 or 14,000ppm during organogenesis was non-toxic to dams or fetuses. A two-generation study in rats fed 1200, 3600 or 12,000ppm EGCG preparation showed no adverse effects on reproduction or fertility. The highest dose reduced the growth rate of offspring, and there was a slight increase in pup loss. A growth effect among pups was also seen at 3600ppm, but in the second generation only. The lowest dose was considered the overall no-observed adverse effect level (NOAEL). As dams consumed twice the amount of feed during the crucial lactation period, the NOAEL was equivalent to 200mg/kg/day EGCG preparation.

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### **Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study.**

**Bettuzzi S, BrausLM, Rizzi F, Castagnetti G, Peracchia G, Corti A.**

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Green tea catechins (GTCs) proved to be effective in inhibiting cancer growth in several experimental models. Recent studies showed that 30% of men with high-grade prostate intraepithelial neoplasia (HG-PIN) would develop prostate cancer (CaP) within 1 year after repeated biopsy. This prompted us to do a proof-of-principle clinical trial to assess the safety and efficacy of GTCs for the chemoprevention of CaP in HG-PIN volunteers. The purity and content of GTCs preparations were assessed by high-performance liquid chromatography [(-)-epigallocatechin, 5.5%; (-)-epicatechin, 12.24%; (-)-epigallocatechin-3-gallate, 51.88%; (-)-epicatechin-3-gallate, 6.12%; total GTCs, 75.7%; caffeine, <1%]. Sixty volunteers with HG-PIN, who were made aware of the study details, agreed to sign an informed consent form and were enrolled in this double-blind, placebo-controlled study. Daily treatment consisted of three GTCs capsules, 200 mg each (total 600 mg/d). After 1 year, only one tumor was diagnosed among the 30 GTCs-treated men (incidence, approximately 3%), whereas nine cancers were found among the 30 placebo-treated men (incidence, 30%). Total prostate-specific antigen did not change significantly between the two arms, but GTCs-treated men showed values constantly lower with respect to placebo-treated ones. International Prostate Symptom Score and quality of life scores of GTCs-treated men with coexistent benign prostate hyperplasia improved, reaching statistical significance in the case of International Prostate Symptom Scores. No significant side effects or adverse effects were documented. To our knowledge, this is the first study showing that GTCs are safe and very effective for treating premalignant lesions before CaP develops. As a secondary observation, administration of GTCs also reduced

lower urinary tract symptoms, suggesting that these compounds might also be of help for treating the symptoms of benign prostate hyperplasia.

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# Green Tea Polyphenol Epigallocatechin-3-gallate (EGCG) Differentially Inhibits Interleukin-1B-Induced Expression of Matrix Metalloproteinase-1 and -13 in Human Chondrocytes

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## ABSTRACT

### ABSTRACT

Interleukin-1/3 (IL-1/3)-induced inflammatory response in arthritic joints include the enhanced expression and activity of matrix metalloproteinases (MMPs), and their matrix degrading activity contribute to the irreversible loss of cartilage and may also be associated with sustained chronic inflammation. We have earlier shown that green tea (*Camellia sinensis*) polyphenol epigallocatechin-3-gallate (EGCG) was non-toxic to human chondrocytes [Singh R, Ahmed S, Islam N, Goldberg VM, and Haqqi TM (2002) *Arthritis Rheum* 46: 2079-2086] and inhibits the expression of inflammatory mediators in arthritic joints [Haqqi TM, Anthony DD, Gupta S, Ahmed N, Lee MS, Kumar GK, and Mukhtar H (1999) *Proc Natl Acad Sci USA* 96: 4524-4529]. Here we show that EGCG at micromolar concentrations was highly effective in inhibiting the IL-1B-induced glycosami-

noglycan (GAG) release from human cartilage explants in vitro. EGCG also inhibited the IL-1B-induced mRNA and protein expression of MMP-1 and MMP-13 in human chondrocytes. Importantly, EGCG showed a differential, dose-dependent inhibitory effect on the expression and activity of MMP-13 and MMP-1. A similar differential dose-dependent inhibition of transcription factors NF-KB and AP-1 by EGCG was also noted. These results for the first time demonstrate a differential dose-dependent effect of EGCG on the expression and activity of MMPs and on the activities of transcription factors NF-KB and AP-1 and provide insights into the molecular basis of the reported anti-inflammatory effects of EGCG. These results also suggest that EGCG or compounds derived from it may be therapeutically effective inhibitors of IL-1B-induced production of matrix-degrading enzymes in arthritis.

Osteoarthritis and rheumatoid arthritis are a group of diseases with different profiles and unknown etiology, but sustained chronic production of inflammatory mediators is an important characteristic of both the diseases. The pro-inflammatory cytokine IL-1/3, produced in an arthritic joint by activated synovial cells and infiltrating macrophages, is considered to be one of the most potent catabolic factors in arthritis (Kraan and van den Berg, 2000). IL-1B induce the enhanced production of several mediators of cartilage degra-

dition such as NO and matrix metalloproteinases (MMPs) by activating a diverse spectrum of signaling cascades in human chondrocytes (Mengshol et al., 2000; van den Berg, 2000) and by inhibiting the concentration of inhibitor of MMPs (TIMP) in arthritic joints (Amin and Abramson, 1998; Mengshol et al., 2000). The expression of IL-1 receptor (IL-1r) is high on chondrocytes isolated from arthritic joints suggesting that they are more sensitive to the action of this cytokine (Ismail, 1992; Martel-Pelletier, 1992). IL-1/3 also suppress the biosynthesis of type II collagen and aggrecan (Goldring et al., 1994; Gouze et al., 2001) and proliferation of chondrocytes (Blanco and Lotz, 1995) thus inhibiting the repair process in the cartilage. Additional evidence pointing to the involvement of IL-1B in cartilage degradation emerged from studies showing that intra-articular administration of IL-1B into rabbit and

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**ABBREVIATIONS:** IL, interleukin; NO, nitric oxide; MMP, metalloproteinase; MAPK, mitogen-activated protein kinase; JNK, c-Jun NH<sub>2</sub>-terminal kinase; AP-1, activating protein-1; EGCG, epigallocatechin-3-gallate; DMMB, 1,9-dimethylmethylene blue; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; TAMRA, 5-carboxytetramethylrhodamine; FAM, 5-carboxyfluorescein; NF-KB, nuclear factor-KB; RLU, relative light units; TIMP-1, tissue inhibitor of metalloproteinase 1; GAG, glycosaminoglycan; ANOVA, analysis of variance; ROS, reactive oxygen species; PAGE, polyacrylamide gel electrophoresis; SEAP, secreted alkaline phosphatase; OA, osteoarthritis.

mouse joints results in loss of proteoglycans from the cartilage (Pettipher et al., 1986; Van de Loo and van den Berg, 1990) and inhibition of IL-1 action by IL-1r antagonist or by IL-1-neutralizing antibodies protects cartilage in an arthritic joint (Oligino et al., 1999; Neidhart et al., 2000).

MMPs are a large group of enzymes that play a crucial role in tissue remodeling as well as in the destruction of cartilage and bone in an arthritic joint due to their ability to degrade a wide variety of extracellular matrix components (Mengshol et al., 2002). Production and release of MMPs is microenvironmental and induced by several factors including the pro-inflammatory cytokine IL-1B (Kraan and van den Berg, 2000). Among the various MMPs, MMP-13 is of particular importance because it is found elevated in joint disorders (Mitchell et al., 1996) and can cleave type II collagen, the major component of the cartilage matrix, more efficiently. Studies have documented that in arthritic joints, degradation of type II collagen is excessive due to increased cleavage by MMPs (Billinghurst et al., 2000). Other studies have shown that excessive activity of MMP-13 can produce the type of pathology seen in arthritic joints (Neuhold et al., 2001). Pro-inflammatory cytokine-induced expression of MMP-13 in human chondrocytes and in animal models of arthritis is dependent on the activation of the MAPK subgroup JNK and the transcription factor AP-1 (Han et al., 2001; Liacini et al., 2002). In chondrocytes, activation of JNK pathway and C/EBP $\beta$  are required for the activation of MMP-13 promoter activity (Mengshol et al., 2001), and indeed, physical interaction between transcription factors C/EBP $\beta$  (Runx-2, C/EBP $\beta$ ) and AP-1 was shown to be necessary for the activation of MMP-13 promoter (D'Alonzo et al., 2002). An important role of JNK in the pathogenesis of arthritis is also evident from studies showing that inhibitors of JNK protect from arthritis in animal models (Han et al., 2001).

Green tea is a rich source of catechins, and several epidemiological and animal model studies have shown that green tea consumption was associated with health benefits including inhibition of inflammation (Higdon and Frei, 2003). Most of the beneficial health effects of green tea are mimicked by its most prevalent catechin epigallocatechin-3-gallate (EGCG) at micromolar concentrations. EGCG influence a number of cellular mechanisms and has been shown to inhibit the activities of MMP-2 and MMP-9 (Garbisa et al., 2001; Cheng et al., 2003). In addition, EGCG is also an inhibitor of the metallo-elastase and serine-elastase activity and down-regulates the levels of several markers of oxidative stress (Benelli et al., 2002; Dona et al., 2003). We have previously shown that EGCG inhibit the activation of the cytokine-activated JNK and AP-1 pathways in human chondrocytes (Singh et al., 2002). In the present study, we evaluated the potential of EGCG to protect human cartilage explants from IL-1/3-induced release of cartilage matrix proteoglycans and the induction and expression of MMP-1 and MMP-13 in human chondrocytes.

## Materials and Methods

**Reagents.** All the culture medium and reagents for molecular biology were obtained from either Cellgro (Mediatech Inc., Herndon, VA) or Invitrogen (Carlsbad, CA). EGCG was purchased from Alexis Biochemicals (San Diego, CA), and recombinant human IL-1/3 was purchased from R & D Systems (St. Paul, MN). 1,9-Dimethylmeth-

ylene blue (DMMB) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Polyclonal goat anti-human MMP-1 and polyclonal goat anti-human MMP-13 antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Horseradish peroxidase-conjugated anti-goat IgG was purchased from Pierce Biotechnology (Rockford, IL).

**Culture of Human OA Cartilage and Chondrocytes.** Human OA cartilage samples were procured through the Cooperative Human Tissue Network, with prior approval of the Institutional Review Board of University Hospitals of Cleveland. Full-thickness cartilage slices (20-25 mg) were dissected from the cartilage using sterile scalpel blade (Feather Safety Razor Co., Osaka, Japan). Four to five cartilage pieces (approximately equal in size and weight) were transferred to each well of a 24-well, flat-bottomed plate (NUNC A/S, Roskilde, Denmark) containing DMEM supplemented with antibiotics and 10% fetal calf serum. The cartilage explants were treated with IL-1B alone or with IL-1B + EGCG or EGCG for 72 h. Explants cultured in the absence of IL-1/3 and EGCG were used as controls. Total glycosaminoglycan present in the culture supernatant was estimated as described below.

**Quantitation of Glycosaminoglycans.** At the end of the culture period, the culture medium was collected from each group [controls, IL-1/3 only (10 ng/ml), IL-1/3 + EGCG (100  $\mu$ M), and IL-1B + EGCG (200  $\mu$ M)]. A 50- $\mu$ l aliquot of the collected supernatant from each sample was used to estimate the total glycosaminoglycan concentration by a colorimetric method employing DMMB as previously described (Farndale et al., 1986). Color intensity was read spectrophotometrically at 535 nm, and the values were derived from a standard curve that was prepared using different concentrations of chondroitin sulfate. Results are expressed as micrograms of glycosaminoglycan released per milligram of cartilage tissue.

**Chondrocyte Culture.** Chondrocytes were prepared by the enzymatic digestion of femoral head cartilage as previously described (Ahmed et al., 2003). Chondrocytes were plated (1  $\times$  10<sup>6</sup>/ml) in 35-mm culture dishes (Becton-Dickinson, Franklin Lakes, NJ) in complete DMEM with 10% fetal calf serum and allowed to grow for 72 h at 37°C and 5% CO<sub>2</sub> in a tissue culture incubator. They were serum-starved overnight and then treated with IL-1/3 (5 ng/ml) and IL-1/3 + EGCG (20-100  $\mu$ M) for time periods indicated in each figure. Chondrocytes cultured without IL-1/3 or EGCG served as controls.

**Western Immunoblotting for MMP-1 and MMP-13.** Confluent chondrocyte cultures were washed with Hank's buffered salt solution and treated with IL-1/3 in the serum-free medium for 24 h. At the end of the experiments, medium was removed and 500  $\mu$ l was concentrated using Microcon concentrators (Millipore, Bedford, MA) for 30 min at 25°C. Concentrated samples with equal amounts of protein (25  $\mu$ g) were mixed with 2x reducing sample buffer and resolved by SDS/PAGE, transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA), and the blot was probed with polyclonal goat anti-human MMP-1 and MMP-13 antibodies (Santa Cruz Biotechnology Inc.). Immunoreactive proteins were visualized by enhanced chemiluminescence using HRP-conjugated anti-goat IgG (Pierce). Images were captured and the intensities of the protein bands were analyzed using the Alpha Innotech Imaging System and are expressed as arbitrary optical density units.

**Determination of TIMP-1, MMP-1, and MMP-13 Activity by ELISA.** The activities of TIMP-1, MMP-1, and MMP-13 were determined in culture supernatant from the above experiments using commercially available ELISA kits essentially according to the instructions of the manufacturer (Amersham-Pharmacia, Piscataway, NJ) and expressed as 5Absorbance<sub>405</sub>/h<sup>2</sup>  $\times$  1000.

**Quantitative RT-PCR.** Total cytoplasmic RNA was prepared from human chondrocytes using a commercially available kit according to the instructions of the manufacturer (Qiagen, Valencia, CA). We used real-time quantitative RT-PCR with internal fluorescent hybridization probes using an ABI Prism 7700 detection system (ABI/Perkin Elmer Biosystems, Foster City, CA) as previously de-

scribed (Singh et al., 2002). This allows the sensitive and specific quantification of targeted mRNA transcripts. The target-specific RT primer sets and their fluorescent probes used have been described earlier (Singh et al., 2002). The probes were labeled with 5-carboxyfluorescein (FAM) at the 5' end and with TAMRA at the 3' end (ABI/Perkin Elmer Biosystems). The degradation of the probe during PCR results in increased fluorescence of the probe and allows the detection of the PCR product by monitoring the increase in fluorescence of the reporter dye. To quantitate the expression of MMP-1 and MMP-13 mRNA, single-stranded complementary DNA (cDNA) was synthesized using 100 ng of total RNA prepared from OA chondrocytes as described earlier (Singh et al., 2002). Concentrations of primers and probes were optimized in pilot studies to allow accurate quantitation of the target transcript. The PCR conditions were 1 cycle at 50°C for 2 min, 1 cycle at 95°C, followed by 40 cycles (95°C for 15 s and 60°C for 1 min). To ensure the lack of DNA contamination in the RNA samples, a tube of sample without RT was included as a no-template control. Fourfold serial dilutions of sample cDNAs were used to generate curves of log input versus threshold cycle. Expression of MMP-1 and MMP-13 mRNA was normalized for levels of  $\beta$ -actin mRNA and the results are expressed as mRNA copies of MMP/ $10^6$  copies of  $\beta$ -actin mRNA.

**Transient Transfection Studies.** To study the effect of EGCG on IL-1/3-induced activation of NF- $\kappa$ B and AP-1, human chondrocytes were transiently transfected with reporter plasmids available commercially (Mercury Pathway Profiling System; Clontech, Palo Alto, CA). Briefly, 60 to 80% confluent  $1 \times 10^6$  cells/35-mm plates were transfected with 1  $\mu$ g of pNF- $\kappa$ B-SEAP and pAP-1-SEAP reporter plasmid or the negative control vector pTAL-SEAP according to the instructions of the manufacturer (Clontech). After transfection, chondrocytes were not treated (controls), treated with IL-1B alone (5 ng/ml), or treated with IL-1/3 + different concentrations of EGCG. The secreted alkaline phosphatase (SEAP) in the culture medium was detected using the Great Escape SEAP chemiluminescence detection kit (Clontech). The results were expressed as relative light units (RLU) after subtraction of the values obtained with chondrocytes transfected with the negative control vector.

**Statistical Analysis.** Each experiment was performed three times using cartilage samples from age- and sex-matched donors. Data obtained were pooled, and the level of significance between untreated chondrocytes, chondrocytes treated with IL-1/3 alone, chondrocytes treated with IL-1B + EGCG, or chondrocytes treated with EGCG alone is based on Dunnett's *t* test followed by analysis of variance (ANOVA). Values of  $p < 0.05$  were considered significant.

## Results

**EGCG Inhibited the IL-1B-Induced Cartilage Matrix Degradation in Vitro.** The effect of EGCG on IL-1/3-induced cartilage degradation is shown in Fig. 1. Treatment with IL-1B induced the cartilage degradation, measured as the release of glycosaminoglycan in culture medium from cartilage explants, in the culture medium as previously shown (Adcocks et al., 2002). However, the IL-1B-induced release of GAG was inhibited in a dose-dependent manner by the green tea catechin EGCG (Fig. 1). The difference in the amounts of glycosaminoglycan released from cartilage explants treated with IL-1/3 alone and in the two groups treated with IL-1/3 in the presence of 100 and 200  $\mu$ M EGCG was statistically highly significant ( $n = 3$ ,  $p < 0.005$ ). Untreated cartilage explants also showed a basal level of glycosaminoglycan release but this was also blocked by EGCG indicating that EGCG on its own did not contribute to the enhanced glycosaminoglycan release detected in the group treated with IL-1/3 alone (Fig. 1). Thus, the critical observation of the present studies is that EGCG appeared to be an effective

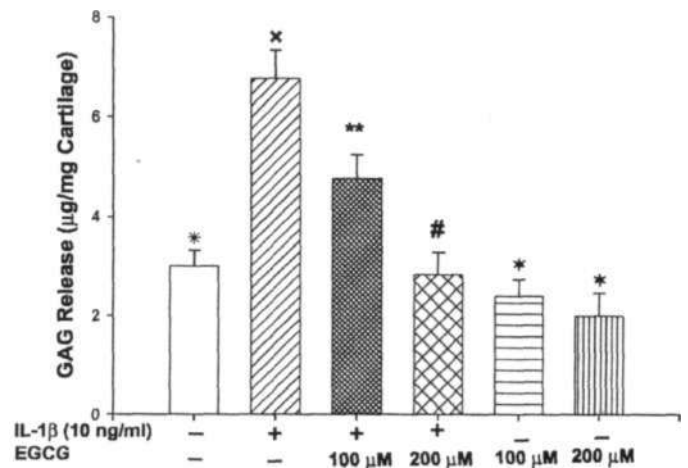


Fig. 1. EGCG inhibited the IL-1/3-induced release of GAG from human cartilage explants in vitro. Approximately equal size and weight cartilage pieces were incubated in medium alone or medium containing either IL-1/3 (10 ng/ml) alone, or IL-1/3 + EGCG (100  $\mu$ M) or IL-1B + EGCG (200  $\mu$ M) for 72 h. Total GAG released from cartilage explants in culture supernatant was quantified using a colorimetric method and the values were derived from a standard curve. This experiment was repeated with age- and sex-matched cartilage samples to ensure for the reproducibility. X,  $p < 0.005$  when compared with controls; \*\*,  $p < 0.005$  compared with IL-1B alone treated group; #,  $p > 0.05$  compared with controls; \*,  $p < 0.05$  compared with controls and \*,  $p < 0.005$  compared with IL-1B alone treated group.

agent for blocking the IL-1/3-induced release of glycosaminoglycan from human cartilage explants, at least in vitro.

### EGCG Inhibited the IL-1B-Induced Expression of MMP-1, MMP-13, and TIMP-1 in Human Chondrocytes.

To evaluate the effect of EGCG on IL-1/3-induced expression of MMPs protein in human chondrocytes, we treated human chondrocytes with IL-1/3 alone and with IL-1/3 + 100  $\mu$ M EGCG for 24 hrs. As shown in Fig. 2, stimulation of human chondrocytes with IL-1B induced the expression and release of MMP-1 and MMP-13 in the culture supernatant as determined by Western immunoblotting. Analysis of the immunoblot image revealed that the levels of the MMPs detected in the supernatant of chondrocytes treated with IL-1B alone

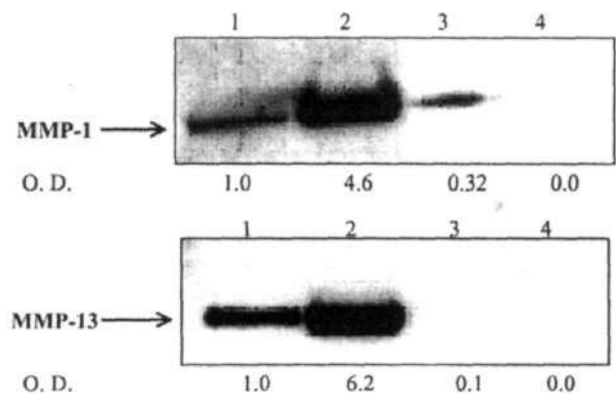
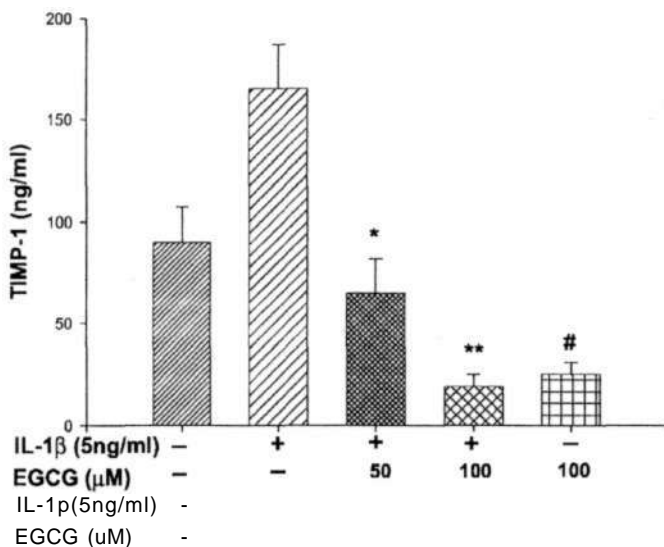


Fig. 2. EGCG inhibited the IL-1/3-induced expression of MMP-1 and MMP-13 in human chondrocytes. Human chondrocytes were not treated (lane 1), treated with IL-1B alone (lane 2), treated with IL-1B + EGCG (100  $\mu$ M) (lane 3), or treated with EGCG alone (100  $\mu$ M) (lane 4) for 24 h. Culture supernatant was concentrated and total proteins (25  $\mu$ g) were resolved by SDS-PAGE and transferred to nitrocellulose. The Western blot was probed with polyclonal antibodies specific for the human MMP-1 and MMP-13 protein. Results shown are representative of two independent experiments.

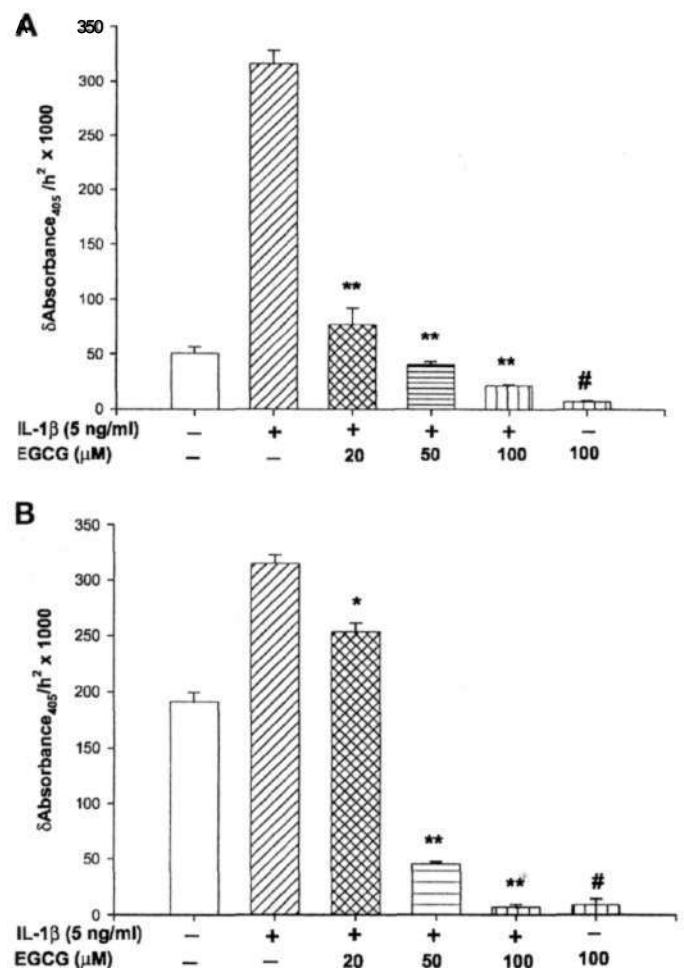
were 4.6-fold higher for MMP-1 and 6.2-fold higher for MMP-13, when compared with the levels detected in untreated chondrocytes (compare lane 1 and lane 2 in each blot). Importantly, cytokine-stimulated increase in the expression of MMP-1 and MMP-13 in the presence of EGCG was inhibited to less than the basal level detected in untreated chondrocyte cultures ( $p < 0.005$ ). Since MMPs are bound to TIMP-1 as a proenzyme, the production of MMPs may also contribute to the enhanced expression of TIMP-1 and inhibition of MMPs may down-regulate the expression of TIMP-1. To address this possibility, we analyzed the effect of EGCG on IL-1/3-induced production of TIMP-1 in culture supernatant. As shown in Fig. 3, IL-1B enhanced the production of TIMP-1 in culture supernatant by 36% when compared with the levels detected in untreated chondrocytes ( $p < 0.05$ ). Prophylactic treatment of chondrocytes with EGCG (100  $\mu\text{M}$ ) significantly ( $p < 0.001$ ) inhibited the IL-1 $\beta$ -induced production of TIMP-1 in culture supernatant. Similarly, treatment with EGCG alone also reduced the endogenously released TIMP-1 in chondrocyte cultures as well ( $p < 0.05$ ). Thus, these data correlate with the down-regulation of IL-1B-induced expression of MMPs in human chondrocytes (Fig. 2) and indicate that the effect of EGCG on TIMP-1 expression may not be direct but most likely reflects the inhibitory effect of EGCG on the expression of MMPs.

**EGCG Down-Regulated the Activities of MMP-1 and MMP-13 in Human Chondrocytes.** We also determined the effect of EGCG on the activities of MMP-1 and MMP-13 using an ELISA kit. To rule out the possibility of detecting cysteine and serine proteinases activity, their inhibitors were added to the conditioned culture medium to abolish their activity prior to the assay. Both MMP-1 and MMP-13 were constitutively active in untreated, control human chondrocytes with the activity level of MMP-1 being at least 3-fold



**Fig. 3.** EGCG inhibited the IL-1B-induced production of TIMP-1 in human chondrocytes. Human chondrocytes (80% confluent) were not treated (1); treated with IL-1B alone (2); treated with IL-1B + EGCG (3-4); or treated with EGCG alone (5) for 24 hr and the TIMP-1 released in the culture medium was measured using an ELISA kit (Biotrak; Amersham). Human chondrocytes stimulated with IL-1/3 in the presence of EGCG had decreased levels of TIMP-1 (\*,  $p < 0.05$ ; \*\*,  $p < 0.001$ ) compared with cultures treated with IL-1B alone. Treatment with EGCG alone decreased the constitutive levels of TIMP-1 (#,  $p < 0.05$ ) when compared with the control values. Values shown are mean  $\pm$  S.D. of three independent experiments, each performed in triplicate using age- and sex-matched samples.

higher than that of MMP-13 (Fig. 4). However, treatment of chondrocytes with IL-1B up-regulated the activities of both the MMP-1 and MMP-13 by 110 and 522% ( $p < 0.001$ ) when compared with their respective control values (Fig. 4). In this experiment, we also analyzed the dose-dependent effect of EGCG on the activity of MMP-1 and MMP-13. Importantly, treatment of human chondrocytes with IL-1/3 in the presence of different doses of EGCG (20, 50, or 100  $\mu\text{M}$ ) also inhibited the activities of MMP-1 and -13. Compared with human chondrocytes treated with IL-1/3 alone, the range of percent inhibition by different doses of EGCG was 20 to 97% for MMP-1 and 75 to 93% for MMP-13. Interestingly, this differential inhibitory effect was most pronounced on the activity of MMP-13 when chondrocytes were treated with 20  $\mu\text{M}$  EGCG (Fig. 4) indicating that low doses of EGCG have a marked selective inhibitory effect on the activity of MMP-13



**Fig. 4.** EGCG inhibited the IL-1/3-induced up-regulation of MMP-13 (A) and MMP-1 (B) activity in human chondrocytes. Human chondrocytes were not treated, treated with 5 ng/ml of IL-1B alone, or treated with IL-1B (5 ng/ml) in the presence of different concentrations of EGCG or with EGCG alone for 24 h and then the supernatant was collected and used to assay for the MMP activity using MMP-1 and MMP-13 Biotrak ELISA kits (Amersham). Results showed that co-treatment with EGCG significantly inhibited the IL-1B-induced up-regulation of both the MMP-13 and MMP-1 activity in a dose-dependent manner (\*,  $p < 0.05$ , \*\*,  $p < 0.001$ ). Treatment with EGCG alone showed a marked reduction in the constitutive activity of both the MMPs when compared with the activity levels detected in untreated chondrocytes (#,  $p < 0.005$ ). Results shown are mean  $\pm$  S.D. of three independent experiments performed with age- and sex-matched samples.

compared with MMP-1, at least in vitro. It is interesting to note that epicatechin or sodium gallate alone or in combination had no inhibitory effect on the activity of either MMP-1 or MMP-13 in this assay (not shown). Additionally, IC<sub>50</sub> values for inhibition by EGCG deduced from regression analysis of the dose-response curve for MMP-1 was 27  $\mu$ M, and for MMP-13 it was 16.5  $\mu$ M, which is within the physiologically achievable range.

**Inhibition of IL-1 $\beta$ -Induced Up-Regulation of MMP-1 and MMP-13 mRNA Expression by EGCG in Human Chondrocytes.** The results of the effects of EGCG on the transcription of MMP genes in human chondrocytes are shown in Fig. 5. The level of MMP-1 and MMP-13 mRNAs was quantified by a highly sensitive and specific quantitative RT-PCR method, and the values obtained were normalized to the level of B-actin mRNA in the samples. Our results showed that human chondrocytes treated with IL-1/3 had different levels of the MMP-1 and MMP-13 mRNA (Fig. 5). In all of the samples analyzed, levels of MMP-1 mRNA were higher compared with the level of MMP-13 mRNA in IL-1 $\beta$ -stimulated human chondrocytes (Fig. 5). However, mRNA levels showed a marked decline in the samples treated with IL-1/3 in the presence of EGCG, and this decline was dose-dependent. Interestingly, and in line with the above results, EGCG was most effective against the induction of MMP-13 even at the lowest dose studied whereas higher doses were needed to have an impact on the expression of MMP-1 mRNA. This is a novel observation and has not been previously reported.

**Transcription Factors NF-KB and AP-1/c-Jun Are Differentially Sensitive to EGCG.** In chondrocytes, the gene expression of MMP-13 is tightly regulated by transcription factors NF-KB and c-Jun, and inhibition of any one or both could result in the attenuation of MMP-13 (Mengshol et al., 2000). In earlier studies, we have shown that EGCG inhibited the IL-1 $\beta$ -induced increase in the nuclear levels of transcription factors NF-KB and AP-1/c-Jun in human chondrocytes by blocking their nuclear translocation and activation (Singh et al., 2002a, 2002b; Ahmed et al., 2003). Induc-

tion and expression of MMP-13 has been shown to be regulated by the activation of both the transcription factors NF-KB and AP-1 (Mengshol, 2000). Using transient transfection with reporter plasmids, we studied whether the inhibition of MMP-1 and MMP-13 mRNA observed in the above experiments could be due to the inhibition of activation and promoter binding activity of NF-KB and AP-1 by EGCG. As shown in Fig. 6 (A and B), IL-1 $\beta$  preferentially and strongly stimulated the NF-KB pathway (approximately 20 times higher) than AP-1 pathway in transiently transfected chondrocytes. Importantly, our results also showed that EGCG differentially inhibits the promoter activation activity of NF-KB and AP-1 and identifies that the activity of transcription factor NF-KB was more sensitive to the effect of EGCG, even to the lower doses. Treatment with EGCG inhibited the activity of NF-KB by 56 to 94% in a dose-dependent manner (Fig. 7A). In contrast, EGCG at a lower dose (20  $\mu$ M) had no

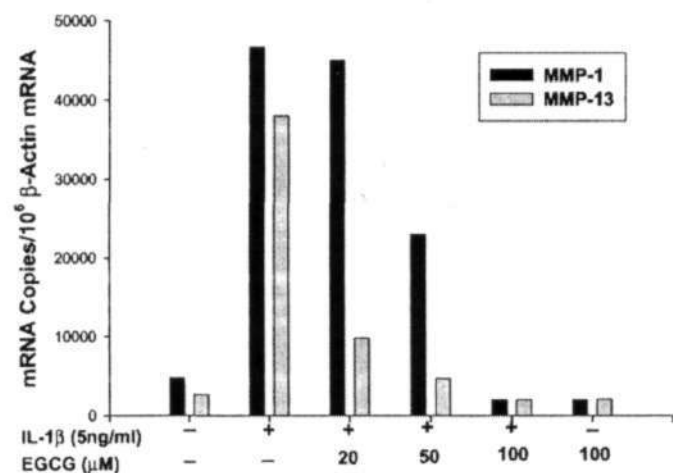


Fig. 5. EGCG inhibited the IL-1/3-induced expression of MMP-1 and MMP-13 mRNA in human chondrocytes. Chondrocytes ( $2 \times 10^6$ ) were stimulated with IL-1 $\beta$  (5 ng/ml) alone or in combination with different concentrations of EGCG for 24 h. Total RNA was prepared, and the expression of MMP-1 and MMP-13 mRNA was determined by real-time quantitative RT-PCR. Values were expressed relative to the levels of B-actin mRNA.

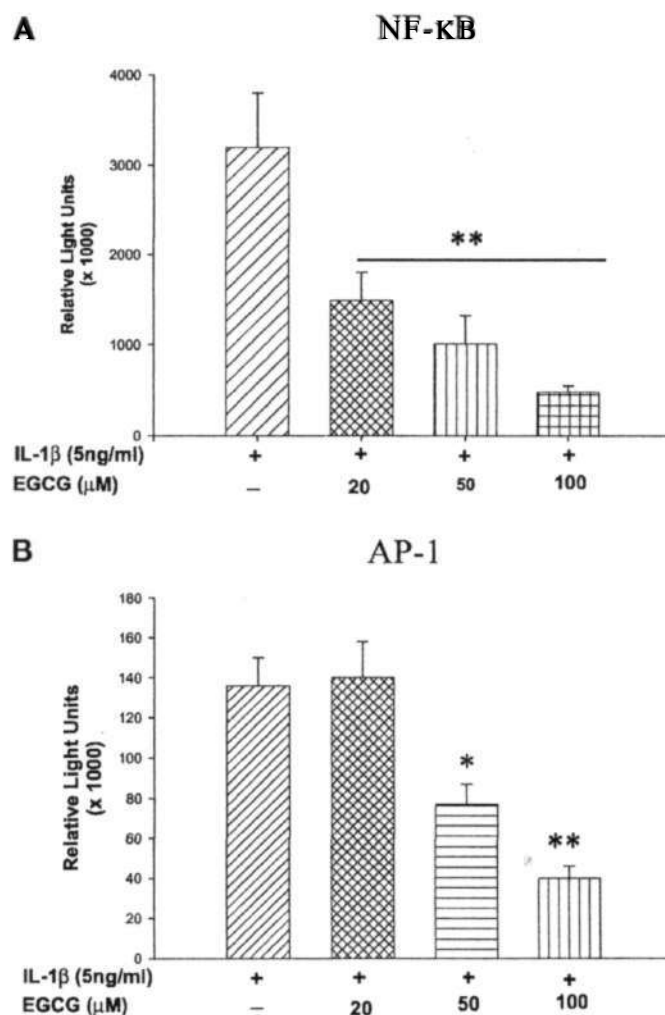


Fig. 6. Differential sensitivity of the DNA binding activity of transcription factors NF-KB and AP-1 to the effects of EGCG in human chondrocytes. Human chondrocytes (60-80% confluent) were transiently transfected with 1  $\mu$ g each of PNF-KB-SEAP (A) and pAP-1-SEAP (B) reported plasmids (Clontech) using the LipofectAMINE reagent. Transfected chondrocytes were then treated with IL-1/3 alone and in the presence of 20, 50, or 100  $\mu$ M of EGCG for 24 h. The SEAP was measured in culture supernatant using Chemiluminescence detection kit. (\*,  $p < 0.05$ ; \*\*,  $p < 0.001$  v/s IL-1 $\beta$ ), and the values are expressed as RLU. Results shown are mean  $\pm$  S.D. of two independent experiments performed with age- and sex-matched samples. (IL = IL-1/3; E = EGCG).

inhibitory effect on promoter activation by AP-1, and higher concentrations of EGCG (50 and 100  $\mu\text{M}$ ) were needed to obtain a 49 to 72% inhibition of AP-1 activity (Fig. 7B). These results correlate with the inhibition of the expression of MMP-1 and MMP-13 mRNAs to the inhibition of NF- $\kappa\text{B}$  and AP-1. However, the differential dose-dependent effect on the activity of the two transcription factors analyzed in this study suggests that the AP-1-dependent gene expression may not be substantially affected by the physiological concentrations of EGCG. These are also novel findings and have not been reported previously.

## Discussion

Previous work from our laboratory has shown that green tea inhibits the development of arthritis in a mouse model (Haqqi et al., 1999), inhibits the degradation of human cartilage proteoglycan and type II collagen (Adcocks et al., 2002), and selectively inhibits the ADAMTS-1, -4, and -5 (Vankemmelbeke et al., 2003). This study addresses the induction of MMP-1 and MMP-13 in human chondrocytes by IL-1/3 and demonstrates the ability of EGCG, the most abundant and biologically active catechin of green tea, to inhibit the IL-1B-induced cartilage proteoglycan degradation and expression of MMP-1 and MMP-13 in human chondrocytes. Our results not only confirm the previous results (Adcocks et al., 2002) that EGCG blocked the IL-1/3-induced cartilage proteoglycan release *in vitro* but extends these further by showing that micromolar concentrations of EGCG effectively inhibit the IL-1B-induced up-regulation of MMP-1 and MMP-13 in the human chondrocytes. Almost complete inhibition of both the MMP-1 and MMP-13 enzyme activity was observed at a concentration of 100  $\mu\text{M}$  EGCG. Although this concentration of EGCG may not be achieved physiologically through oral consumption but may readily be achieved through local administration. Our results showing that the two matrix metalloproteinases, MMP-1 and MMP-13, known to be associated with cartilage degradation in an arthritic joint were differentially inhibited by EGCG is an important and novel finding. These results show that MMP-13 was more sensitive to the inhibitory effect of even lower concentrations of EGCG as determined by an ELISA method (Fig. 4). Additionally, and as previously reported (Vankemmelbeke et al., 2003), the inhibitory effect apparently was not via direct inhibition of the activity of MMP-1 or MMP-13 but most likely reflected the inhibition of IL-1B-induced expression of their mRNAs suggesting that the effect was at the transcriptional level. This aspect was not addressed in the previous studies (Vankemmelbeke et al., 2003). These findings assign a novel property to EGCG, adding to the anti-cancer and anti-inflammatory properties previously described for this compound (Lin, 2002; Higdon and Frei, 2003). Thus, in addition to blocking the release of proteoglycans from cartilage matrix by inhibiting the ADAMTS (Vankemmelbeke et al., 2003), EGCG also inhibits the IL-1B-induced up-regulation of expression and activity of collagenase in physiologically achievable doses in human chondrocytes. Therefore, consumption of green tea or EGCG may inhibit the activities of MMPs involved in the degradation of native collagen, and this may have a suppressive effect on cartilage degradation in arthritic joints.

MMPs are a family of proteolytic enzymes that are nor-

mally required for the timely and controlled breakdown of the ECM under normal physiological conditions (Brinckerhoff and Matrisian, 2002). However, their uncontrolled regulation and enhanced expression has been closely associated with the progression of arthritis (Murphy et al., 2002). Earlier studies have shown that EGCG inhibited the gelatinase subgroup of MMPs (MMP-2 and MMP-9) in various cancerous cell lines when stimulated with ultraviolet B radiations, reactive oxygen species (ROS) and pro-inflammatory cytokines like IL-1B and TNF- $\alpha$ , thereby inhibiting tumor metastasis (Garbisa et al., 2001; Cheng et al., 2003). However, this is the first elaborative study to evaluate the effect of EGCG on collagenase subgroup of MMPs. Screening of potential molecules possessing possible therapeutic efficiency toward the inhibition of MMPs has recently gained considerable attention (Skiles et al., 2001). Limitations of synthetic MMP inhibitors due to their monomodal nature, lack of specificity, and greater side effects suggest a need to develop therapeutic strategies focusing on the prophylactic agents or supplements that could modify or reverse the progression of cartilage degradation in the affected joints. Recent studies have proved the ability of botanicals in targeting multiple downstream events, alone or with current modalities of treatment to provide higher efficacy and minimal toxicity for an effective intervention (Qiu and Kao, 2003). The catechins are bioavailable following the consumption of green tea with a half-life of a few hours (Yang et al., 1998). Therefore, it is likely that consumption of green tea may have a prophylactic effect on cartilage homeostasis. In this regard, as shown in the present study, green tea catechin EGCG certainly holds promise as it consistently and reproducibly inhibited the IL-1B-induced up-regulation of MMPs in human chondrocytes—the only cell type present in the cartilage.

Although these MMPs collectively act in a synchronized manner to degrade articular cartilage, the collagenase subgroup (especially MMP-1 and -13) has been shown to perform a salutary role in chewing the native type II collagen in ECM (Billinghurst et al., 2000). The proteolytic activities of MMPs are precisely regulated during activation from their precursors and inhibition by endogenous inhibitors of metalloproteinases (TIMPs) (Visse and Nagase, 2003). IL-1B-induced factors have been shown to activate the MMPs by the disruption of Cys-Zn<sup>2+</sup> interaction (Brinckerhoff and Matrisian, 2002). Different MMPs have been implicated in different disease conditions, such as gelatinases in cancer metastasis and collagenases in arthritis (Brinckerhoff and Matrisian, 2002). Although, EGCG has been shown to inhibit tumor growth/invasion by inhibiting the gelatinase activity (Garbisa et al., 2001; Cheng et al., 2003), this is the first study showing the effect of EGCG on collagenase gene expression and activity.

There are a number of studies documenting the beneficial health effects of green tea consumption. Most of these studies place emphasis on the anti-cancer properties of green tea, which have now been attributed, at least in part, to the ability of green tea polyphenols to inhibit the gelatinases (Garbisa et al., 2001; Cheng et al., 2003). To this, based on our results, we can add that EGCG in a dose-dependent manner is a potent inhibitor of IL-1B-induced induction of collagenases (MMP-1 and MMP-13) and markedly inhibits collagenase-3 (MMP-13) at a physiologically achievable concentration (20  $\mu\text{M}$ ) whereas higher concentrations (beyond

the concentrations that can be attained simply by drinking green tea) were needed to inhibit the induction and expression of MMP-1 to a meaningful extent. We therefore conclude that inhibition of arthritis following green tea consumption in an animal model (Haqqi et al., 1999) and inhibition of cartilage degradation by EGCG (the present study; Adcocks et al., 2002; Vankemmelbeke, 2003) may be the result of direct inhibition of some of the matrix-degrading enzymes/factors by EGCG through preserving the Cys-Zn<sup>2+</sup> interaction or in combination with down-regulation of these enzymes/factors at the mRNA level through inhibition of transcription factors. Therefore, it is tempting to suggest that green tea polyphenol EGCG or compounds derived from it may serve as lead agents in the design of more potent and effective inhibitors of collagenases for use therapeutically to block the pathogenesis of arthritis.

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# Pharmacokinetics and Safety of Green Tea Polyphenols after Multiple-Dose Administration of Epigallocatechin Gallate and Polyphenon E in Healthy Individuals<sup>1</sup>

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## ABSTRACT

**Purpose:** Green tea and green tea polyphenols have been shown to possess cancer preventive activities in pre-clinical model systems. In preparation for future green tea intervention trials, we have conducted a clinical study to determine the safety and pharmacokinetics of green tea polyphenols after 4 weeks of daily p.o. administration of epigallocatechin gallate (EGCG) or Polyphenon E (a defined, decaffeinated green tea polyphenol mixture). In an exploratory fashion, we have also determined the effect of chronic green tea polyphenol administration on UV-induced erythema response.

**Experimental Design:** Healthy participants with Fitzpatrick skin type II or III underwent a 2-week run-in period and were randomly assigned to receive one of the five treatments for 4 weeks: 800 mg EGCG once/day, 400 mg EGCG twice/day, 800 mg EGCG as Polyphenon E once/day, 400 mg EGCG as Polyphenon E twice/day, or a placebo once/day (8 subjects/group). Samples were collected and measurements performed before and after the 4-week treatment period for determination of safety, pharmacokinetics, and biological activity of green tea polyphenol treatment.

**Results:** Adverse events reported during the 4-week treatment period include excess gas, upset stomach, nausea, heartburn, stomach ache, abdominal pain, dizziness, headache, and muscle pain. All of the reported events were rated as mild events. For most events, the incidence reported in the polyphenol-treated groups was not more than that re-

ported in the placebo group. No significant changes were observed in blood counts and blood chemistry profiles after repeated administration of green tea polyphenol products. There was a >60% increase in the area under the plasma EGCG concentration-time curve after 4 weeks of green tea polyphenol treatment at a dosing schedule of 800 mg once daily. No significant changes were observed in the pharmacokinetics of EGCG after repeated green tea polyphenol treatment at a regimen of 400 mg twice daily. The pharmacokinetics of the conjugated metabolites of epigallocatechin and epicatechin were not affected by repeated green tea polyphenol treatment. Four weeks of green tea polyphenol treatment at the selected dose and dosing schedule did not provide protection against UV-induced erythema.

**Conclusions:** We conclude that it is safe for healthy individuals to take green tea polyphenol products in amounts equivalent to the EGCG content in 8-16 cups of green tea once a day or in divided doses twice a day for 4 weeks. There is a >60% increase in the systemic availability of free EGCG after chronic green tea polyphenol administration at a high daily bolus dose (800 mg EGCG or Polyphenon E once daily).

## INTRODUCTION

Tea (*Camellia sinensis*) is one of the most consumed beverages in the world, especially in Asian countries. Tea consumption may be linked to low incidences of various pathological conditions, including cardiovascular disease, diabetes, obesity, and cancer. Green tea, GTEs,<sup>3</sup> and EGCG have been shown to inhibit carcinogenesis induced by a wide variety of carcinogens in rodent cancer models. Cancer chemopreventive activity of green tea has been demonstrated in the following target organs: colon, duodenum, esophagus, forestomach, large intestine, liver, lung, mammary glands, and skin (reviewed in Refs. 1, 2). The principal active polyphenols in green tea include EGCG, EGC, EC, and epicatechin gallate, with EGCG being the most abundant and possessing the most potent antioxidative activity. The cancer chemopreventive activities of green tea have been attributed, in part, to the antioxidative and free radical scavenging activities of green tea polyphenols (3, 4). Studies have also suggested that the cancer preventive properties of green tea are related to inhibition of tumor promotion and cell

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<sup>3</sup> The abbreviations used are: GTE, green tea extract; EGCG, epigallocatechin gallate; CL/F, oral clearance; EGC, epigallocatechin; EC, epicatechin; AUC, area under the plasma concentration-time curve; MED, minimum erythema dose; HPLC, high-performance liquid chromatography; C<sub>max</sub>, maximum plasma concentration; T<sub>max</sub>, time to reach the maximum plasma concentration; t<sub>1/2</sub>, half-time; VB/F, oral apparent volume of distribution.

proliferation (5), and induction of Phase II detoxification enzymes (6, 7).

At present, epidemiological evidence of the protective effect of tea consumption against the development of human cancers is not conclusive. This may be attributed to variables related to individual differences in tea preparation and consumption patterns, and seasonal and geographic differences in tea production. Controlled prospective human intervention trials to evaluate the chemopreventive activity of ingestion of tea or tea components are clearly necessary. Because it is not easy to change the dietary habits of an individual, ingesting green tea products in oral formulations may be more acceptable for chronic use in healthy populations. We have reported recently the pharmacokinetics and safety of two oral green tea polyphenol formulations (EGCG and Polyphenon E, a defined mixture of green tea polyphenols) after single-dose administration (8). Peak plasma EGCG levels of 200-400 ng/ml (0.4-0.8  $\mu$ M) can be achieved, after the administration of these formulations at doses equivalent to the EGCG content in 8-16 cups of green tea (depending on the cup size). Here, we report results from a follow-up study designed to determine the safety and pharmacokinetics of oral tea polyphenol products after 4 weeks of daily administration. In an exploratory fashion, the study has also determined the effect of chronic green tea polyphenol administration on UV-induced erythema response. This study provides the fundamental knowledge needed to conduct future intervention trials using oral green tea polyphenol products.

## MATERIALS AND METHODS

**Study Drugs.** EGCG, Polyphenon E, and placebo capsules were supplied by the Chemoprevention Agent Development Research Group, National Cancer Institute (Bethesda, MD). On average, each EGCG capsule contained 200 mg EGCG and pharmaceutical excipients consisting of pregelatinized starch, colloidal silicon dioxide, and magnesium stearate. Each Polyphenon E capsule contained 200 mg EGCG, 37 mg EGC, 31 mg EC, other green tea polyphenols, and pharmaceutical excipients consisting of pregelatinized starch, colloidal silicon dioxide, and magnesium stearate. Placebo capsules contained only pharmaceutical excipients consisting of microcrystalline cellulose, pregelatinized starch, colloidal silicon dioxide, and magnesium stearate. Caffeine was not present in any of the formulations. The study medications were stored at room temperature and protected from environmental extremes. On the basis of the content analysis performed every 6 months, green tea polyphenols were found to be stable under the above storage condition.

**Participants.** Forty healthy men and women >18 years of age with Fitzpatrick skin type II or III participated in the study. Individuals with skin type II have skin that burns and peels easily after short initial sun exposure and tends to develop a light tan. Individuals with skin type III typically develop a slight tender burn after short initial sun exposure and a moderate tan. These skin types allow evaluation of UV-induced erythema response without resulting painful burn. The participants were in performance status 0-1 (determined by Southwest Oncology Group Performance Status Criteria) and have normal liver and renal function. Participants were excluded if they were pregnant,

had cancers of any type within the past 5 years, had severe metabolic disorders or other life-threatening acute or chronic diseases, had weight loss >10%, or had gastric ulcer within the last 6 months. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all of the participants.

**Study Design.** During the initial clinic visit, study participants completed a medical history form and underwent a brief physical examination. A fasting blood was collected and subjected to a complete blood count with differential leukocyte count and the following blood chemistry analyses: glucose, urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, total protein, albumin, globulin, cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, calcium, phosphorus, alkaline phosphatase, 7 glutamyl transpeptidase, alanine amino transferase, aspartate amino transferase, lactate dehydrogenase, total bilirubin, and iron. Eligible subjects were required to refrain from the ingestion of tea, tea products, dietary supplements, and herbal products 2 weeks before the placebo run-in period and throughout the entire green tea polyphenol treatment period. Study subjects were randomly assigned to receive one of the five treatments (8 subjects/group): 800 mg EGCG formulation once daily, 400 mg EGCG formulation twice daily, 800 mg EGCG as Polyphenon E once daily, 400 mg EGCG as Polyphenon E twice daily, or placebo once daily.

All of the subjects underwent a 2-week placebo run-in period in which they were instructed to ingest the placebo capsules once a day or twice a day depending on their assigned dosing schedule. Subjects with >80% compliant during placebo run-in based on pill count were entered into the green tea polyphenol treatment period. The baseline UV light-induced MED was determined before the initiation of the polyphenol/placebo treatment phase. On the first treatment day, a fasting blood was collected before ingestion of the study medication. Subsequently, subjects took 800 or 400 mg dose of the assigned study capsules with a standardized light breakfast. Blood samples (5-7 ml each) were collected at 0.5, 1, 2, 3.5, 5, 6.5, 8, and 24 h after drug administration. After the first study day, study subjects were provided with a 4-week supply of the assigned study agent and instructions on how to take the study drug. Study subjects were also provided with a medication intake calendar to write down the time and quantity of any medication usage (including the study medication). A daily diary form was provided to record side effects experienced during this period with documentation of time of onset and resolution, severity, and remedial measures taken. At the end of treatment period, study subjects underwent a post-treatment MED evaluation. On the last treatment day, most subjects underwent procedures similar to that described for the first treatment day for determination of plasma pharmacokinetics of green tea catechins. For subjects assigned to receive the placebo capsules, only a fasting blood sample was collected on the last treatment day. The fasting blood sample collected on the last treatment day was subjected to post-treatment clinical laboratory evaluation. All of the study participants were followed for 4 weeks for any potential adverse events related to the study procedure or the study agent.

**Sample Collection and Processing.** Blood samples were collected into Vacutainer tubes containing sodium heparin. Once collected, blood samples were kept in the refrigerator and centrifuged at 4°C within 2 h of collection. Plasma was aliquoted into cryotubes containing a small aliquot of ascorbate-EDTA solution [0.4 M NaH<sub>2</sub>PO<sub>4</sub> buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)] and stored at -80°C until analysis.

**Tea Polyphenol Concentration Measurements.** EGCG, EC, and EGC concentrations in plasma samples were determined within 1 month of collection using a published HPLC procedure (9). In brief, for determination of free green tea polyphenols, plasma samples or spiked plasma standards were extracted with methylene chloride to remove lipid components. The remaining aqueous phase was extracted with ethyl acetate. The ethyl acetate layer was mixed with a small aliquot of 0.1% ascorbic acid before drying by vacuum centrifugation. The dried residue was redissolved in 15% acetonitrile and injected onto HPLC. For determination of the total of free and glucuronic acid/sulfate conjugates of tea polyphenols, plasma samples were mixed with an aliquot of B-glucuronidase and sulfatase in the presence of ascorbate-EDTA solution. After pretreatment, the samples were extracted as described above for the free polyphenols.

The HPLC system consisted of an ESA Model 540 refrigerated autosampler, an ESA Model 580 two-pump solvent delivery system, an ESA 5600 coulochem electrode array system, and a Supelcosil C<sub>18</sub> reversed-phase column (150 X 4.6 mm; particle size, 5 µm; Supelco Inc.). The autosampler and column temperatures were maintained at 6°C and 35°C, respectively. This assay used a gradient of two mobile phases. Buffer A consisted of 30 mM NaH<sub>2</sub>PO<sub>4</sub> buffer, acetonitrile, and tetrahydrofuran in the volume ratio of 98.13:1.75:0.12 (pH 3.35). Buffer B consisted of 15 mM NaH<sub>2</sub>PO<sub>4</sub> buffer, acetonitrile, and tetrahydrofuran in the volume ratio of 41.5:58.5:12.5 (pH 3.45). The column was eluted with 96% buffer A and 4% buffer B from 0 to 7 min. Then the linear gradient was changed progressively to 17% buffer B at 25 min, 28% buffer B at 31 min, 33% buffer B at 37 min, and 98% buffer B at 38 min. It was maintained at 98% buffer B from 38 to 43 min and finally changed back to 4% buffer B at 44 min for the analysis of the next sample. The flow rate was maintained at 1 ml/min. The eluent was monitored by the coulochem electrode array system with potential settings at -90, -10, 70, and 150 mV.

**Determination of MED.** Erythema was induced by applying UV radiation at six doses ranging from 10 to 42 mJ/cm<sup>2</sup> to six sites of 1-cm in diameter in a horizontal row on mid-buttock skin. A multipoint solar UV simulator (Model 600; Solar Light Co., Philadelphia, PA) was used for the UV irradiation. The simulator was equipped with a 150W Xenon lamp emitting a continuous spectrum of radiation beginning at 240 nm through the infrared spectrum and maximally peaking at 360 nm. UVC and visible wavelengths were reduced with a liquid filter and 1-mm Schott WG 320 filter. Spectroradiometric assessment of the lamp indicated that relative emission in the UVA (320-400 nm), UVB (290-320 nm), and UVC (200-290 nm) wavebands was 32%, 61%, and 7%, respectively. The lamp was housed in a black plastic tube with six apertures, 1-cm in diameter. The apparatus was calibrated before each use. The UV irradiation

time lasted for 1 min. The MED was determined visually 22-24 h after irradiation and defined as the lowest UV dose causing uniform redness filling the irradiated site. The evaluator was blinded to the treatment assignment of the subject.

**Data Analysis.** Plasma EGCG concentration-time data were analyzed with the model-independent approach (10). Data in the terminal, log-linear phase were analyzed by linear regression to estimate terminal elimination rate constant ( $\lambda$ ) and  $t_{1/2} = 0.693/\lambda$ . In general, there were four data points in the terminal log-linear phase. The AUC<sub>0-∞</sub> after the first dose was determined by trapezoidal rule up to the last measured concentration-time value to which was added the terminal area. The terminal area was calculated by dividing the concentration at the last time point by  $\lambda$ . To correct for drug accumulation because of repeated dosing and to allow for comparison with the AUC obtained after the first dose, the AUC after the last catechin dose (AUC<sub>last dose</sub>) was calculated by trapezoidal rule up to 24 h and 12 h after dosing for the once daily and twice daily dosing schedules, respectively. Concentrations at 12 h after the last catechin dose for the twice daily treatment group were interpolated from the regression line. C<sub>max</sub> and T<sub>max</sub> were obtained by visual inspection of the plasma concentration *versus* time profile. CL/F (systemic clearance/oral bioavailability) was estimated from the quotient of dose and AUC<sub>0-∞</sub> or AUC<sub>last dose</sub> after the first or last tea catechin dose, respectively. V<sub>p</sub> estimated from the oral data are also influenced by the F value [V<sub>p</sub>/F = (CL/F)/ $\lambda$ ]. AUC<sub>0-∞</sub> and AUC<sub>last dose</sub> of total (free and glucuronic acid/sulfate conjugates) EGC and EC were also estimated with the model-independent approach.

The pharmacokinetic parameters obtained after the first dose were compared among treatment groups using one-way ANOVA followed by a Bonferroni adjusted t test for the pairwise multiple comparisons. The pharmacokinetic parameters obtained after repeated treatment were compared with those obtained after the first dose using a paired t test. The baseline MEDs were compared among treatment groups using one-way ANOVA. Ratios of the post-treatment MED measurements to those obtained at baseline were calculated and used to compare the treatment effect using one-way ANOVA. A *P* < 0.05 was considered statistically significant.

## RESULTS

Table 1 summarizes the demographic data of the study participants. A total of 40 subjects (8/group) completed the study. There were no significant differences in the average age, weight, and height among treatment groups. There were between 2 and 4 male participants in each treatment group.

Adverse events reported during the 4-week treatment period are summarized in Table 2. Data are presented as the number of events reported during the 4-week green tea polyphenol/placebo treatment period, and values in parentheses represent the number of individuals experienced the event. The reported events include excess gas, upset stomach, nausea, heartburn, stomachache, abdominal pain, dizziness, headache, and muscle pain. All of the reported events have been rated as mild events (grade 1). For most events, the incidence reported in the treatment groups was not significantly more than that in the placebo group. Mild nausea was more frequent after the 800-mg

Table 1 Subject demographic data by treatment group

	Placebo	800 mg EGCG once daily	800 mg EGCG as Polyphenon E once daily	400 mg EGCG twice daily	400 mg EGCG as Polyphenon E twice daily
Number of subjects	8	8	8	8	8
Fraction of male participants	3/8	2/8	3/8	4/8	2/8
Age (yr)	34.5 ± 10.6 <sup>a</sup>	39.5 ± 10.4	32.4 ± 10.1	29.4 ± 10.7	34.1 ± 11.9
Height (inches)	67.1 ± 2.7	64.9 ± 4.6	67.9 ± 3.2	66.1 ± 5.3	65.1 ± 3.1
Weight (lbs)	169 ± 36	165 ± 52	167 ± 30	160 ± 40	150 ± 19

Mean ± 1 SD.

Table 2 Adverse events reported during the 4-week green tea polyphenol/placebo treatment period

Data are presented as the number of events reported and values in parenthesis represent the number of individuals experienced the event.

	Placebo (n = 8)	800 mg EGCG once daily (n = 8)	800 mg EGCG as Polyphenon E once daily (n = 8)	400 mg EGCG twice daily (n = 8)	400 mg EGCG as Polyphenon E twice daily (n = 8)
Headache	3(1)	2(2)	0	1(1)	1(1)
Stomach ache	0	1(1)	1(0)	1(1)	1(1)
Upset stomach	0	0	0	1(1)	0
Heartburn	2(1)	0	0	0	0
Abdominal pain	2(1)	2(2)	1(1)	0	0
Excess gas	1(0)	0	0	0	1(1)
Nausea	1(1)	5(2)	3(1)	0	1(1)
Dizziness	0	1(1)	0	0	0
Muscle pain	0	1(1)	0	0	0

once daily treatment than that in the placebo group (5, 3, and 1 occurrence for 800 mg EGCG once daily, 800 mg EGCG as Polyphenon E once daily, and placebo, respectively). Complete blood count and a panel of blood chemistry profiles were obtained before and after 4 weeks of daily administration of the study agent. No significant changes were observed in these clinical laboratory measurements (data not shown).

Because EGCG was present mostly in the free form in the systemic circulation [ $>92\%$  as the free form, based on the AUC ratio of free *versus* total (free and conjugated) EGCG], Figs. 1 and 2 illustrate the average plasma concentration-time profiles of free EGCG after EGCG or Polyphenon E administration. The average pharmacokinetic parameters of free EGCG after p.o. administration of tea polyphenols are summarized in Table 3. Before repeated tea polyphenol treatment, pharmacokinetic parameters of EGCG were similar among the different treatment groups. The AUC and  $C_{max}$  of EGCG after the administration of 800-mg dose of EGCG or Polyphenon E were higher than those obtained after 400-mg dose of the respective formulation, but the differences did not reach statistical significance. After repeated tea polyphenol treatment, the AUC of EGCG obtained after the 800-mg dose of Polyphenon E was significantly higher than that obtained from the 400-mg dose of either product. The AUC of EGCG obtained after the 800-mg dose of EGCG was significantly higher than that after the 400-mg dose of EGCG. Four weeks of repeated tea polyphenol administration at 800 mg once daily resulted in significant changes in the AUC of free EGCG, whereas repeated administration at 400 mg twice daily did not result in significant changes in the pharmacokinetics of free EGCG. The AUC of free EGCG increased from  $95.6 \pm 46.8$  to  $145.6 \pm 85.1$  min  $\mu\text{g/ml}$  ( $P < 0.05$ ) and from  $98.1 \pm$

$46.5$  to  $158.4 \pm 89.8$  min  $\mu\text{g/ml}$  ( $P < 0.05$ ) for the 800 mg once daily EGCG and Polyphenon E treatment, respectively. A decreasing trend was observed in the CL/F and VB/F of EGCG after repeated treatment at 800 mg once daily; however, the changes did not reach statistical significance. Repeated administration of green tea polyphenols did not result in significant changes in  $C_{max}$ ,  $T_{max}$ , and  $t_{1/2}$  of EGCG.

The AUCs of total EGC and EC after p.o. administration of Polyphenon E before and after 4 weeks of treatment are summarized in Table 4. Because the concentrations of free EGC and EC were below the limit of quantification in most plasma samples [0-4% present as the free form, based on the AUC ratio of free *versus* total (free and conjugated) catechins], AUCs presented represent mostly the levels of conjugated metabolites of EGC and EC. Four weeks of tea polyphenol treatment with either dosing schedule did not result in significant changes in the levels of conjugated tea catechins.

Table 5 shows the changes in MED after 4 weeks of green tea polyphenol/placebo treatment. The data are presented as the ratios of MED determined after repeated treatment over that obtained before treatment. As shown in the data, the ratios of MED approximated unity for all of the study groups, suggesting that the intervention did not change the UV-induced erythema response.

## DISCUSSION

There have been no reports of clinical toxicity when green tea is consumed as a beverage throughout the day. Consumption of up to 20 cups of green tea per day is not uncommon in certain populations. Oral pills of GTE or green tea polyphenol products

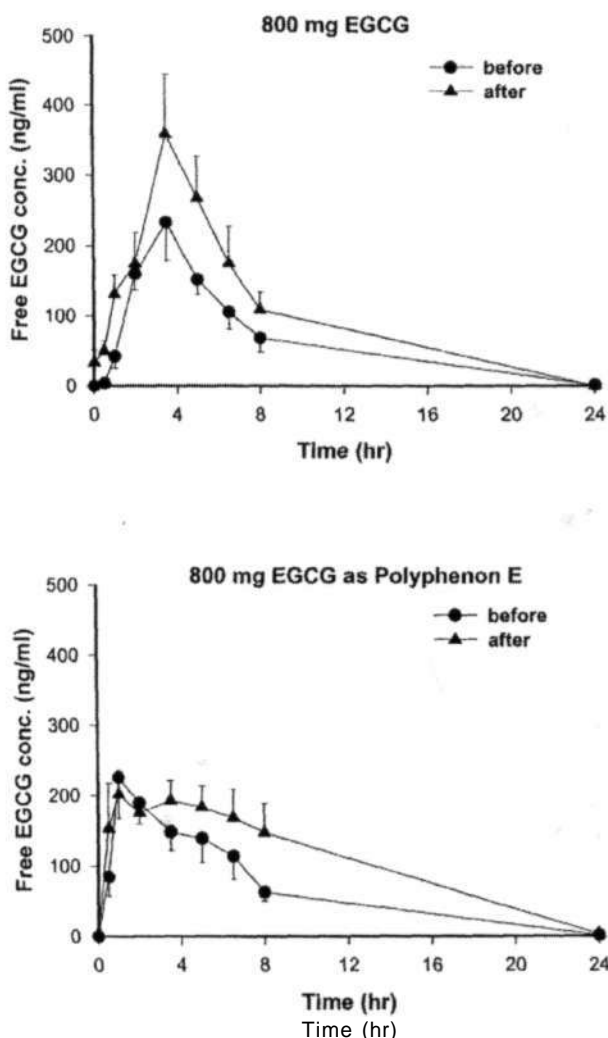


Fig. 1 Average plasma concentration-time profiles of free EGCG after an 800-mg dose of EGCG as EGCG or Polyphenon E formulation. The profiles were obtained before and after 4 weeks of green tea polyphenol treatment with a dosing schedule of 800 mg once daily. Each point represents the mean of data from 7 or 8 subject, bars,  $\pm$ SE.

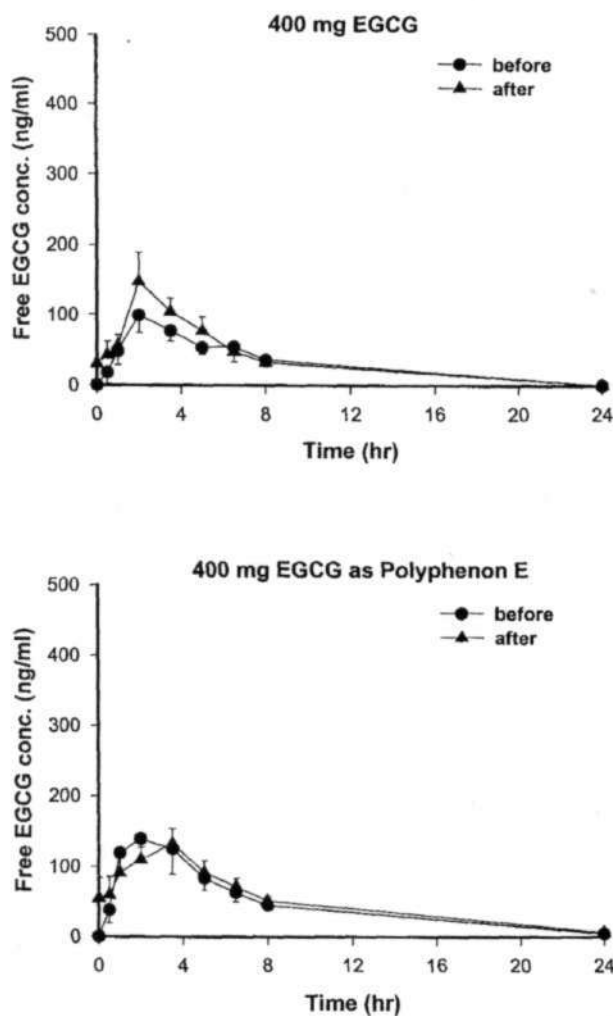


Fig. 2 Average plasma concentration-time profiles of free EGCG after a 400-mg dose of EGCG as EGCG or Polyphenon E formulation. The profiles were obtained before and after 4 weeks of green tea polyphenol treatment with a dosing schedule of 400 mg twice daily. Each point represents the mean of data from 8 subjects; bars,  $\pm$ SE.

are available commercially as dietary supplements. Use of standardized oral products can facilitate the conduct of controlled human intervention trials to evaluate the biological activity of green tea or green tea components. However, safety data based on human consumption cannot be extrapolated to chronic consumption of large amounts of an isolated component (e.g., EGCG) or an enriched extract (e.g., Polyphenon E) at regimens that would gain better compliance (e.g., once or twice daily dosing) in intervention trials. A recent study has determined the toxicity of oral GTE administered once daily or three times daily in adult patients with solid tumors (11). Dose levels of 0.5 to 5.05 g/m<sup>2</sup> once daily and 1.0 to 2.2 g/m<sup>2</sup> three times daily were explored. The study found dose-limiting side effects of gastrointestinal complaints (abdominal bloating, dyspepsia, flatulence, nausea, and vomiting) and central nervous system stimulation (agitation, dizziness, insomnia, tremors, and restlessness). These side effects are likely to be related to the 7% caffeine content in

the study formulation. The study reported that a dosing regimen of 1.0 g/m<sup>2</sup> three times daily is well tolerated for at least 6 months. To achieve this dose, study participants have been required to swallow 7-10 capsules each time and three times a day. This dose level is roughly equivalent to drinking 7-8 Japanese-style cups (one cup = 120 ml) of green tea three times daily (a total of 21-24 cups of tea/day). In the current study, standardized, defined, and decaffeinated green tea polyphenol oral products in amounts similar to the EGCG content in 16 Japanese-style cups of green tea were consumed once daily or in divided doses twice daily (4 capsules/day) for 4 weeks. On the basis of the reported adverse events and clinical laboratory data, the study agents and dosing schedules have been found to be safe and well tolerated by the study participants for at least 1 month. The reported adverse events were rated as mild events. The more common events include headache, stomach ache, abdominal pain, and nausea, which have been reported in sub-

Table 3 Pharmacokinetic parameters of free EGCG obtained after p.o. administration of EGCG or Polyphenon E before and after 4 weeks of green tea polyphenol treatment

	800 mg EGCG (n = 7) <sup>a</sup>		800 mg EGCG as Polyphenon E (n = 8)		400 mg EGCG (n = 8)		400 mg EGCG as Polyphenon E (n = 8)	
	First dose	On the last treatment day	First dose	On the last treatment day	First dose	On the last treatment day	First dose	On the last treatment day
AUC <sup>b</sup> (min·µg/ml)	95.6 ± 46.8 <sup>c</sup>	145.6 ± 85.1 <sup>d</sup>	98.1 ± 46.5	158.4 ± 89.8 <sup>d</sup>	46.9 ± 21.4	43.9 ± 25.6 <sup>e,f</sup>	71.2 ± 36.9	54.8 ± 23.7 <sup>e</sup>
C <sub>max</sub> (ng/ml)	234.9 ± 140.9	390.3 ± 231.4	263.8 ± 135.7	287.6 ± 124.2	137.6 ± 66.5	161.4 ± 100.5 <sup>f</sup>	179.9 ± 114.3	155.4 ± 61.9 <sup>f</sup>
T <sub>max</sub> (min)	224.4 ± 33.4	210.0 ± 73.5	112.5 ± 65.6	248.5 ± 184.9	183.9 ± 117.1	135.6 ± 48.4	150.8 ± 109.7	146.6 ± 102.2
CL/F (liter/min)	10.5 ± 4.4	7.3 ± 3.4	9.6 ± 3.4	8.0 ± 7.1	11.2 ± 5.2	13.1 ± 8.6	7.0 ± 3.4	9.0 ± 4.2
V <sub>d</sub> /F (liter)	1910 ± 866	1686 ± 1241	2760 ± 1901	1551 ± 795	2516 ± 750	3456 ± 3200	2139 ± 716	3759 ± 2089
t <sub>1/2</sub> (min)	136.7 ± 54.4	158.9 ± 78.7	200.4 ± 94.7	163.0 ± 56.2	183.0 ± 75.3	170.5 ± 50.2	241.2 ± 115.6	296.6 ± 152.9

<sup>a</sup> Data from only 7 subjects were used in the analysis because of incomplete sample collection in 1 study participant.

<sup>b</sup> AUC<sub>0-∞</sub> was calculated after the first catechin dose. After repeated dosing, AUC<sub>0-24h</sub> was calculated after administration of 800-mg dose of the study agent on the last treatment day, and AUC<sub>0-12h</sub> was calculated after administration of 400-mg dose of the study agent on the last treatment day.

<sup>c</sup> Mean ± SD.

<sup>d</sup> Significantly different from that of the first dose, *P* < 0.05.

<sup>e</sup> Significantly different from that after 800-mg dose of EGCG as Polyphenon E on the last treatment day, *P* < 0.05.

<sup>f</sup> Significantly different from that after 800-mg dose of EGCG on the last treatment day, *P* < 0.05.

Table 4 AUC<sup>g</sup> (ug·min/ml) of total (free and conjugated) EGC and EC obtained after p.o. administration of Polyphenon E before and after 4 weeks of Polyphenon E treatment

	800 mg EGCG as Polyphenon E (n = 8)		400 mg EGCG as Polyphenon E (n = 8)	
	First dose	After repeated dosing	First dose	After repeated dosing
Total EGC	103.4 ± 41.4 <sup>h</sup>	95.9 ± 16.2	50.4 ± 19.1 <sup>e</sup>	63.6 ± 47.2
Total EC	130.4 ± 72.2	154.5 ± 37.6	55.4 ± 16.6 <sup>e</sup>	77.8 ± 81.5

\* AUC<sub>0-∞</sub> was calculated after the first catechin dose. After repeated dosing, AUC<sub>0-24h</sub> was calculated after administration of 800-mg dose of Polyphenon E on the last treatment day, and AUC<sub>0-12h</sub> was calculated after administration of 400-mg dose of Polyphenon E on the last treatment day.

<sup>g</sup> Mean ± SD.

<sup>h</sup> Significantly different from that at the 800-mg dose level, *P* < 0.05.

Table 5 Changes in MED after 4 weeks of green tea polyphenol/placebo treatment

Data are expressed as ratios of the post-treatment values to those obtained at baseline.

MED (after/before)	Placebo (n = 8)	800 mg EGCG once daily (n = 8)	800 mg EGCG as Polyphenon E once daily (n = 8)	400 mg EGCG twice daily (n = 8)	400 mg EGCG as Polyphenon E twice daily (n = 8)
		1.09 ± 0.08 <sup>a</sup>	1.15 ± 0.11	1.11 ± 0.14	1.07 ± 0.08

<sup>a</sup> Mean ± SD.

jects receiving green tea polyphenol treatment as well as in subjects receiving placebo. There were no significant changes in blood counts and blood chemistry profiles after 4 weeks of green tea polyphenol treatment.

On the basis of the observed plasma half-lives of EGCG, we do not expect EGCG to accumulate in the body after repeated dosing at a once daily schedule. The accumulation ratio was calculated based on the half-life and dosing interval (12), and was found to be < 1.05. Consistently, EGCG was not detected or was detected at low levels in the predose sample collected on the last treatment day for the once daily schedule. Nevertheless, small amounts of EGCG are expected to accumulate after repeated dosing at a twice daily schedule with an average accumulation ratio of 1.07-1.24. This is reflected by the presence of measurable concentrations of EGCG in most of the

predose samples collected on the last treatment day. Average predose EGCG levels of 29.9 and 54.8 ng/ml were observed after repeated dosing of EGCG and Polyphenon E, respectively, at the twice daily schedule. Some of the subjects had a short elapsed time from the time the predose sample was collected to the time the previous dose was taken, which could additionally contribute to the predose EGCG levels.

On average, there was a >60% increase in the AUC of free EGCG after 4 weeks of tea polyphenol treatment at a dosing schedule of 800 mg once daily. The observed increase in the systemic exposure of free EGCG is not related to drug accumulation after repeated dosing, because the AUC calculation has corrected for this factor (see "Data Analysis" for details). A dosing schedule of 400 mg twice daily did not result in significant changes in the AUC of free EGCG. Because the AUC

calculation has corrected for the accumulation factor, comparisons of the EGCG AUC after the first dose *versus* that on the last treatment day do not reveal the expected small accumulation effect. The relative proportions of free *versus* total (free and conjugated) EGCG did not change consistently after 1 month of treatment. Neither dosing schedule resulted in significant changes in the AUC of EGC and EC. EGC and EC are present in plasma mostly as the conjugated form, and the relative proportions of free *versus* total EGC or EC did not change consistently after 1 month of treatment. These data suggest that the conjugation process is not affected by repeated treatment of EGCG/Polyphenon E. Because EGCG is the only component in the EGCG formulation and the major component in the Polyphenon E formulation, it is likely that the 800-mg dose of EGCG/Polyphenon E resulted in significantly elevated EGCG levels in the gastrointestinal tract and subsequently inhibited presystemic elimination of EGCG but not EGC or EC. The 400-mg twice daily regimen apparently did not result in tea catechin concentrations that would exert a significant inhibitory effect. The mechanism(s) responsible for the observed increase in the AUC of free EGCG after chronic treatment of EGCG/Polyphenon E at a high daily bolus dose remain(s) to be studied. Inhibitions in nonenzymatic degradation, intestinal flora metabolism, methylation, and/or intestinal efflux of EGCG are plausible contributing factors. It is not known whether ingestion of green tea polyphenols at a high daily bolus dose for >4 weeks will result in additional enhancement in the systemic exposure of EGCG.

In animal model systems, topical treatment or p.o. administration of green tea or green tea polyphenols has been shown to inhibit UV radiation-induced skin tumorigenesis, formation of cutaneous edema, and depletion of antioxidant-defense system (13-15). Katiyar *et al.* (16) have shown that topical application of green tea polyphenols to human skin before UV irradiation significantly reduced the UV-induced erythema response and pyrimidine dimer formation. In a follow-up study, topical application of EGCG to human skin before UV irradiation markedly decreased UV-induced changes in markers of oxidative stress and antioxidant enzymes (17). In these studies, EGCG/green tea polyphenols prepared in acetone were applied topically 20 min before UV irradiation. This route of administration with acetone as the vehicle is likely to give rise to high levels of green tea polyphenol in epidermis and/or dermis during UV irradiation. In the current study, no significant changes in MED were observed after 4 weeks of p.o. EGCG/Polyphenon E administration at a daily dose of 800 mg of EGCG. Interestingly, some of the study participants indicated that they have experienced less-intensive sunburn reactions when receiving the green tea polyphenol treatment. One of the disparities between our study and that reported by Katiyar *et al.* (16) is the route of tea polyphenol administration. Topical application of EGCG/tea polyphenols in acetone is likely to have resulted in high local concentrations of green tea polyphenols, whereas p.o. administration of green tea polyphenols may not result in accumulation of high levels of green tea polyphenols in the skin during UV irradiation. This would be an important factor of consideration if protection against UV-induced erythema response requires the presence of high concentrations of green tea polyphenols at the target site. Insufficient treatment duration could also potentially

contribute to our observations, because UV-induced erythema on dorsal skin has been shown not to be affected after 4 weeks of p.o. administration of carotenoids and vitamin E in healthy human subjects, but diminished significantly 8-12 weeks after treatment (18). Incorporation of other sensitive markers of photodamage should also be considered in future trials. In addition, the green tea polyphenol products used in the current study are decaffeinated products. Studies have compared the inhibitory effects of green tea and decaffeinated green tea in UV-induced skin carcinogenesis models and found that the decaffeinated products were either effective but less active or not effective (13, 14, 19), suggesting that caffeine contributes to the biological activity of green tea. However, the decreased effectiveness of decaffeinated green tea may be because the decaffeinated process also reduces the levels of green tea polyphenols.

We conclude that p.o. administration of EGCG or Polyphenon E at a daily dose of 800 mg (based on the EGCG content) for 4 weeks is safe and well tolerated in healthy human subjects. Repeated green tea polyphenol administration at a high daily bolus dose (800 mg once daily) results in a >60% increase in the systemic exposure of EGCG, possibly because of inhibition of presystemic elimination of this catechin. Repeated administration of EGCG and Polyphenon E at a daily dose equivalent to the EGCG content in 16 Japanese-style cups of green tea for 4 weeks did not provide protection against UV-induced erythema.

#### ACKNOWLEDGMENTS

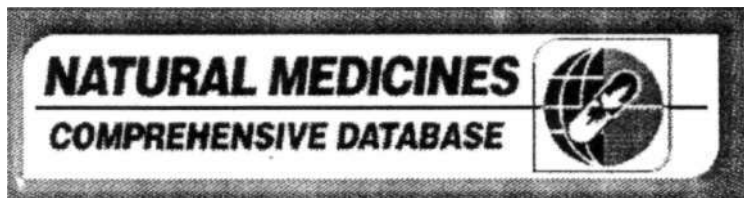
We thank Angelica Villalobos and Wade Chew for excellent technical assistance.

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### PAPAIN



#### Also Known As

#### Also Known As:

#### Scientific Names

Papainum Crudum, Plant Protease Concentrate, Vegetable Pepsin.  
CAUTION: See separate listings for Bromelain, Papaya, and American Pawpaw.

#### People Use This For

#### Scientific Name:

#### Safety

Carica papaya.  
Family: Caricacea.

#### Effectiveness

#### People Use This For:

#### Mechanism of Action

Orally, papain is used for inflammation and edema following trauma and surgery, as a digestive aid, for treating parasitic worms, inflammation of the throat and pharynx, herpes zoster symptoms, chronic diarrhea, hay fever, nasal drainage, and psoriasis. Papain is also used as an adjuvant treatment for tumors.

#### Adverse Reactions

Topically, it is used to treat infected wounds, sores, and ulcers.

#### Herb Interaction

In manufacturing, papain is a component of cosmetics, dentifrices, enzymatic soft contact lens cleaners, meat tenderizers, and meat products. It is also used for stabilizing and chillproofing beer.

#### Drug Interaction

#### Safety:

#### Food Interaction

LIKELY SAFE ...when used orally in amounts commonly found in foods. Papain has Generally Recognized as Safe (GRAS) status in the US (4912).

#### Lab Test Interaction

POSSIBLY SAFE ...when used orally and appropriately for medicinal purposes (964,968,969).

#### Disease Interaction

POSSIBLY UNSAFE ...when used orally in large amounts. In excessive doses, papain can cause significant side effects including esophageal perforation (6) ...when raw papain is used topically. Raw papain or papaya latex is a severe irritant and vesicant (6).

#### Dosage

#### Comments

**PREGNANCY:** POSSIBLY UNSAFE ...when used orally. There is some concern that crude papain is teratogenic and embryotoxic (6).

#### Print Version

**LACTATION:** Insufficient reliable information available; avoid using.

#### Patient Handout

#### Effectiveness:

#### References

POSSIBLY EFFECTIVE

#### Brand Names

**Herpes zoster (shingles).** Taking papain orally may improve the symptoms of herpes zoster (965).

#### Suggest Changes

**Pharyngitis.** Taking papain orally, in combination with other agents, may relieve pharyngeal inflammation and swelling (964,963,969).

There is insufficient reliable information available about the effectiveness of papain for its other uses.

#### Mechanism of Action:

Papain is actually a mixture of the proteolytic enzymes papain, chymopapain A, chymopapain B, and papaya peptidase A isolated from the fruit of Carica papaya. Chymopapain A and B have a similar proteolytic spectrum to papain but are less potent (6). There is some evidence that a multi-enzyme preparation containing papain can increase the release of reactive oxygen species (ROS) by polymorphonuclear cells (PMNs). ROS are thought to have tumoricidal effects (962). The multi-enzyme preparation also seems to induce the cytokines tumor necrosis factor (TNF)-alpha, interleukin-1 (IL-1)-beta, and interleukin-6 (IL-6) in a time and dose dependent manner (1384).

#### Adverse Reactions:

Orally, large amounts of papain can cause esophageal perforation (fi). Ingestion of

Access Agreement: papaya latex (raw papain) can cause severe gastritis. Topically, papaya latex can cause severe irritation and blisters (6). Topical use of papain can cause itching (966).

Positions Open: Severe allergic reactions have been reported in sensitive individuals (6,967). One case report suggests that there may be cross-sensitivity between papain, fig, and kiwi (963).

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#### Interactions with Herbs & Supplements:

**HERBS WITH ANTICOAGULANT/ANTIPLATELET POTENTIAL:** Concomitant use of herbs that have constituents that might affect platelet aggregation could theoretically increase the risk of bleeding in some people. These herbs include angelica, clove, danshen, garlic, ginger, ginkgo, Panax ginseng, red clover, turmeric, and others.

#### Interactions with Drugs:

None known.

#### Interactions with Foods: -

**FIG, KIWI:** Cross sensitivity to papain may occur in individuals sensitive to fig and kiwi (963).

**POTATO PROTEIN:** May inhibit papain proteolytic activity (958).

#### Interactions with Lab Tests:

**INTERNATIONAL NORMALIZATION RATIO (INR):** Concomitant use of papaya extract (papain) and warfarin may increase INR (613).

#### Interactions with Diseases or Conditions:

**CLOTTING DISORDERS:** Avoid; theoretically, may increase bleeding risk (2).

#### Dosage/Administration:

**ORAL:** 1500 mg (2520 FIP units) per day have been used in clinical trials to treat inflammation and swelling following trauma and surgery (2).

**TOPICAL:** No typical dosage.

#### Comments:

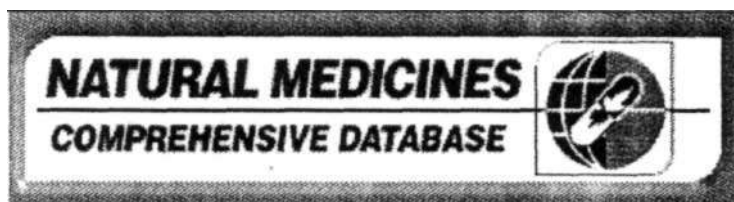
None.

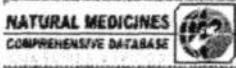
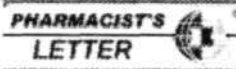
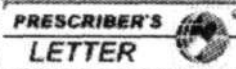

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# **Papain**

Papainum crudum

*Papain*

Published August 25, 1994

## *Name of Drug*

Papainum crudum, papain.

## *Composition of Drug*

Raw papain is latex from *Carica papaya* L (pawpaw) [Fam. Caricaceae] that has been dried using various methods; where necessary it is decontaminated mechanically or by filtration.

Papain is the enzyme mixture extracted using various means from raw papain; it contains, along with papain (EC 3.4.22.2), chymopapain A and B and papaya peptidase A.

## *Pharmacological Properties, Pharmacokinetics, Toxicology*

There is no extensive, satisfactory scientific experimental material available on the effects of raw papain/papain. The results on the analgesic and antiinflammatory effects are contradictory. Experiments have shown that papain has an edema-reducing effect. The fibrinogenous effect has not been sufficiently proven.

On the basis of animal experiments papain is said to demonstrate an absorption rate of 3 - 4 percent when taken orally. There is no research material available on the human pharmacokinetics of the drug. There is no extensive, satisfactory scientific experimental material available on the toxicology of papain/raw papain. Papain is not embryo-toxic or teratogenic; there are positive results in the case of raw papain.

There is no material available on the mutagenicity and carcinogenicity of papain.

## *Clinical Data*

### *1. Uses*

- a) Indications established through research: None.
- b) Reported indications for therapeutic use and grounds for rejection: Infestation with ascarids, oxyurids, and trichocephalus nematodes.

Papain/raw papain is used in combination in preparations for the treatment of inflammatory conditions of the mouth, throat and pharynx and of the upper respiratory tract; for influenza-type infections; loss of appetite; satiety; flatulence; Roemheld syndrome; putrefying-fermenting dyspepsias; enzyme deficiency; gastrointestinal digestion complaints; inflammations and ulcers in the gastroduodenal area; pancreas excretion insufficiency; dyskinesia of the liver and of the gallbladder ducts; chronic constipation; congestion of the liver; viral infections; anal thrombosis; concomitant therapy of malignant tumors; metastases; relapse prophylaxis; side effects of radiation treatment; lymphatic congestion following surgery and radiation treatment; palliative treatment of tumor patients;

carcinomas, sarcomas, Hodgkin's disease, leukemia; circulatory complaints, arteriosclerosis, vascular disease, thrombophlebitis, thrombosis, hemorrhoids, varicose ulcers, poorly healing wounds, burns, abscesses, fistulas, traumatic edema, hematoma, acute and chronic inflammations, bronchitis, adnexitis, urethritis, rheumatic and degenerative complaints; conditions of aging, exhaustion, and exhaustion syndrome, in convalescence; vitamin, mineral and metabolic substance deficiency, metabolic illnesses, dyscrasia, neurosthenia, neuritis, physical and mental exhaustion and depression.

The efficacy of the drug in the above conditions is insufficiently proven with the exception of some effect of papain in the treatment of traumatic and postoperative edema.

There are other more effective substances available for the treatment of worm infestation..

## 2. Risks

An increase in the tendency to bleed in people with clotting disorders cannot be excluded. Allergic reactions may occur.

## Evaluation

Due to the insufficiently proven efficacy of its use in the treatment of worm infestation and the risks associated, as well as the availability of treatment alternatives, the use of raw papain/papain cannot be recommended.

The efficacy of Papain in combination with other drugs used in the treatment of inflammations, edema and swelling following trauma and surgery needs to be specifically proven. Various experiment-based studies as well as clinical research indicate that Papain may be effective in high doses (daily dose = 1500 mg corresponding to 2520 FIP units).

## Papaya leaf

Caricae papayae folium

*Melonenbaumblätter*

Published October 15, 1987

## Name of Drug

Caricae papayae folium, papaya leaf.

## Composition of Drug

Papaya leaf consists of fresh or dried leaf of *Carica papaya* L. [Fam. Caricaceae], harvested before fruit development, as well as preparations thereof.

## Uses

Papaya leaf preparations are used singly or in combinations for prophylaxis and therapy of diseases and discomforts of the gastrointestinal tract, for infections with intestinal parasites, as an anthelmintic for oxyurids, strongyloides, ascarides, ancylostoma, such as *Necator americanus*, and other nematodes, and also for a sedative and diuretic.

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Review

## Bromelain as a Treatment for Osteoarthritis: a Review of Clinical Studies

Sarah Brien<sup>1,\*</sup>, George Lewith<sup>1</sup>, Ann Walker<sup>2</sup>,  
Stephen M. Hicks<sup>2</sup> and Dick Middleton<sup>3</sup>

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## Abstract

Bromelain, an extract from the pineapple plant, has been demonstrated to show anti-inflammatory and analgesic properties and may provide a safer alternative or adjunctive treatment for osteoarthritis. All previous trials, which have been uncontrolled or comparative studies, indicate its potential use for the treatment of osteoarthritis. This paper reviews the mechanism of its putative therapeutic actions, those clinical trials that have assessed its use in osteoarthritis to date, as well as considering the safety implications of this supplement for osteoarthritis and reviewing the evidence to date regarding the dosage for treating this condition. The data available at present indicate the need for trials to establish the efficacy and optimum dosage for bromelain and the need for adequate prospective adverse event monitoring in such chronic conditions as osteoarthritis.

Keywords: bromelain - herbal - osteoarthritis - proteolytic enzymes - review

## Introduction

In recent years, a number of clinical studies have appeared to substantiate one of the traditional therapeutic uses of extracts of bromelain, namely, in the treatment of inflammatory disorders of the musculoskeletal system. This paper sets out to review the clinical evidence for the use of bromelain in osteoarthritis.

Osteoarthritis is the most common form of arthritis in Western countries; in the USA prevalence of osteoarthritis ranges from 3.2% to 33% dependent on the joint (1). Its prevalence increases with age, and sex differences are evident (2). It can also create substantial disability (2). The risk of disability attributable to knee osteoarthritis alone is greater than any other medical disorder in the elderly (3), apart from cardiac diseases. Risk factors associated with both the development (e.g. heredity, age, female sex, obesity, trauma) and progression of the disease [e.g. obesity, low bone density, non steroidal anti-inflammatory drug (NSAID) use] have been identified (4); obesity is considered a major risk factor for both the development and progression of osteoarthritis (5,6). As allopathic medicine is unable to halt this progression conventional medical treatment is aimed at decreasing pain and improving function by the use of NSAIDs, other analgesics, steroidal joint injections and, as a last resort, joint

replacement. Because the high incidence of adverse events, especially gastrointestinal, associated with both non-selective and COX-2-selective NSAID use is high (7-9), effective but safer alternative treatments would be of benefit to osteoarthritis sufferers.

## Bromelain

Bromelain is a food supplement that may provide an alternative treatment to NSAIDs for patients with osteoarthritis. Bromelain is a crude, aqueous extract obtained from both the stem and fruit of the pineapple plant, which contains a number of proteolytic enzymes (10,11) and has shown potentially beneficial effects due to its anti-inflammatory and analgesic properties. Currently, bromelain is used for acute inflammation and sports injuries. It is not a licensed medical product and is freely available to the general public in health food stores and pharmacies in the USA and Europe.

### Mechanism of Action

The mechanisms of action have been reviewed (10-12). Bromelain has been shown to have a number of beneficial properties including anti-inflammatory and analgesic actions in addition to its anti-oedematous, antithrombotic and fibrinolytic effects (11). Experimental evidence suggests that bromelain's action as an anti-inflammatory is mediated via the following factors: (i) by increasing serum fibrinolytic activity (13), reducing plasma fibrinogen levels (14) and decreasing bradykinin levels (which results in reduced vascular permeability) and hence reducing oedema and pain (15); (ii) by mediating prostaglandin levels (by decreasing levels of PGE<sub>2</sub> and thromboxane A<sub>2</sub>); and (iii) through modulation of certain immune cell surface adhesion molecules (16-20), which play a role in the pathogenesis of arthritis (21). However, many of these studies are of poor quality and further data is needed to clarify definitive mechanisms of its action.

Data have also indicated that bromelain has analgesic properties, for example in inflammatory pain in humans (22), human urogenital inflammation (23), and in various animal inflammatory models (13,23). Its analgesic properties are thought to be a result of its direct influence on pain mediators such as bradykinin (15). as well as its indirect effects through its anti-inflammatory actions (e.g. reduction in oedema, debris and immune complexes), which reduce pain.

## Clinical Studies

Bromelain was first reported to be of value as an analgesic/anti-inflammatory for use in both rheumatoid arthritis and

osteoarthritic patients in 1964 (24). Clinical trials have assessed the effectiveness of bromelain most frequently using preparations containing differing complexes of proteolytic enzymes and differing concentrations of bromelain. Three complexes have been used: (i) Phlogenzyme™ (PHL), which contains the proteolytic enzymes bromelain (90 mg/tab), trypsin and rutin; (ii) Wobenzyme (WOB) which contains bromelain (45 mg/tab), papain, trypsin, chymotrypsin, pancreatin, lipase and amylase; and (iii) Wobenzym N™ (WOB-N) which contains bromelain (45 mg/tab), trypsin, papain, chymotrypsin, pancreatin and rutin. Bromelain has been assessed in the treatment of osteoarthritis of two joints, i.e. the knee (24-30) and the shoulder (as assessed under the global term periarthritis humeroscapularis) (31,32). Tables 1 and 2 summarise those studies that have investigated the effect of bromelain in knee and shoulder osteoarthritis, respectively.

View this table: Table 1 Summary of studies assessing the effectiveness of bromelain as a [in this window] treatment for osteoarthritis of the knee [in a new window]

View this table: Table 2 Summary of studies assessing the effectiveness of bromelain as a [in this window] treatment for osteoarthritis of the shoulder (periarthritis humero [in a new window] scapularis)

The majority of studies assessing bromelain for osteoarthritis have been either open studies (24,30) or equivalence studies designed to assess comparative effectiveness and safety against standard NSAIDs treatment (25-29, Klein, 1994, unpublished data.). A number of these studies are unpublished [as reviewed by Leipner *et al.* (25)], including two placebo controlled studies designed to assess the efficacy of bromelain in knee osteoarthritis. The following sections will review the studies that have been carried out to date. Direct comparison between these trials is difficult as different dosages or preparations of bromelain have been administered. The majority of the studies have methodological issues that make it difficult to draw definite conclusions.

### Bromelain for Knee Osteoarthritis

Ten studies have been identified that have assessed bromelain in osteoarthritis of the knee (Table 1). The earliest reported studies investigating bromelain were a series of case reports on 28 patients, with moderate or severe rheumatoid or osteoarthritis, described by Cohen and Goldman (24). The studies reported indicated that the use of bromelain, at varying doses (these doses were relatively low as compared to subsequent studies) and differing duration, had positive clinical effects in 18 patients (as measured by assessment of reduction in soft tissue swelling, pain and/or joint stiffness)

and no adverse events associated with the medication were reported in any of these case reports. This data therefore provided a plausible basis for the further assessment of bromelain in musculoskeletal disorders.

Four unpublished studies: two placebo-controlled, randomised trials and two controlled and randomised studies were reported in the review by Leipner *et al.* (25). These studies were designed to assess the comparative effectiveness of bromelain with a standard treatment, the NSAID diclofenac (DF). No significant improvement in outcome was observed in either of the two placebo-controlled trials but both are of poor methodological quality. The outcome measure for one of the unpublished trials may have been inappropriate and both studies may have been inadequately powered (sample size in both studies was  $n = 60$ ). In addition, in common with the majority of studies assessing bromelain for this indication, the treatment period was short (3 weeks duration) as compared to normal herbal practice where this preparation may be prescribed for 2-3 months in the first instance. Definitive conclusions cannot therefore be drawn from these two efficacy studies. However the safety and tolerability in both these studies appeared adequate as only minor (mainly gastrointestinal) adverse events were reported and dropout rates were low (5% in both studies). Klein and Kullich's (27) double blind, randomised, controlled trial of 73 patients with osteoarthritis of the knee compared a commercial proteolytic enzyme preparation (Phlogenzym®) containing bromelain (among other proteolytic enzymes) with a dose of DF (100-150 mg/day) (24). They report an equivalent reduction in pain indices of 80% for the two treatments during 3 weeks of therapy and 4 weeks of follow-up with few adverse reactions to either treatment. The two unpublished comparative trials identified that treatment with bromelain (540 mg/day as part of the complexes PHL or WOB) reduced osteoarthritis symptoms and that the reduction was comparable to standard treatment. However, again the treatment period in both these studies was short and it is not possible to identify if the study was adequately powered as no sample size calculations are available. Tolerability was good with both PHL and WOB; however, a high rate of adverse drug reactions (none serious) was reported in the WOB study, with a rate of reporting of 50% of subjects in the WOB and the DF treatment groups. These unpublished reports therefore show equivocal evidence in support of bromelain in osteoarthritis, but highlight the potential safety issue.

Four published studies reported trials to assess the effectiveness of bromelain for knee osteoarthritis (26-29). These studies used similar treatment periods (3 or 4 weeks) and similar daily doses of a standard treatment, DF (150-100 mg/day); however, different doses of bromelain were tested (range from 540 to 1890 mg/day). The first study reported by Singer and Oberleitner (26) assessed bromelain at a dose of 945 mg/day (which is higher than that used in most studies) versus DF after 4 weeks of treatment, and although assessment of equivalence was not reported, both groups showed similar reductions in the primary outcome. However, there were more adverse drug reactions (mainly gastrointestinal: 13 in the WOB group versus nine in the DF group) and drop-outs (20% WOB versus 10% DF) as compared to the standard treatment group, which raises concerns about the safety and tolerability of bromelain at this higher dose. These safety and

tolerability issues were not replicated in the study by Tilwe *et al.* (29) who administered a daily bromelain dose of 1890 mg/day (in the form of the complex PHL) against the DF comparative group. Equivalence was not tested in this study, but both groups showed reduced symptoms of pain and swelling (comparable across groups), and also joint tenderness (the improvement was significantly better in the PHL group). Tolerability was deemed good (there were no drop-outs), and no significant safety issues were raised in this study despite the high dose employed. The final comparative study was reported by Singer *et al.* (28) who compared bromelain (in the complex PHL) at a dose of 540 mg/day against DF in 68 subjects. This study demonstrated that bromelain showed significantly better improvement in both the primary outcome (Lequesne index,  $P= 0.017$ ) and summary pain scores ( $P= 0.047$ ) as compared to DF. Tolerability and safety were acceptable and levels were similar in both treatment groups. In summary, the four comparative trials indicate that bromelain appears to be as effective as the standard treatment in osteoarthritis of the knee, but higher doses may be associated with safety concerns.

Finally, Walker *et al.* (30) recently described an open study of one month treatment intervention of bromelain using two dose regimes (200 and 400 mg) in otherwise healthy adults ( $n = 77$ ) with acute knee pain with no medical diagnosis. The data identified a significant clinical improvement compared to baseline in both the primary outcome [symptoms assessed by the Western Ontario McMaster University Arthritis Index, WOMAC (32)] and in the secondary outcomes (overall psychological wellbeing), at both doses. Furthermore, mean improvements in total symptom score, stiffness and physical function and psychological well-being were significantly greater in the high-dose compared with the low-dose group. However, definitive conclusions cannot be drawn from this study since there are a number of methodological shortcomings. These include the issue of power, which was not addressed: there was no control group (and therefore bias cannot be eliminated) and these patients did not have a formal diagnosis of their knee pain.

In conclusion, bromelain appears to have potential for the treatment of knee osteoarthritis. However it is important to note that there are a number of methodological issues that are common to the studies reported, including the possibility of inadequate power, inadequate treatment periods, inadequate or non-existent follow-up to monitor possible adverse drug reactions. Furthermore, the optimum dosage for this condition remains unclear. A phase II clinical trial would be beneficial to identify the optimal dosage and to systematically monitor safety issues before a definitive efficacy study could be completed.

#### Bromelain for Osteoarthritis of the Shoulder

Two studies have assessed *the* use of bromelain in osteoarthritis of the shoulder (31. Klein, 1994, unpublished data) (Table 2). Both studies have assessed the complex PHL, which has been used at the same daily dose (equivalent of 540 mg bromelain per day) and for the same treatment period of 3 weeks with no follow-up. The first study (by Klein, 1994) is an unpublished report of a double blind placebo controlled trial assessing PHL in 60 patients. No significant difference in treatment

groups was observed after treatment. The level of adverse drug reactions and rate of drop out was low. However, there are a number of methodological caveats. It is unclear if the study was adequately powered to detect treatment group differences and, as with the knee osteoarthritis studies, the treatment period and lack of follow-up period are inadequate and the optimum dosage is not clear. The second study by Klein *et al* (31) was designed to compare PHL against the standard DF treatment (100 mg/day) in *n* - 40 patients with this condition. No group differences in the primary outcome measures (summary pain score) were observed and safety and tolerability were adequate at this dose. However, this study also suffers from possibly being inadequately powered, a brief treatment period and limited follow up.

In conclusion the data from these two studies do not provide support for the effectiveness and safety of bromelain in osteoarthritis of the shoulder; further studies are needed that are adequately powered to identify the optimal dose and optimal treatment period for this condition.

### Summary of Clinical Trials Assessing Bromelain for Osteoarthritis

The use of bromelain for the treatment of osteoarthritis looks promising. However, a number of methodological caveats indicate that further studies are warranted, in particular phase II clinical trials to identify the optimum dosage, followed by a definitive randomised placebo-controlled trial to confirm its efficacy in the treatment of osteoarthritis.

## Bromelain and Adverse Events

Bromelain has been used as treatment for a number of disease conditions, in addition to osteoarthritis of the knee and shoulder joints (Table 1). No serious adverse events have been reported with the consumption of either bromelain or pineapples in these studies. Adverse events that have been reported are mainly gastrointestinal (i.e. diarrhoea, nausea and flatulence), but have also included headache, tiredness, dry mouth, skin rash and allergic reactions (not specified).

The trials assessing bromelain in osteoarthritis have used doses of bromelain in the range 540-1890 mg/day. Safety and tolerability for bromelain at the lower dose appears good with similar if not better safety profiles as compared to standard treatment. However, the studies that have used a higher daily dose of bromelain [945 mg/day (26); 1890 mg/day (29)] appear to be conflicting. The authors employing the highest dose reported that the medication was well tolerated; the dose of 945 mg/day, however, showed a higher incidence of adverse drug reactions and drop-outs as compared to the profiles from the standard NSAID treatment group. A formal phase II study is needed to identify safety and efficacy/effectiveness of bromelain. In addition, it is conceivable that patients

would clinically receive bromelain for longer treatment periods than have been assessed by the current osteoarthritis studies. Further work is therefore needed to evaluate the long-term safety of this supplement. Finally, there are also a number of other potential safety issues that need to be addressed. These include investigating the possibility of renal effects (because of modulation of biosynthesis of prostaglandins), potentiating effects on the action of anticoagulants [e.g. warfarin (33)] and enhanced absorption of antibiotics (11).

### Dosage in human studies

The review by Maurer (11) identified that bromelain has been used in the daily dosage range of 200-2000 mg, with therapeutic action shown at 160 mg/day. The trials assessing bromelain in osteoarthritis have used bromelain at a higher therapeutic dose, in the range of 540-1890 mg/day. Safety and tolerability at the lower dose appears to be good; the data indicates that bromelain at this dose appears to be as effective as standard treatment with at least similar safety and tolerability profiles. The two studies employing a higher daily dose [945 mg/day (26) and 1890 mg/day (29), both comparative trials] showed that the dose of 945 mg/day showed similar outcomes to DF, whereas 1890 mg/day appeared to be superior to DF in one of the primary outcome measures (joint swelling). As yet there have been no formal phase II studies to assess the optimal dose. However, the recent study by Walker *et al.* (30) in acute knee pain showed a significant dose-dependent effect between the two doses of 200 and 400 mg per day, over a period of one-month therapy. Further study is needed to identify the optimal dose for the treatment of chronic joint inflammation over longer periods of time (e.g. 3-4 months) within a blinded and randomised trial.

## Summary

The currently available data do indicate the potential of bromelain in treating osteoarthritis. However, further studies are needed before a definitive conclusion can be drawn. Specifically, there is a need for trials to establish efficacy, and dose ranging studies to identify the optimum dosage (with adequate prospective adverse event monitoring). Finally, future work should focus on the dose-response parameters and efficacy of long-term bromelain use in chronic conditions such as osteoarthritis.

## Conflict of Interest

Dick Middleton is consultant to Lichtwer Pharma UK Ltd who manufacture bromelain. Steven Hicks was funded by Lichtwer for a post-graduate fellowship from 1998 to 2002.

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## Footnotes

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# Bromelain: A Literature Review and Discussion of its Therapeutic Applications

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## Abstract

First introduced as a therapeutic compound in 1957, bromelain's actions include: (1) inhibition of platelet aggregation; (2) fibrinolytic activity; (3) anti-inflammatory action; (4) anti-tumor action; (5) modulation of cytokines and immunity; (6) skin debridement properties; (7) enhanced absorption of other drugs; (8) mucolytic properties; (9) digestive assistance; (10) enhanced wound healing; and (11) cardiovascular and circulatory improvement. Bromelain is well absorbed orally and available evidence indicates that its therapeutic effects are enhanced with higher doses. Although all of its mechanisms of action are still not completely resolved, it has been demonstrated to be a safe and effective supplement. (Alt Med Rev 1996;1(4):243-257)

## Description

Pineapple has been used as a medicinal plant in several native cultures and bromelain has been known chemically since 1876. In 1957, bromelain was introduced as a therapeutic compound when Heinicke found it in high concentrations in pineapple stems.

Bromelain is a general name for a family of sulfhydryl proteolytic enzymes obtained from *Ananas comosus*, the pineapple plant. It is usually distinguished as either fruit bromelain or stem bromelain depending on its source, with all commercially available bromelain being derived from the stem.<sup>1</sup> The term bromelain will be used to refer to stem bromelain in the remainder of this article.

Bromelain's primary component is a sulfhydryl proteolytic fraction. Bromelain also contains a peroxidase, acid phosphatase, several protease inhibitors, and organically bound calcium. When the proteolytic fraction of bromelain is purified and extracted, the result is a potent proteolytic enzyme *in vitro*; however, this component has been shown to be physiologically inactive *in vivo* for many of the conditions where bromelain has a beneficial effect.<sup>2</sup> It appears that a great deal of the physiological activity of bromelain is not accounted for in its proteolytic fraction and it is likely that the beneficial effects of bromelain are due to multiple factors, not to one single factor that can be isolated.

To date, eight basic proteolytically active components have been detected in the stem. The two main components have been labeled F4 and F5. The proteinase considered to be the

most active fraction has been designated as F9, which comprises about 2% of the total proteins. It is estimated that 50% of the proteins in F4 and F5 are glycosylated, whereas F9 was found to be unglycosylated. The optimal pH for the F4 and F5 fractions is between 4.0 and 4.5 and for F9 close to a neutral pH.<sup>3</sup> The entire extract of bromelain has been shown to exhibit its activity over a pH range of 4.5 to 9.8.<sup>4</sup>

Since bromelain is derived from a natural source, different sources can exhibit variability in their physiological activity, even when their proteolytic activity is the same. Bromelain is not heat stable so its physiological activity can be further reduced by improper processing or storage conditions.

## Absorption and Availability

Bromelain is absorbed intact through the gastrointestinal tract of animals, with up to 40% of the high molecular weight substances detected in the blood after oral administration. The highest concentration of bromelain is found in the blood 1 hour after administration; however, its proteolytic activity is rapidly deactivated,<sup>5</sup> probably by the normal plasma protease controls and serum alpha2-macroglobulin.

A variety of designations have been used to indicate the activity of bromelain; with published research varying in the designation utilized. Rorer units (R.U.), gelatin dissolving units (G.D.U.), and milk clotting units (M.C.U.) are the most commonly used measures of activity. One gram of bromelain standardized to 2000 M.C.U. would be approximately equal to 1 gram with 1200 G.D.U. of activity or 8 grams with 100,000 R.U. of activity.

## Platelet Aggregation, Fibrinolysis and Anti-Inflammatory Activity

The first conclusive evidence that bromelain prevents aggregation of blood platelets was reported in 1972. Bromelain was administered orally to 20 volunteers with a history of heart attack or stroke, or with high platelet aggregation values. Bromelain decreased aggregation of blood platelets in 17 of the subjects and normalized values in 8 of the 9 subjects who previously had high aggregation values.<sup>6</sup> In vitro studies have demonstrated that bromelain inhibits platelet aggregation stimulated by ADP or epinephrine, as well as by prostaglandin precursors, in a dose-dependent manner.<sup>7</sup>

Bromelain is an effective fibrinolytic agent in vitro and in vivo; however, its effect is more evident in purified fibrinogen solutions than in plasma. This is probably due to the antiproteases present in plasma. A dose-dependent reduction of serum fibrinogen level is seen in rats following administration of bromelain, and at the highest concentrations of bromelain, both prothrombin time (PT) and activated partial thromboplastin time (APTT) are markedly prolonged.<sup>8</sup> Bromelain's fibrinolytic activity has been attributed to the

enhanced conversion of plasminogen to plasmin, which limits the spread of the coagulation process by degrading fibrin.<sup>9</sup>

Bromelain seems to have both direct as well as indirect actions involving other enzyme systems in exerting its anti-inflammatory effect. Both etodolac and bromelain inhibit the inflammatory pain in rats in a dose-dependent manner.<sup>10</sup> Bromelain was the most potent of nine anti-inflammatory substances tested on experimentally-induced edemas in rats;<sup>11</sup> while prednisone and bromelain have been shown to be comparable in their ability to reduce inflammation in rats.<sup>12</sup> Treatment with bromelain and emorfazone has been shown to decrease significantly the heat-evoked immunoreactive substance P release and subsequent edema in a rat model.<sup>13</sup>

## Mechanism of Action

Surface contact, by collagen or platelets, activates the kinin system and the clotting cascade by stimulating the conversion of Hageman factor to an active protease (factor XIIa). Factor XIIa then activates the kinin system by converting plasma prekallikrein into kallikrein, and continues the intrinsic path of the clotting cascade by converting factor XI to its active form. Kallikrein, in an autocatalytic loop, accelerates the activation of Hageman factor, which continues to potently activate both the kinin system and the clotting cascade. In addition, Kallikrein cleaves (HMWK) to produce bradykinin, a potent stimulator of both increased vascular permeability and pain. The activation of the clotting cascade will culminate in the conversion of fibrinogen to fibrin (see Figure 1). Fibrin then forms a protective matrix around the injured area. This matrix inhibits tissue drainage, promotes edema and blocks circulation of blood flow.

In order to determine the effects of bromelain on the plasma kallikrein system, bradykinin levels and plasma exudation at the inflammatory site were examined in rats. Bromelain (5 and 7.5 mg/kg) caused a dose-dependent decrease of bradykinin levels at the inflammatory site and a parallel decrease of the prekallikrein levels in sera. Plasma exudation was also reduced dose dependency. Bradykinin-degrading activity in sera was elevated after treatment with bromelain, although it was unchanged in the pouch fluid.<sup>14</sup> The levels of high molecular weight (HMW) kininogen and pre-kallikrein in rat plasma were markedly reduced after single injection of bromelain (10 mg/kg, i.v.) and gradually recovered over a 72 hour period. The level of low molecular weight (LMW) kininogen was not changed during this period.<sup>15</sup>

Bromelain-treated rats also show a reduction in Factor X and prothrombin, both of which are needed for the activation of fibrinogen to fibrin through the common pathway of the intrinsic and extrinsic cascade.<sup>16</sup> This indicates that bromelain's action is in part a result of inhibiting the generation of bradykinin at the inflammatory site via depletion of the plasma kallikrein system, as well as limiting the formation of fibrin by reduction of clotting cascade

intermediates. These actions result in significant reduction in pain and edema, as well as enhanced circulation to the injured site.

After the formation of a clot, vessel repair begins with the conversion of plasminogen to plasmin, which then acts to degrade fibrin into smaller components which can be removed by monocytes and macrophages. In rats, bromelain has been shown to stimulate the conversion of plasminogen to plasmin, resulting in increased fibrinolysis. This minimizes venous stasis, facilitates drainage, increases permeability and restores the tissue's biological continuity.<sup>16</sup>

The therapeutic effect of bromelain may also be due to its ability to selectively modulate the biosynthesis of thromboxanes and prostacyclins; two groups of prostaglandins with opposite actions which ultimately influence activation of cyclic-3,5-adenosine (cAMP), an important cell-growth modulating compound.

The binding of epinephrine, collagen, or thrombin to platelets activates the enzymes phospholipase C and phospholipase A2 which release arachidonic acid from membrane phospholipids (phosphatidylcholine and phosphatidylinositol). Table 1 lists the inflammatory actions of arachidonic acid metabolites.

Plasminogen, which is activated to plasmin by the oral administration of bromelain, has been shown to inhibit the release of arachidonic acid from cell membranes, resulting in decreased platelet aggregation and modulation of the series 2 prostaglandins.<sup>17</sup> It is also hypothesized that bromelain therapy leads to a relative increase of the endogenous prostaglandins, PGI2 and PGE2 over thromboxane A2.<sup>18</sup>

Non-steroidal anti-inflammatory drugs inhibit cyclooxygenase, which is required for the synthesis of series 2 prostaglandins, resulting in a decrease in both pro and anti-inflammatory prostaglandins. Rather than blocking the arachidonic acid cascade at the enzyme cyclooxygenase, like NSAIDs, bromelain may selectively decrease thromboxane generation and change the ratio of thromboxane/prostacyclin (PGI2) in favor of prostacyclin (see Figure 2). Bromelain, similar to NSAIDs, has been shown to inhibit PGE2, however, its action is significantly weaker.<sup>16</sup> Table 2 lists bromelain's impact on selected mediators of inflammation.

## Antitumor

The first documented use of oral bromelain on cancer patients was in 1972. Twelve patients with ovarian and breast tumors were given 600 mg of bromelain daily for from 6 months to several years, with reported resolution of some of the cancerous masses and a decrease in metastasis.<sup>19</sup> Bromelain in doses of over 1000 mg daily has been combined with chemotherapeutic agents such as 5-FU and vincristine, and has been reported to result in tumor regression.<sup>19,20</sup>

Bromelain has also decreased lung metastasis of Lewis lung cancer cells implanted in mice in a dose-dependent manner. This antimetastatic potential was demonstrated by both the active and inactive bromelain, with or without proteolytic and anticoagulant properties.<sup>21,22</sup>

## Cytokine Induction

The successful initiation of an immune response depends on T cells and macrophages, along with the polypeptide factors they produce, called cytokines, which play a key role in communication during normal immunological response as well as infectious, inflammatory, and neoplastic disease states. Table 3 lists cytokines and their activities.

Bromelain, papain, and amylase have all been demonstrated to induce cytokine production in human peripheral blood mononuclear cells. Treatment leads to the production of tumor necrosis factor-alpha (TNF-alpha), interleukin-1-beta (IL-1 beta), and interleukin-6 (IL-6) in a time and dose-dependent manner. Interferon-alpha (IFN-alpha) and interferon-gamma (IFN-gamma), which had no effect alone, synergistically increased TNF-alpha production when applied together with the enzymes.<sup>23,24</sup> The tryptic but not the autolytic fractions of papain and bromelain have a higher (10- to 40-fold) inducing capacity for TNF production than the untreated enzyme.<sup>25</sup> Trypsin alone had only a small inducing effect.

The ability to induce cytokine production may explain the antitumor effects observed after oral administration of polyenzyme preparations.

## Immunity

Bromelain has been shown to remove T-cell CD44 molecules from lymphocytes and to affect T-cell activation. The highly purified bromelain protease F9 was tested on the adhesion of peripheral blood lymphocytes (PBL) to human umbilical vein endothelial cells (HUVEC). Both bromelain and protease F9 reduced the expression of CD44, but F9 was about 10 times more active than bromelain; having about 97% inhibition of CD44 expression. The results indicate that F9 selectively decreases the CD44 mediated binding of PBL to HUVEC.<sup>26</sup>

## Debridement

Bromelain applied topically as a cream (35% bromelain in a lipid base) can be beneficial in the elimination of burn debris and in acceleration of healing. A non-proteolytic component of bromelain is responsible for this effect. This component, referred to as escharase, has no hydrolytic enzyme activity against normal protein substrates or various glycosaminoglycan

substrates and its activity varies greatly from preparation to preparation.<sup>27</sup>

Topical bromelain has achieved complete debridement on experimental burns in rats in an average of 1.9 days as compared to collagenase, which required an average of 10.6 days for similar results.<sup>28</sup>

Topical bromelain separates eschar at the interface with living tissue. It is hypothesized that bromelain activates collagenase in living tissue which then attacks the denatured collagen in the eschar. This produces a demarcation between living and dead tissue. With very little scraping, using a tongue depressor, all of the eschar can be removed and a bed suitable for grafting results. By using bromelain, grafting can occur as soon as 24 hours after the accident. Utilizing bromelain cream in the treatment of burns usually results in minimal or no scar tissue formation.

The applicability of topical bromelain in frostbite eschar removal was extrapolated and investigated. In the initial trial, no debridement other than that of the superficial layers of the eschar was noted. Although third degree burn injuries debrided to a graftable bed after two topical applications of bromelain, frostbite injuries remained unaffected.<sup>29</sup>

## Potential of Antibiotics

Antibiotic potentiation is one of the primary uses of bromelain in several foreign countries. Bromelain can modify the permeability of organs and tissues to different drugs. It prolongs sleeping time in mice administered pentobarbital<sup>30</sup> and increases spinal levels of penicillin and gentamycin in rats. In humans, bromelain has been documented to increase blood and urine levels of antibiotics 16 and results in higher blood and tissue levels of tetracycline and amoxicillin when they are administered concurrently with bromelain.<sup>31</sup>

Treatment of 18 women with 80 mg of bromelain concurrently with amoxicillin or tetracycline resulted in increased serum levels and concentrations of both antibiotics in uterus, ovarian tubes, and ovaries as compared with controls. This effect was not generated by indomethacin, an anti-inflammatory drug which acts as a cyclooxygenase inhibitor, which indicates that bromelain has some undetermined activity that enhances absorption and tissue distribution of antibiotics.<sup>32</sup> A three-fold increase in the level of tetracycline in serum after oral ingestion of 540 mg of enterically-coated bromelain has also been demonstrated in a double blind test.<sup>33</sup>

Combined bromelain and antibiotic therapy was instituted for 53 hospitalized patients with the following conditions; pneumonia, bronchitis, cutaneous staphylococcus infection, thrombophlebitis, cellulitis, pyelonephritis and perirectal and rectal abscesses. Twenty three of the patients had been on antibiotic therapy without success. Bromelain was administered four times a day along with the following antibiotics either alone or in combination; penicillin, chloramphenicol, erythromycin or novobiacin. A control group of 56 patients was

treated with antibiotics alone. Of the 23 patients who had been unsuccessfully treated with antibiotics, 22 responded favorably to the combined treatment. In every disease state studied there was a significant reduction in morbidity when the combination of bromelain and antibiotics was used as opposed to antibiotics alone. Another group of 106 cases was treated with bromelain alone, with results comparable to those obtained with antibiotic treatment.<sup>34</sup>

Forty eight patients with acute sinusitis were placed on standard therapy, which included antihistamines and analgesic agents, along with antibiotics if indicated. Twenty three of the patients received bromelain four times daily, while the remaining 25 received a placebo. Of the patients receiving bromelain, 83% had complete resolution of nasal mucosal inflammation compared with only 52% in the placebo group. Improvement in breathing occurred in 78% of those receiving bromelain as compared to 68% in those receiving placebo. In the patients not receiving antibiotic treatment, 85% of patients receiving bromelain had complete resolution of inflammation of the nasal mucosa and complete resolution of breathing difficulties. Only 40% of the placebo group had a similar outcome with respect to inflammation, while 53% reported resolution of breathing difficulty.<sup>35</sup>

The potentiation of antibiotics and other medicines by bromelain may be due to enhanced absorption, as well as increased permeability of the diseased tissue which enhances the access of the antibiotic to the site of the infection. It is also thought that the use of bromelain may provide a similar access to specific and non-specific components of the immune system, therefore, enhancing the body's utilization of its own healing resources.

## Mucolytic Properties

The topical use of the enzymes, bromelain or papain, to remove excessive cervical mucus was demonstrated in 1954. Observations following its use demonstrated that pseudo and actual space-occupying lesions could be more positively identified, and inflammatory changes of the canal and its glands could be visualized with greater accuracy.<sup>36</sup>

Effects of bromelain on rabbit sputum consistency were investigated in vitro and in vivo. Of the enzymes tested, bromelain exerted the most potent lowering effect on sputum viscosity and also showed a tendency to increase the sputum volume.<sup>37</sup>

In a clinical study of 124 patients hospitalized with chronic bronchitis, pneumonia or bronchopneumonia, bronchiectasis, or pulmonary abscess, those receiving bromelain orally showed a decrease in the volume and purulence of the sputum. 17 These results support the effectiveness of bromelain in decreasing the viscosity of sputum so that it can be more easily cleared from the respiratory tract.

## Digestive Aid

Bromelain has been used successfully as a digestive enzyme following pancreatectomy, in cases of exocrine pancreas insufficiency and in other intestinal disorders.<sup>38</sup> Because of its wide pH range, bromelain has activity in the stomach as well as the small intestine. It has also been shown to be an adequate replacement for pepsin and trypsin in cases of deficiency. The combination of ox bile, pancreatin and bromelain is effective in lowering stool fat excretion in patients with pancreatic steatorrhoea. In addition, this combination resulted in a gain in weight in most cases as well as an enhanced subjective feeling of well being. Symptomatic improvement was also noted in relation to pain, flatulence and stool frequency.<sup>39</sup>

Bromelain has been reported to heal gastric ulcers in experimental animals.<sup>40</sup> In an extensive study of the effect of bromelain on the gastric mucosa, it was found that bromelain increased the uptake of radioactive sulfur by 50% and glucosamine by 30 -90%. Increased uptake of these substances may allow the gastric mucosa to heal more rapidly under the influence of bromelain.<sup>41</sup>

In a study designed to examine the effect of bromelain on enterotoxin receptor activity in porcine small intestine, orally administered bromelain inhibited enterotoxin attachment to pig small intestine in a dose-dependent manner. Attachment was negligible after treatment. Serum biochemical analysis and histopathological examination of treated piglets showed no adverse effects with the bromelain treatment. Administration of bromelain may therefore be useful for preventing enterotoxin-induced diarrhea.<sup>42</sup>

## Surgical Procedures and Musculoskeletal Injuries

Bromelain also has therapeutic effects in the treatment of inflammation and soft tissue injuries. An early clinical trial on bromelain was conducted on 74 boxers with bruises on the face and haematomas of the orbits, lips, ears, chest and arms. Bromelain was given four times a day for 4 days or until all signs of bruising had disappeared. A control group of 72 boxers were given a placebo. In 58 of the boxers taking bromelain, all signs of bruising cleared completely in four days, with the remaining 16 requiring 8-10 days for complete clearance. In the control group, only 10 had complete clearance within four days, with the remainder requiring seven to fourteen days for resolution.<sup>43</sup>

The edema-reducing property of bromelain was investigated in traumatically-induced hindleg edema in rats. After enteral application of bromelain a significant reduction of the edema could be observed, however, parenteral application only resulted in a minimal therapeutic effect. Although enterally-applied enzymes are thought to be degraded in the gut, the better results were obtained after oral administration of bromelain, supporting the observation that bromelain can be absorbed by the gut without losing its biological properties.<sup>11</sup>

Fifty-five pre-surgical patients were divided into two groups. Group one, consisting of 22

patients, took bromelain four times a day for 48-72 hours prior to surgery and continued for 72 hours after surgery. Group two, consisting of 33 patients, took bromelain starting on the day of surgery, with the first dose administered one hour prior to surgery. Fifty percent of group one and 42.4% of group two had complete disappearance of pain and inflammation within 72 hours. Pain and inflammation persisted past 72 hours in only one member of the group supplemented with bromelain for three days prior to surgery, as opposed to five members of the group that started supplementation one hour prior to surgery. In a separate study, supplementation of bromelain starting 48-72 hours prior to surgery reduced the average number of days for complete disappearance of pain from 3.5 to 1.5, and disappearance of inflammation from 6.9 to 2.0 days, as compared with controls receiving no bromelain.<sup>44</sup>

Sixteen patients undergoing oral surgery were given bromelain four times a day starting 72 hours prior to surgery. At 24 hours after surgery, 75% of these patients were evaluated as having mild or no inflammation, in contrast to only 19% of a group receiving a placebo. Twenty-four hours after surgery, pain was either absent or mild in 38% of bromelain-treated patients, as opposed to 13% receiving placebo. After 72 hours, this increased to 75% of those in the bromelain group, as compared to only 38% in the placebo group.<sup>45</sup>

In an observation study involving 59 patients with blunt injuries to the musculoskeletal system, the efficacy and tolerability of high-dose bromelain, in addition to the usual therapeutic measures, was investigated. Treatment with bromelain resulted in a clear reduction in all four parameters tested; swelling, pain at rest and during movement, and tenderness.<sup>46</sup>

## Cardiovascular and Circulatory Applications

Research has indicated that bromelain prevents aggregation of human blood platelets in vivo and in vitro, prevents or minimizes the severity of angina pectoris and transient ischemic attacks (TIA), is useful in the prevention and treatment of thrombosis and thrombophlebitis, may break down cholesterol plaques, and exerts a potent fibrinolytic activity. If administered for prolonged time periods, bromelain also exerts an anti-hypertensive effect in experimental animals.<sup>247</sup>

Administration of 400-1000 mg/day of bromelain to 14 patients with angina pectoris resulted in the disappearance of symptoms in all patients within 4 to 90 days.<sup>48</sup> Similar results have been observed in patients taking between 500-700 mg/day of bromelain. After discontinuing bromelain, angina attacks reappear after a variable period of time, often triggered by stressful experiences.<sup>2</sup>

A drastic reduction in the incidence of coronary infarct after administration of potassium and magnesium orotate along with 120-400 mg of bromelain per day has also been reported.<sup>49</sup>

In a study involving 73 patients with acute thrombophlebitis, bromelain, in addition to analgesics, was shown to decrease all symptoms of inflammation; including, pain, edema, tenderness, skin temperature, and disability.<sup>40</sup>

The ability of bromelain to influence these conditions may be due to its ability to breakdown fibrinous plaques. Bromelain has been shown to dissolve arteriosclerotic plaque in rabbit aorta in vivo and in vitro.<sup>2</sup> It is likely that bromelain also increases vessel wall permeability to oxygen and nutrients while increasing blood fluidity, both of which aid in these conditions.

## Toxicity, Side Effects and Allergic Reactions

Bromelain is considered to have very low toxicity, with an LD50 greater than 10 kg . Toxicity tests on dogs, with increasing levels of bromelain up to 750 mg/kg administered daily, showed no toxic effects after six months. Dosages of 1.5 g/kg/day administered to rats show no carcinogenic or teratogenic effects.<sup>51</sup>

In human clinical tests, side effects have not been observed. Bromelain supplementation up to 460 mg has been shown to have no effect on heart rate or blood pressure; however, increasing doses up to 1840 mg have been shown to increase the heart rate proportionately. In some cases an increase of up to 80% of the baseline has been reported, which may be a result of bromelain's influence on IL-1 and TNF production. Maximum effects were seen at 2 hours but some residual effect remained at 24 hours. At doses above 700 mg, palpitations and subjective discomfort have been reported. Blood pressure changes have not been demonstrated in humans at any dosage level.<sup>52</sup>

The allergenic potential of proteolytic enzymes should not be underestimated, for they cause, in particular, IgE-mediated respiratory allergies of both the immediate type and the late-phase of immediate type with predominantly respiratory symptoms. Allergy to bromelain has been reported in workers of a blood-grouping laboratory, and investigation indicates that (1) bromelain is a strong sensitizer, (2) sensitization usually occurs due to inhalation and not to ingestion, (3) bromelain allergy is occupationally acquired, and adequate precautions are necessary.<sup>53</sup> The risk of sensitization to enzymes due to inhalation as a result of occupational exposure is very high (up to 50%).<sup>54</sup>

Bromelain has been shown to cross-react with the sera in about 28% of persons with IgE allergic response to honeybee venom.<sup>55</sup> Bromelain, along with horseradish peroxidase and ascorbate oxidase are recognized by the IgE of sera from patients who are hypersensitive to olive tree pollen.<sup>56</sup>

Bromelain and papain, due to their use as a meat tenderizer and to clarify beer, are considered as potential ingestive allergens and may represent an unrecognized cause of an allergic reaction following a meal. As with other food substances, a small segment of the

population, particularly those with a sensitivity to pineapple, may be sensitive to oral supplementation with bromelain. As contact allergens, the enzymes play a minor role; however, it is thought that skin testing with isolated proteases like bromelain may induce systemic reactions in susceptible individuals, even at very high dilution.<sup>53,57</sup>

## Indications for the Use of Bromelain

There are several compelling reasons for supplementation with oral bromelain.

1. It inhibits blood platelet aggregation, favorably modulates prostaglandin formation and minimizes risk of coronary atherosclerotic disease.
2. It continues to provide a desired physiological action for as long as it is administered, with no evidence indicating that a tolerance develops.
3. It is considered to be non-toxic and lacking in side effects, so it can be used without concern in doses from 200 to 2000 mg for prolonged periods of time.
4. It is a protein and seems to be as easily metabolized as other dietary proteins.
5. It is well absorbed and seems to have greater therapeutic impact when administered orally as opposed to intravenously
6. While effective for inflammation and injury, it is even more effective if administered prior to a traumatic event, i.e. surgery or athletic competition.
7. It seems to enhance the absorption of and improve the action of other substances when they are administered in combination.
8. Because of its impact on the cytokine system, particularly IL-1 and TNF, which stimulate fever and acute phase response, and its demonstrated ability to increase the heart rate, bromelain may assist in generating an acute-stage healing response.

Bromelain has a wide range of conditions for which it has well documented therapeutic efficacy (see Table 4).

## Dosage and Prescription Instructions

Available research does not demonstrate an enhanced efficacy of bromelain when it is administered between meals. It is generally recommended that bromelain be taken away from food unless it is being used as a digestive aid, because it is believed that otherwise, it

will tend to act as a digestive enzyme and its therapeutic benefit may be diminished. While this may in fact be the case, the clinical studies conducted on bromelain have not followed this protocol.

Bromelain has shown therapeutic benefits in doses as small as 160 mg/day; however, it is thought that, for most conditions, best results occur starting at a dose of 750-1000 mg/day. Most research on bromelain has been done utilizing divided doses, usually four per day, and findings indicate that results are dose-dependent. See Table 5 for a summary of prescription instructions.

## Conclusion

Bromelain has been used for a variety of clinical applications for more than 35 years. Although its mechanisms of action has not been completely resolved, bromelain has demonstrated a beneficial effect on the kinin system, the coagulation cascade, the cytokine system, and prostaglandin synthesis. Bromelain is believed to enhance the absorption of flavonoids and has been shown to increase absorption of glucosamine, so bromelain supplementation should be considered when these nutrients are given. It may also enhance absorption and utilization of many other substances; however, to date research in this area has focused primarily on antibiotics. Bromelain has been shown to exert a beneficial effect at doses as low as 160 mg/day, however, there is a general consensus among researchers that the best results occur when bromelain is given in doses above 500 mg per day and that results improve in a dose-dependent manner with higher levels of bromelain supplementation. Bromelain has been demonstrated to be well absorbed after an oral dose and has been shown to be safe at high doses for prolonged periods of time. For the conditions discussed in this review, bromelain has shown itself to be an effective supplement.

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**Return to Table of Contents**