Table 1 DIPS改変による健康食品と医薬品の併用における有害事象の因果関係判定評価分類票

No	質問項目	はい	いいえ	不詳
1	過去に，ヒトにおいて信頼できる当該健康食品と当該医薬品の併用における相互作用の報告がありますか？	+1	−1	0
2	観察された有害事象は，当該健康食品の既知の相互作用の特徴と一致しますか？	+1	−1	0
3	観察された有害事象は，併用した医薬品の既知の相互作用の特徴と一致しますか？	+1	−1	0
4	有害事象の経過には，妥当な時間的関連がありますか？（発現および消失，あるいはそのどちらか一方）	+1	−1	0
5	併用した医薬品を変更せずに，当該健康食品の摂取中止により有害事象の程度は軽くなりましたか？（もし投与を中止していないなら「不詳」とし，質問6に進んでください）	+1	−2	0
6	併用した医薬品の連用の下で当該健康食品が再摂取されたとき，有害事象は再現しましたか？	+2	−1	0
7	その有害事象に関して妥当な，相互作用以外の原因はありますか？*)	−1	+1	0
8	併用した医薬品の血中濃度や他の体液濃度は，その相互作用を引き起こすレベルでしたか？	+1	0	0
9	有害事象は，併用した医薬品の作用と矛盾しない客観的証拠によって確認されましたか？（質問8による薬物濃度以外）	+1	0	0
10	当該健康食品の摂取量を増やしたとき有害事象の程度は重く，または当該健康食品の摂取量を減らしたときに有害事象の程度は軽くなりましたか？	+1	−1	0

*)基礎疾患の病態，他の併用薬，年齢などを考慮．「いいえ」という答えは，どのような代替案を考慮したとしても，十分な情報が存在しない場合を前提（不確かなときは，不詳）とする．

＜合計点による評価判定スコア＞ highly probable（非常に確からしい）9点以上，probable（確からしい）5〜8点，possible（可能性がある）2〜4点，doubtful（疑わしい）2点未満

関係を判定した報告は，国内外の文献を検索するかぎりみられない．そこで本研究では，DIPSを健康食品と医薬品との相互作用における有害事象の因果関係評価に応用し，実際の臨床文献における有害事象報告に適用することにより，その信頼性を検討することとした．

方　法

Hornらによる DIPSを健康食品と医薬品との相互作用に対応できるように改変し，因果関係判定評価分類票を和文で作成した(Table 1)．DIPS改変にあたっては，「医薬品と医薬品との相互作用」を「健康食品と医薬品との相互作用」に置き替え，それにより文脈上の齟齬が生じないように文章を修正した．DIPSの10項目の評価内容および評価点の判定の重み付けについては修正を加えなかった．また，評価点の合計に基づく4段階の判定スコアの配点基準についても原著どおり，highly probable（非常に確からしい）9点以上，probable（確からしい）5〜8点，possible（可能性がある）2〜4点，doubtful（疑わしい）2点未満とした．

今回対象とした健康食品は，国内外を通じて医薬品との相互作用の報告が多いセントジョーンズワート(St. John's wort)と，同じく国内外を通じて使用頻度が高い健康食品の1つであるイチョウ葉エキス（ginkgo biloba extracts）を選んだ[8]．それぞれの臨床文献の検索は，MEDLINEおよび医学中央雑誌をデータベースとして利用して症例報告（case reports）を検索し，さらにThe Cochrane Library，独立行政法人国立健康・栄養研究所ホームページも渉猟した．検索語としては，MEDLINEではMeSH termとして，セントジョーンズワートに対してはhypericum，イチョウ葉エキスはginkgo bilobaを選び，それぞれの健康食品に対し，MeSH termのadverse effects，drug interactions，reduced reactionsについて網羅的に検索した．医学中央雑誌ではセントジョーンズワート，イチョウ葉エキスそれぞれに対し，相互作用，副作用について検索した．次いで検索した相互作用および有害事象報告を臨床文献から収集後，4名の評価者（日本臨床薬理学会指導医1名，薬剤師3名）が今回作成した因果関係判定評価分類票を用いて，独立に，かつ文献を読む順番を単純ランダム化して有害事象を評価し，次いで評価者間信頼性を検討した．

評価者間信頼性の解析は，評価者4名および医師を除いた薬剤師3名のそれぞれにおいて実施した．報告間の変動と評価者間の変動を変量効果とみなした二元配置分散分析モデルをあてはめ，評価点を連続変数とみなして評価者間信頼性係数（級内相関係数：intraclass correlation coefficient；ICC）とその95%信頼区間を推定した．また，判定スコアについてSchoutenの多評者間κ係数および重みつきκ係数をそれぞれ推定した．なおκ係数の解析においては，評価者の1人でも，評価項目10項目のうち評価適用外と判定した

Table 2　DIPS改変因果関係判定評価分類票による各評価者の評価内分け

番号	併用薬名	相互作用・有害事象名	DIPS評価点(合計) 医師	薬剤師1	薬剤師2	薬剤師3	判定スコア 医師	薬剤師1	薬剤師2	薬剤師3	調査文献名 年;巻:開始ページ
S1	methylphenidate	併用薬の薬効減弱	2	1	4	2	2	1	2	2	Med Hypotheses 2007;68:1189
S2	fluoxetine	無顆粒球症、骨髄壊死	0	1	−2	3	1	1	1	2	Acta Medica 2005;48:91
S3	ethinylestradiol	避妊効果の減弱	1	2	3	2	1	2	2	2	Br J Clin Pharmacol 2003;55:112
S4	fentanyl	麻酔作用の減弱	4	3	2	3	2	2	2	2	Anesthesiology 2002;96:1025
S5	buspirone	セロトニン症候群	6	2	5	6	3	2	3	3	J Psychopharmacol 2002;16:401
S6	tacrolimus	腎毒性	4	7	3	7	2	3	2	3	Transplantation 2002;73:1009
S7	olanzapine	髪、眉の脱毛	−1	2	3	3	1	2	2	2	Can J Psychiatry 2001;96:77
S8	cyclosporine	併用薬の血中濃度低下	6	5	6	6	3	3	3	3	Med Klin 2001;96:480
S9	clonazepam	セロトニン症候群	4	0	1	1	2	1	1	1	Can J Psychiatry 2001;46:77
S10	cyclosporine	血清クレアチニン値の上昇	6	7	6	6	3	3	3	3	Am J Kidney Dis 2001;38:1105
S11−1	cyclosporine	拒絶反応	6	6	6	6	3	3	3	3	Prog Transplant 2001;11:116
S11−2	cyclosporine	拒絶反応	6	7	6	6	3	3	3	3	Prog Transplant 2001;11:116
S12	fentanyl	血圧下降	5	5	0	5	3	3	1	3	J Clin Anesth 2000;12:498
S13	cyclosporine	拒絶反応	6	6	6	6	3	3	3	3	J Hepatol 2000;33:853
S14	cyclosporine	併用薬の血中濃度低下	8	5	8	6	3	3	3	3	Nephrol Dial Transplant 2000;15:1473
S15−1	nortriptyline	躁状態	3	NA	−1	2	2	NA	1	2	J Clin Psychopharmacol 2000;20:115
S15−2	lithium	妄想	0	−1	2	1	1	1	2	1	J Clin Psychopharmacol 2000;20:115
S16	cyclosporine	拒絶反応	4	6	6	6	2	3	3	3	Lancet 2000;355:548
S17−1	caffein	光線過敏症	3	1	4	2	2	1	2	2	Ann Pharmacother 2000;34:1013
S17−2	cyclosporine	併用薬の薬効減弱	6	6	6	4	3	3	3	2	Ann Pharmacother 2000;34:1013
S18	lithium	躁状態	NA	NA	0	NA	NA	NA	1	NA	Biol Psychiatry 1999;46:1707
G1	acarbose	肝障害	−2	−3	−2	−2	1	1	1	1	Ann Pharmacother 2006;40:151
G2	phenitoin	痙攣	4	5	6	3	2	3	3	2	J Anal Toxicol 2005;29:755
G3	alendronate sodium hydrate	硝子体出血	4	3	2	3	2	2	2	−2	Br J Ophthalmol 2005;89:1378
G4	diclofenac	術後出血	1	NA	2	NA	1	NA	2	NA	Anaesthesia 2005;60:725
G5	aspirin	出血傾向	4	5	4	5	2	3	2	3	J Arthroplasty 2005;20:125
G6	lidocaine	斑状出血	2	2	1	1	2	2	1	1	Br J Plast Surg 2005;58:100
G7	lidocaine	眼球出血	4	2	2	2	2	2	2	2	Postgrad Med J 2003;79:531
G8	ibuprofen	脳出血	4	5	2	3	2	3	2	2	Atherosclerosis 2003;167:367
G9	aspirin	出血傾向	5	6	5	6	3	3	3	3	Transpl int 2002;15:377

略号: S(St. John's wort)、G(Ginkgo biloba)
DIPS評価:NA: Not Applicable(評価不能)
判定スコア:1(doubtful)、2(possible)、3(probable)
CI:confidence Interval(信頼区間)

評価者間信頼性係数:
(4名全員)0.745[95%CI(0.601, 0.858)]
(薬剤師3名)0.741[95%CI(0.575, 0.861)

多評価者間 κ 係数:
(4名全員)0.495[95%CI(0.218, 0.772)]
(薬剤師3名)0.532[95%CI(0.223, 0.767)]

項目があった文献は，解析対象から除外した．

結　果

収集した評価対象報告は，セントジョーンズワート21件（文献18件），イチョウ葉エキス9件（文献9件），計30件であった（Table 2）．それぞれの健康食品における相互作用の内訳は，セントジョーンズワートでは，併用したcyclosporineの薬効減弱8件，抗うつ薬や抗精神病薬の副作用増強6件，その他7件（麻酔薬の効果減弱，避妊薬の効果減弱等）であり，イチョウ葉エキスでは，出血傾向7件（非ステロイド性消炎鎮痛薬併用での副作用増強等），その他2件（抗てんかん薬の効果減弱等）であった．

評価対象30件の評価点における評価者間信頼性係数は，4名の評価者全員では0.745［95%信頼区間（0.601, 0.858）］であった．一方，判定スコアにおける多評価者間 κ 係数は0.495［95%信頼区間（0.218, 0.772）］，一次重みつき κ 係数は0.574，二次重みつき κ 係数は0.663であった（κ 係数については，評価項目の中で評価不能と評価された項目があった3件を除いた27件で解析）．

一方，評価者から医師を除いた薬剤師3名のデータを解析した結果では，評価者間信頼性係数は0.741［95%信頼区間（0.575, 0.861）］であり，多評価者間 κ 係数は0.532［95%信頼区間（0.223, 0.767）］，一次重みつき κ 係数は0.600，二次重みつき κ 係数は0.674であった．

考　察

今回，drug-drug interactionにおける有害事象の因果関係評価であるDIPSを健康食品用に改変し，臨床文献に適用することにより信頼性評価を行った．評価項目の特徴としては，10項目という比較的シンプルなチェック項目の中に，有害事象の因果関係判定に必要とされる一般的な評価項目すなわち，臨床的既知報告の存在，時間的な関連性，中止・減量による改善，偶然の再摂取による症状の再現・増悪，他の要因の除外，体液中濃度等の客観的指標による確証，用量依存性の反応に関する評価を含み，かつ相互作用を検討するうえで必要な内容として，健康食品と併用医薬品それぞれにおける生物学的作用機序との整合性を検討したことが挙げられる．

今回の信頼性評価の結果では，評価者間信頼性係数と κ 係数でみるかぎり，従来の医薬品における有害事象判定の信頼性評価と比べ遜色ない結果を示した[6]．しかしながら，対象とした有害事象報告はデータベースから検索した既出文献からであり，その意味では因果関係において確からしい症例がすでに選ばれていると考えられ，一般の臨床現場からの有害事象報告とは異なる可能性は否定できない．今後は文献報告だけでなく，医療現場から直接報告される有害事象を評価分類することで信頼性評価を行う必要があると考える．

有害事象の因果関係判定においては，評価者の臨床的経験や判断能力等の高度な専門性が評価に影響を与える一方で，臨床現場では医療従事者の誰もが使用できるような，簡便でより汎用性の高いものが期待されている．今回，評価者は少ないながら，医師を含めた評価者4名と薬剤師3名での評価で，ICCおよび κ 係数がほぼ同じ結果が得られたことは，今回の判断基準が医師，薬剤師の職種に関わらず使用できる可能性を持つと考えられる．今後より多くの評価者により，この判断基準が臨床現場に応用可能であるかを検討していきたいと考える．

以上，DIPSを健康食品と医薬品との相互作用における有害事象の因果関係評価に応用し，実際の臨床文献における有害事象報告に適用して信頼性評価を行うことにより，臨床現場への応用の可能性を示した．今後は文献報告だけでなく，医療現場から直接報告される有害事象を検討することで今回の結果を検証し，さらに妥当性の検討を加える必要があると考える．

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ダイエット関連事例

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肥満抑制を目的としたダイエット用健康食品は，昨今，非常に多く市場に出回り，さらにインターネット等を介して一般の消費者が容易に入手できる状況にある。ダイエット用健康食品の多くは有効性のエビデンスが明らかでないままに消費者が安易に摂取しており，またそれによる健康被害も後を絶たない。本稿ではダイエット用健康食品による健康被害の中で，社会的に大きな問題となった，エフェドラ，アマメシバ，シブトラミン含有製品等の摂取による有害事例を提示し，その発症機序について概説するとともに，ダイエット用健康食品を使用する上での注意点を喚起する。

キーワード　ダイエット，健康食品，エフェドラ，アマメシバ，シブトラミン

はじめに

肥満抑制を目的としたダイエット用健康食品は，昨今，非常に多く市場に出回っており，海外で製造販売されている健康食品においてもインターネット等を介して一般の消費者が容易に入手できる状況にある。肥満が国民的な問題となっている米国の例を挙げると，ダイエット用サプリメント（dietary supplement）は50種以上あり，さらにdietary supplementと他のサプリメントを組み合わせた複合健康食品は125種以上にも及ぶという[1]。ダイエット用健康食品の多くは，有効性のエビデンスが明らかでないままに消費者が安易に摂取しており，またそれによる健康被害も後を絶たない[2-4]。本稿では今までに報告されてきたダイエット用健康食品による健康被害の中で，社会的に大きな問題となった，エフェドラ，アマメシバ，シブトラミン含有製品等の摂取による有害事例を提示し，その発症機序について概説するとともに，ダイエット用健康食品を使用する上での注意点を喚起することとする。

エフェドラ（Ephedra sinica）

1）過去に起きた有害事例

2003年2月17日，エフェドラ含有の栄養補助食品を常用していた米大リーグ（ボリチモア オリオールズ）のSteve Bechler投

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手が，フロリダの春のトレーニングキャンプ中に突然死した。血液中に多量のエフェドラが検出され，検察官はエフェドラを含むサプリメントの摂取が突然死に影響したと断定した。Bechler投手を始め，米国では年間1,000万人以上の人々がエフェドラを使用していたが，2004年米国食品医薬品局（FDA, Food and Drug Administration）は，エフェドラによる心血管系の副作用での死亡例が続出したことを重く見て販売禁止に踏み切った。これは，米政府が栄養補助食品を販売禁止とした最初の事例となった。

2）メカニズム

エフェドラは，中国原産のハーブから作られる漢方薬「麻黄（ma-huang）」由来のエフェドリンが主成分である。エフェドリンは交感神経興奮作用を有し，代謝促進や覚醒の働きがある一方，動悸，高血圧等の心血管系および精神異常等の中枢神経系の副作用があり，さらに重症になると心筋梗塞や致死的不整脈を来し死亡した例も報告されている[5]。日本における事例では，28歳女性がエフェドラとカフェインを含むdietary supplement containing ephedra and caffeine（DSEC）を大量摂取し，重症の心室細動を起こした例が報告されており，その発症機序として，エフェドリンによる心血管系への直接作用に加え，カフェインの相乗作用と低カリウム血症の関与が考えられている[6]。ダイエット用健康食品は強壮剤やハーブティーとの複合健康食品として販売されることも多く，その場合には含有されるカフェインの過剰摂取や相互作用にも注意を払う必要がある。

[redacted]us androgynus）

1）過去に起きた有害事例

アマメシバの摂取による有害事例は，1996年にLancet誌上に報告された台湾での事例に遡る[7]。最初の事例は1994年8月に報告され，55歳の女性が40数日間アマメシバを摂取後，呼吸困難と不眠，食欲不振等を来した。その事例後，1995年6月から8月にかけて，台湾各地からアマメシバの摂取による中毒が疑われる事例が多数報告された。事例の多くは肥満の若い女性で，呼吸困難を主症状とし，既知の呼吸器疾患やアレルギー歴，喫煙・職業・環境等の影響は否定的であり，アマメシバの摂取による閉塞性細気管支炎が強く疑われた。

日本では，2003年4月にアマメシバの摂取によると思われる閉塞性細気管支炎の初発例が報告された[8]。事例は40歳の女性で，進行性の呼吸困難を訴え，低酸素血症と気管支拡張剤に全く反応しない閉塞性換気障害が認められた。その後の全国調査で報告された8事例の集計では，アマメシバ摂取期間は3～10カ月，主症状は進行性の呼吸困難と咳であり，低酸素血症と肺機能検査における著明な1秒量の低下および呼気時胸部CT検査での低吸収域のモザイクパターンが特徴的であった[9]。治療反応性は乏しく肺障害の多くは不可逆的であり，2年経過後の予後では，呼吸不全のため1例が死亡，1例が肺移植手術，さらに2名が在宅酸素療法を受けていることが示された。アマメシバ加工食品は，改正食品衛生法（第4条の2）に基づき販売禁止となった健康食品の第1号となった。

2）メカニズム

アマメシバによる閉塞性細気管支炎の発症機序は，原因化学物質も含め未だ明らかになっていない。アマメシバは，マレーシア等東南アジアで葉を加熱調理して食材とする習慣が古くからあるが，食する量は今回の事例よりはるかに少なく，また同様の健康被害の報告もない。そのことから今回

の有害事例が起きた背景には，アマメシバを生鮮未調理で，ジュースや粉末あるいは錠剤などに加工後，健康食品として継続的に大量に摂取したこととの関連が推定されている[7-9]。また，日本での事例2家系4例が親子であったことから，その発症に宿主側の免疫細胞応答の影響等が考えられている。

(sibutramine)含有製品等 -医薬品の添加-

1）過去に起きた有害事例

ダイエット用健康食品による健康被害には，無承認無許可医薬品の添加によるものが多く，それによる健康被害が後を経たないことが社会的に大きな問題となっている[3]。2002年7月に日本国内で，中国製ダイエット用健康食品を利用していた女性が重篤な肝障害で死に至るという事件が起きたが，その後，同様の肝障害の報告は国内で600例を超え，3名の死亡例を出すまでに至った。肝障害を来したダイエット用健康食品からは，食欲抑制剤であるN-ニトロソフェンフルラミン，フェンフルラミンおよび甲状腺末等の医薬品が含有されていた[10]。ダイエット用健康食品に含まれる医薬品は，その後も，緩下剤（センノシド），利尿薬（ヒドロクロロチアジド，フロセミド），中枢性食欲抑制薬（マジンドール）等，数多く検出され枚挙に暇がない状況である。

最近，ダイエット用健康食品に日本では認可されていない肥満抑制薬であるシブトラミンが使用されたことによる健康被害が増加しており，その中には死亡例も報告されている。シブトラミンを含むダイエット用健康食品としては，「御芝堂減肥こう嚢」，「天天素清脂こう嚢」，「終極瘦身（J-minus "瘦極身"）」，「Venus line 21」，「Slim」，「Jelimel sliming capsules（瘦身救兵）」，「Super Fat Burning」，「コリアンスリム」といった多くの商品があり，中国製および韓国製のものが多い。厚生労働省では，シブトラミンや，その活性代謝物である脱N-ジメチルシブトラミン，脱N-メチルシブトラミンを含むダイエット食品に対し著しい健康被害の恐れがあるとして，購入および摂取を中止するように警告を発している。

2）メカニズム

シブトラミンは，脳内神経伝達物質のセロトニンおよびノルアドレナリン等モノアミンの再取り込みを阻害することにより満腹感を亢進し，食欲抑制をもたらすとともに，エネルギー消費促進作用を併せ持っている。海外臨床試験では用量依存的かつプラセボと比較して有意な体重減少・維持効果が示され，1997年に肥満抑制薬として米国FDAが認可している。日本では治験中であるが，現在承認申請の段階に入っている。

シブトラミンの主な副作用は，不眠，頭痛，吐き気，口内乾燥，便秘等があり，また重篤な心血管系の副作用として血圧および心拍数の増加，さらに不整脈を来して死亡した例もある[11,12]。米国内で許可されてから2003年8月までに54例の死亡例が報告され，そのうち30例は心血管疾患が原因とされている。また相互作用として，うつ病あるいはパーキンソン病の治療薬であるMAO（monoamine oxidase）阻害薬や選択的セロトニン再取込み阻害薬（SSRI, selective serotonin reuptake inhibitor）の併用により副作用が増強する可能性があるので，併用時には注意が必要である。

以上，ダイエット用健康食品による健康被害の中で社会的に大問題となった，エフェドラ，アマメシバ，シブトラミン含有製品等の有害事例を概略した。ダイエットに

限らず健康食品により健康被害が起きる原因としては，健康食品の成分自体によるものと，違法な医薬品成分の添加によるもの等がある[3,4]。したがって医療従事者は，それぞれの発症機序を十分に考慮しながら健康食品による健康被害に対処する必要がある。また，エフェドラやアマメシバが販売禁止となったとはいえ，その成分がハーブティーの中に複合健康食品として混在していたり，インターネットオークション等を介して消費者が海外から個人輸入して使用してしまう可能性は依然として存在するため，その点にも注意を払う必要がある。ダイエット用健康食品による健康被害を未然に防ぐためには，“痩せたいために”という理由で消費者が安易に健康食品を使用しないように働き掛けることが非常に重要である。肥満抑制の基本は，健康的なバランスの取れた食事と適度の運動，そして暴飲暴食等の不健康な生活行動を是正することであり，決してダイエット用健康食品で安易に達成できるものではない。そのことを医療従事者は，消費者に強調して，し過ぎることはない。

プロフィール

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現在，健康食品や医薬品の有効性や安全性の評価に関する研究に従事。専門は，臨床薬理学，神経内科学，内科学。

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Enhancement by Grapefruit Juice of Morphine Antinociception

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The aim of this study was to investigate the effect of grapefruit juice intake on the antinociception of morphine in rats. The antinociception of morphine (30 mg/kg, *per os* (*p.o.*)) was significantly enhanced by the oral administration of grapefruit juice (2 ml/rat). Further, the effect of grapefruit juice was examined in morphine-tolerant rats. The repeated administration of morphine (100 mg/kg *p.o.*) for 5 d caused a marked decrease in the antinociception, indicating the development of morphine-tolerance. In the morphine-tolerant rats, oral administration of grapefruit juice potentiated significantly the antinociceptive effect of morphine. To examine the pharmacokinetics of morphine after the repeated treatment with morphine for 5 d, microdialysis probes were implanted into the jugular vein and spinal intrathecal space in rats. The morphine concentrations in the blood and intrathecal cerebrospinal fluid (CSF) were gradually decreased by the repeated treatment with morphine. The grapefruit juice treatment significantly increased the blood concentration of morphine in morphine-tolerant rats. These results suggest that oral administration of grapefruit juice enhances the morphine antinociception by increasing the intestinal absorption of this agent.

Key words morphine; grapefruit juice; tolerance; microdialysis

Drug–food interactions are increasingly recognized as noteworthy clinical events that should be considered in order to avoid adverse effects. Indeed, the intake of grapefruit juice has been demonstrated to elevate serum concentrations of several drugs including calcium channel blockers such as felodipine, nifedipine and nisoldipine, verapamil, cyclosporine, tacrolimus and midazolam.[2–6] The main mechanism for the interaction with grapefruit juice is considered to be the inhibition of cytochrome P450 3A4 (CYP3A4), the major drug metabolism enzyme in the intestine. Recent investigations have shown that grapefruit juice inhibits not only CYP3A4 but also drug transporters like P-glycoprotein,[7–9] which plays important roles in the intestinal barrier function in a coordinated manner with CYP3A4.[10] The inhibitory effect of grapefruit juice on the intestinal barrier function may enhance the oral bioavailability of drugs, which has been associated with a higher incidence of side effects. From the point of view of beneficial use of dietary constituents, the enhancement of bioavailability can potentiate the therapeutic effect of drugs. Until now, few studies have focused on the beneficial use of interaction between dietary constituents and drugs.

Morphine is the most commonly used opioid analgesic for the treatment of cancer pain. Morphine is a substrate of P-glycoprotein,[11] and its antinociceptive effect is enhanced by knockout of the P-glycoprotein gene in mice and the administration of P-glycoprotein inhibitor in rats.[12–14] In humans, it has been reported that the absorption of morphine is regulated by intestinal P-glycoprotein.[15] Further, P-glycoprotein may be partially associated with morphine tolerance.[16] The tolerance limits the clinical use of morphine. We speculated that the intake of grapefruit juice inhibits the intestinal P-glycoprotein-mediated efflux of morphine and subsequently enhances the antinociceptive effect after an oral administration by increasing the drug's bioavailability.

In this study, we examined the effects of grapefruit juice intake on oral morphine antinociception in morphine-naïve and morphine-tolerant rats. The antinociceptive effect and concentrations of morphine in the blood and intrathecal cerebrospinal fluid (CSF) were monitored in rats treated repeatedly with morphine.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 250 g were housed three to four per cage with free access to food and water and maintained on a 12-h light/dark cycle in a room with controlled temperature (24±1 °C) and humidity (55±5%) throughout a whole experimental period. This study was conducted according to guidelines approved by the Experimental Animal Ethical Committee of University of Shizuoka. Morphine hydrochloride was purchased from Takeda Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were purchased from commercial sources.

Grapefruit juice (Sunkist®) was orally administered at a volume of 2 ml per rat 30 min prior to the drug administration in accordance with previous report.[17] In the single administration experiment, rats received 2 ml of water, grapefruit juice (Sunkist®) or quinidine (30 mg/kg *per os* (*p.o.*)), a P-glycoprotein inhibitor,[18] and 30 min later, morphine (30 mg/kg). The tail-flick latency test was used to quantify antinociception, with a thermal stimulus being applied to the tail.[19] Before the drug administration, baseline antinociceptive testing was performed. The antinociceptive testing was performed at 60, 120 and 180 min after the morphine treatment. A maximum tail-flick latency of 10 s was used to minimize the tissue damage to the tail. The tail-flick latency values were converted to a percentage of the maximum possible effect (%MPE): %MPE=(postdrug latency−predrug latency)/(maximum latency−predrug latency)×100.

In the repeated administration experiment, development of morphine tolerance was measured according to a method[20] described previously with a slight modification. Briefly, rats received morphine (100 mg/kg) orally once a day for 5 d, and

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antinociception was determined by tail-flick test as described above once a day (120 min after morphine administration) to avoid tissue damage by repeated measurements. On the day (day 6) after the last treatment, 2 ml of grapefruit juice (Sunkist®) was orally administered to rats 30 min before another administration of morphine (100 mg/kg *p.o.*), and the tail-flick latency test was performed as described above.

The concentration of morphine in the blood and spinal CSF was determined by the microdialysis method. The spinal intrathecal dialysis probe was constructed from Cuprophan hollow fibers (inside diameter (i.d.), 0.2 mm; MW cut-off, 12500; RENAK-E, RE-10M, Kawasumi Chemical Industries Ltd., Tokyo, Japan).[21,22] The fibers were coated with epoxy glue, except for a 4-cm region in the middle. A Nichrome wire (outside diameter (o.d.), 0.1 mm; Unique Medical Co., Ltd., Tokyo, Japan) was then passed through the fiber and both ends of the fiber were attached to pieces of polyethylene tube (PE-10; Natsume Seisakusho Co., Ltd., Tokyo, Japan). Rats were anesthetized with ketamine (188 mg/kg intramuscular injection (i.m.)) and their heads were placed in a stereotaxic apparatus (SR-6, Narishige Scientific Instrument Lab., Tokyo, Japan). The probe was inserted through an incision in the cisternal membrane and slowly passed caudally 9 cm into the intrathecal space to leave the uncoated section of the catheter at the Th11–L2 spinal segments. The two PE-10 ends of the dialysis probe were externalized on the top of the head. Rats were allowed to recover from the surgery for 3 d. They were then anesthetized with ether and a dialysis probe for vessels (TP-100-10, Eicom Corp., Tokyo, Japan) was implanted into the jugular vein. The dialysis probes implanted into the jugular vein and spinal intrathecal space were perfused at a constant rate of 5 μl/min with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.4 mM $CaCl_2$, pH 7.3) containing antipyrine as a reference of probe recovery.[23] After the oral administration of morphine (100 mg/kg) in rats, collection of the dialysate was started. The blood and spinal CSF dialysate samples were collected every 60 min for 300 min, and each sample was kept at −20 °C until the analysis. The dialysate concentration of morphine was measured by HPLC with fluorimetric detection.[24] The HPLC system consisted of a pump (880-PU, Japan Spectroscopic Co. (Jasco), Tokyo, Japan), a fluorescence detector (RF-535, Shimadzu, Tokyo, Japan) and an integrator (C-R6A, Shimadzu, Tokyo, Japan). The analytical column was composed of Nucleosil C18 ODS (4.6 mm×250 mm, 5 μm particle size, GL Sciences). Gradient elution was carried out at room temperature at a constant flow rate of 1.0 ml/min. Solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in 40% acetonitrile. The initial concentration of acetonitrile was 6.4%. After the injection of sample, the system was pumped isocratically for 2 min, followed by a gradient from 6.4 to 20% acetonitrile over 10 min, and then a gradient from 20 to 40% acetonitrile over 2 min to wash the column. The column elute was monitored fluorometrically at excitation and emission wavelengths of 280 and 335 nm, respectively. The concentration of morphine in blood (C_{blood}) or spinal CSF (C_{CSF}) was estimated from the dialysate concentration (C_d) using antipyrine as a reference.[23]

$$C_{blood} \text{ or } C_{CSF} = C_d/\{1-\exp(-R_{d,ref}PA_{vitro}/F)\}$$

F is the dialysate flow rate and PA_{vitro} is the *in vitro* permeability rate constant, which can be estimated from the *in vitro* recovery of the microdialysis probe.[23] $R_{d,ref}$ is the effective dialysis coefficient of the reference compound, antipyrine, which is the ratio of the *in vivo* and *in vitro* probe recovery.

The statistical analysis of the data was performed with Student's *t*-test for single comparisonss. Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

In the single administration experiment, the morphine caused increases in latency in the tail-flick test in rats. The oral administration of grapefruit juice (2 ml/rat *p.o.* 30 min before the morphine administration) significantly increased the antinociception at 60 min after the morphine was administered (Fig. 1). The area under the effect–time curve (*AUE*) in grapefruit juice-treated rats was 1.5-times greater than that in control rats. The oral administration of quinidine, a P-glycoprotein inhibitor, increased markedly antinociception of morphine as shown by 2.8-fold greater *AUE* compared that in control rats (Fig. 2). On the other hand, the administration of grapefruit juice without morphine did not cause antinociceptive effects (data not shown). These results suggest that grapefruit juice enhances the antinociceptive effect of morphine in rats, though the antinociception increase by grapefruit juice was smaller than that by quinidine.

In the repeated administration experiment, the rats received morphine (100 mg/kg) orally once a day for 5 d. An antinociception was measured by the tail-flick test 120 min after receiving morphine. The antinociception was 100%

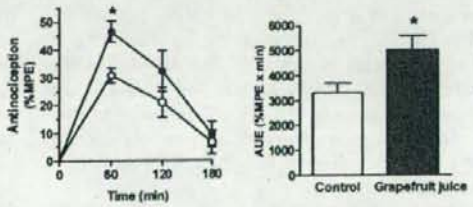

Fig. 1. Effects of Grapefruit Juice Administration on Antinociception of Morphine in Rats

Rats received water (control) (○) or grapefruit juice (2 ml/rat) (●) 30 min before morphine administration (30 mg/kg *p.o.*). The tail-flick test was conducted 60, 120 and 180 min after the morphine administration. The area under the effect–time curve (*AUE*) for antinociception of morphine was calculated by a trapezoidal role. Each point and column represents the mean±S.E. for five rats. * $p<0.05$ *vs.* control.

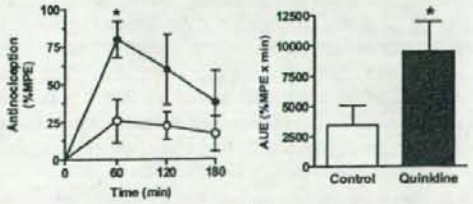

Fig. 2. Effects of Quinidine Administration on Antinociception of Morphine in Rats

Rats received water (control) (○) or quinidine (30 mg/kg *p.o.*) (●) 30 min before morphine administration (30 mg/kg *p.o.*). The tail-flick test was conducted 60, 120 and 180 min after the morphine administration. The area under the effect–time curve (*AUE*) for antinociception of morphine was calculated by a trapezoidal role. Each point and column represents the mean±S.E. for four rats. * $p<0.05$ *vs.* control.

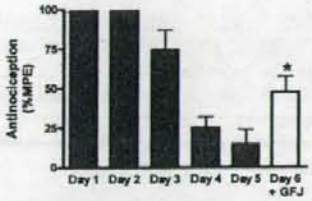

Fig. 3. Effects of Repeated Oral Administration of Morphine on the Antinociceptive Action of This Agent in Rats

Morphine (100 mg/kg) was given orally once a day for 1—5 d. After 120 min, rats were subjected to the tail-flick test. On the day (day 6) after the 5-d-treatment with morphine, rats received grapefruit juice (GFJ) (2 ml/rat) 30 min before receiving morphine. The data are presented as % MPE. Each column represents the mean±S.E. for four rats. * $p<0.05$ *vs.* day 5.

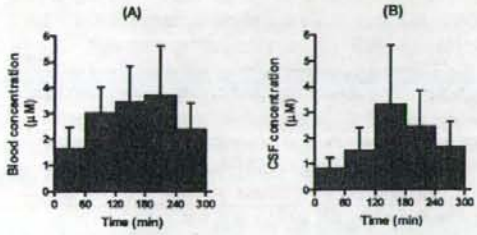

Fig. 4. The Concentration of Morphine in the Blood (AUC_{blood}) (A) and Spinal CSF (AUC_{CSF}) (B) of Morphine after Oral Administration on Day 1 in Rats

Microdialysis probes were implanted into the jugular vein and intrathecal space. Rats received orally morphine (100 mg/kg). After the morphine administration, dialysate samples were collected every 60 min for 300 min, and the concentrations were measured. Each column represents the mean±S.E. for four rats.

MPE on day 1 and 2, and 75, 25 and 14% MPE, respectively, on day 3, 4 and 5 (Fig. 3). Tolerance developed with the repeated oral administration of morphine. The day (day 6) after the 5-d-treatment with morphine, rats were administered grapefruit juice (2 ml/rat *p.o.*) 30 min before receiving morphine. The pretreatment with grapefruit juice significantly enhanced the antinociception of morphine from 14% MPE (day 5) to 48% MPE.

To determine the pharmacokinetics of morphine during the development of morphine-tolerance, a microdialysis method was applied to the jugular vein and spinal intrathecal space. The concentrations of morphine in blood and spinal CSF on day 1 increased with time and reached maximum levels at 180—240 min in blood and 120—180 min in spinal CSF, respectively (Fig. 4). The concentrations of morphine in blood and spinal CSF gradually decreased during the oral treatment (Fig. 5). The day (day 6) after the 5-d-treatment with morphine, rats were administered grapefruit juice (2 ml/rat *p.o.*) 30 min before receiving morphine. The AUC_{blood} was significantly (1.9 times) greater on day 6 than day 5 (Fig. 5A). The AUC_{CSF} was increased 1.3 times by the grapefruit juice, but not significantly (Fig. 5B). The concentration ratio of AUC_{CSF} to AUC_{blood} (AUC_{CSF}/AUC_{blood}) was 0.62, 0.45, 0.67 and 0.50 on day 1, 3, 5 and 6, respectively. The increases in the plasma concentration of morphine caused by grapefruit juice may contribute at least partly to the enhancement of morphine antinociception.

Grapefruit juice and its constituents are considered to affect the functions of drug transporters such as P-glycoprotein,[7—9] multidrug resistance protein 2 (MRP2)[25] and organic anion transporting polypeptide (OATP),[26] in addition to the drug metabolism enzyme CYP3A4. Of these enzymes and transporters, P-glycoprotein can affect morphine's disposition, because morphine is a substrate for P-glycoprotein, but not CYP3A4 or MRP2.[11—14] It was reported that the inhibition of intestinal P-glycoprotein by oral administration of quinidine enhances the absorption and pharmacological effect of morphine in humans.[15] Oral administration of quinidine also elevated the antinociception of orally-administered morphine in rats. Grapefruit juice extracts cause a four-fold increase in the transport of [^{3}H]vinblastine, a P-glycoprotein substrate, from apical to basolateral sides across human intestinal Caco-2 cells.[8] De Castro *et al.*[9] have reported that 6′,7′-epoxybergamottin, 6′,7′-dihydroxybergamottin, naringin and naringenin in grapefruit juice inhibit the P-glycoprotein-mediated transport of talinolol in human intestinal Caco-2 cells with IC_{50} values of 0.7, 34, 236 and 2409 μM, respectively. They have suggested that these furanocoumarins and flavonoids are able to inhibit intestinal P-glycoprotein-mediated transport because they are present in grapefruit juice in the same concentration ranges.[27,28] Taken together, oral administration of grapefruit juice may potentiate the antinociceptive effect of morphine by increasing the intestinal absorption possibly *via* the inhibition of intestinal P-glycoprotein.

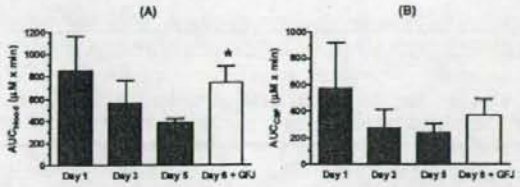

Fig. 5. Area under the Concentration Curve of Morphine in the Blood (AUC_{blood}) (A) and Spinal CSF (AUC_{CSF}) (B) of Morphine after Oral Administration for 1—5 d in Rats

Microdialysis probes were implanted into the jugular vein and intrathecal space. Rats received orally morphine (100 mg/kg) once a day. On the day (day 6) after the 5-d-treatment with morphine, rats received grapefruit juice (GFJ) (2 ml/rat) 30 min before receiving morphine. The concentrations of morphine in the dialysates were measured on day 1, 3, 5 and 6. Each column represents the mean±S.E. for three to four rats. * $p<0.05$ *vs.* day 5.

P-glycoprotein at the blood–brain barrier modulates the antinociceptive effect of morphine by regulating its transport from the blood into the central nervous system.[12—14] However, the spinal CSF to blood concentration ratio of morphine was not changed by grapefruit juice, suggesting little or insignificant inhibition of P-glycoprotein at the brain barrier. Although flavonoids and furanocoumarins are partially absorbed from the intestine, flavonoids such as naringin are most likely hydrolyzed by intestinal enzymes[29,30] and bergamottin has very low permeability through CYP3A4-expressing Caco-2 cell monolayers.[31] In addition, bergamottin and dihydroxybergamottin strongly bind to human serum albumin. These dispositional properties may explain in part why grapefruit juice inhibits intestinal CYP3A4 rather than hepatic CYP3A4 *in vivo*.[6] The unbound concentrations of these flavonoids and furanocoumarins in blood may be too low to inhibit P-glycoprotein at the luminal membrane of the blood–brain barrier.

Aquilante *et al.*[16] have reported that repeated morphine

administration causes a two-fold increase in the P-glycoprotein level in rat brain associated with the decrease in the antinociceptive effect. In morphine-tolerant rats, intestinal P-glycoprotein-mediated transport may be stimulated and therefore more susceptible to the inhibition of intestinal P-glycoprotein. Thus, inhibitors of intestinal P-glycoprotein such as grapefruit juice may partly overcome morphine-tolerance, though little clinical evidence has been presently reported on enhancement of effects of morphine by grapefruit juice. In fact, it may be difficult to control the intestinal P-glycoprotein activity using grapefruit juice, because the amounts of flavonoids and furanocoumarins may differ with area, season, and production process.[27,28] In addition, it has been suggested that grapefruit juice–drug interaction is caused by additive or synergistic effects of several flavonoids and furanocoumarins in the juice.[32] Thus, further quantitative analysis will be required to clarify the mechanism underlying enhancement of the antinociception of morphine by grapefruit juice.

In conclusion, grapefruit juice is suggested to potentiate the antinociception of morphine associated with an increase in intestinal absorption. This enhancement may partially overcome morphine-tolerance.

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Antinociceptive Effects of St. John's Wort, *Harpagophytum Procumbens* Extract and Grape Seed Proanthocyanidins Extract in Mice

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Hypericum perforatum **extract (St. John's wort, SJW),** ***Harpagophytum procumbens*** **extract (HPE) and Grape seed proanthocyanidin extract (GSPE) have a broad spectrum of biological activities including antidepressant, anti-inflammatory or anti-oxidant effects. The aim of this study was to clarify antinociceptive properties of SJW, HPE and GSPE in mice with mechanisms that might potentially underlie these activities. Also, the effects of these herbal extracts on the antinociception and plasma and brain concentrations of morphine were examined. Oral pretreatment with SJW (100—1000 mg/kg) and HPE (30—300 mg/kg) attenuated significantly times of licking/biting both first and second phases of formalin injection in mice in the dose-dependent manner, and GSPE (10—300 mg/kg) suppressed second phase. Naloxone (5 mg/kg, s.c.) significantly attenuated antinociceptive effect of HPE but not SJW and GSPE. Formalin injection resulted in significant increase in the content of nitrites/nitrates (NO_x) in mouse spinal cord. The rise of spinal NO_x content by formalin was significantly attenuated by HPE and SJW. The pretreatment with SJW significantly potentiated an antinociceptive effect of morphine (0.3 mg/kg, s.c.), although concentrations of morphine in plasma and brain were not significantly changed by these herbal extracts. In conclusion, the present study has shown that SJW, HPE and GSPE exert significant antinociceptive effects in the formalin test of mice. In addition, opioidergic system seems to be involved in the antinociceptive effect of HPE but not SJW and GSPE. Furthermore, SJW potentiates morphine-induced antinociception possibly by pharmacodynamic interaction.**

Key words antinociceptive effect; St. John's wort; *Harpagophytum procumbens* extract; Grape seed proanthocyanidin extract; morphine; interaction

Currently, the consumption of dietary supplement containing botanical products and foods is growing at a remarkable speed, in terms of the promotion of health or prevention and treatment of diseases. The extract from *Hypericum perforatum* (St. John's wort, SJW) possess clinical efficacy in the therapy of mild to moderate depression.[1,2] The most important constituents of SJW are phloroglucinols such as hyperforin and pseudohyperforin, naphthodianthrones such as hypericin and pseudohypericin, in addition to flavonoids such as rutin, quercetin, quercitrin. Several *in vitro* studies have indicated that SJW and hyperforin may act *via* a blockade of reuptake of serotonin, noradrenaline and dopamine in similar manner as most of the current antidepressants such as tricyclic antidepressants,[3—5] which have been known to exhibit antinociceptive properties by monoamine reuptake blockade.

Harpagophytum procumbens extract (HPE) and Grape seed proanthocyanidins extract (GSPE) have been reported to exert anti-inflammatory activity in rodents.[6—8] *Harpagophytum procumbens* commonly known as Devil's claw is an herbaceous plant, growing specifically in Southern Africa. Preparations of its secondary roots contain iridoid glycosides, mainly harpagoside, harpagogide and procumbide. HPE have been shown to possess clinical efficacy in the treatment of degenerative rheumatoid arthritis, osteoarthritis and tendonitis.[6—8] In addition, experimental study has revealed anti-inflammatory activity of HPE in Freund's adjuvant-induced arthritis model.[9] Proanthocyanidins are naturally occurring polyphenolic compounds widely available in fruits, vegetables, nuts, seeds, flowers and bark. Grape seed proanthocyanidins, a combination of biologically active polyphenolic flavonoids including oligomeric proanthocyanidins, have been shown to exert a novel spectrum of biological, pharmacological, therapeutic and chemoprotective properties against oxygen free radicals and oxidative stress. GSPE protects against free radicals models and has exhibited superior antioxidant performance as compared to vitamin C, E and β-carotene.[10]

Previous studies with antidepressant activity of SJW and anti-inflammatory effects of HPE and GSPE have led to the idea that these herbal products exert antinociceptive action. Thus, the aim of this study was to clarify the antinociceptive properties of SJW, HPE and GSPE after oral administration to mice with mechanisms that might potentially underlie these activities. The effects of these herbal extracts on the antinociception and plasma and brain concentrations of morphine were also examined.

MATERIALS AND METHODS

Drugs SJW and HPE were kindly donated by Indena (Milan, Italy). SJW was standardized to the content of hypericin (0.3%) and hyperforin (3.2%) and HPE was also standardized to the content of harpagoside (1.9%). GSPE was kindly supplied by Kikkoman Co. (Chiba, Japan), and standardized to the content of proanthocyanidin (83.9%). Morphine hydrochloride was purchased from Takeda Pharmaceutical Co. (Osaka, Japan). All other drugs and materials were obtained from commercial source. Morphine, naloxone and formalin were dissolved in 0.9% NaCl. SJW was suspended

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in distilled water and sonicated for 10 min before oral administration. HPE and GSPE were dissolved in distilled water.

Animals Male ICR mice (Japan SLC Inc., Shizuoka, Japan) weighting 20—30 g were used. Animals were housed under a 12-h light/dark cycle in a room with controlled temperature (24±1 °C) and humidity (55±5%). They were allowed free access to food and water prior to the experiments. All animal procedures were in strict accordance with the guideline approved by the Experimental Animal Ethical Committee of University of Shizuoka.

Formalin Test In the formalin test, mice were adapted in open Plexiglas observation cambers at 1 h before injection of formalin. Formalin (20 μl of a 2.5% solution in saline) was injected subcutaneously into the dorsal surface of right hind paw of mice using a Hamilton microsyringe with a 30-gauge needle, as previously described.[11] Each mouse was immediately returned to the observation chamber after formalin injection. A mirror was placed behind the chamber to allow the unhindered observation of formalin-injection paw. The time spent for licking or biting of injected paw (nociceptive response) was measured with stopwatch at 5 min intervals until 40 min post formalin injection and considered as a quantitative indication of nociception. The sum of time of licking/biting from 0 to 5 min was considered as the first phase, whereas the second phase was taken as the sum of time for licking/biting from 10 to 30 min. SJW (100—1000 mg/kg), HPE (30—300 mg/kg), GSPE (10—300 mg/kg) or vehicle (control group) were orally administrated to different groups of mice 60 min before formalin injection. Naloxone (5 mg/kg), yohimbine (3 mg/kg) and methysergide (3 mg/kg) were subcutaneously administered just before oral administration of these herbal extracts and the formalin test was performed 60 min after the administration of herbal extracts.

To examine effects of these herbal extracts on the antinociception of morphine, animals received SJW (300 mg/kg), HPE (30 mg/kg), GSPE (30 mg/kg) or vehicle at 45 min before the treatment with morphine (0.3 mg/kg, s.c.). Formalin test was performed at 15 min after morphine administration.

Tail-Flick Test Tail flick latency was measured using the automated tail flick analgesia meter (MK-330B, Muromachi Kikai, Tokyo, Japan), as previously described[12] with minor modification. A noxious beam of light was focused on the tail about 4 cm from the tip, and the tail flick latency was recorded automatically to the nearest 0.1 s. The intensity of the radiant heat source was adjusted to yield baseline latencies between 2 and 4 s. Each mouse was given one test to determined baseline latency to tail-flick with a cutoff of 10 s set to avoid tissue damage. Animals were administrated SJW (1000 mg/kg), HPE (1000 mg/kg), GSPE (1000 mg/kg) or zvehicle, and tail flick latencies were determined at 30, 60, 90, 120, 150 and 180 min after administration.

Measurement of Locomotor Activity The locomotor activity of mice was assessed in the open-field test as described previously.[13] Open field was a 30×30 cm acrylic area with 30 cm high black walls surrounding the field. Thin black stripes were painted across the floor dividing the field into 9 squares of equal area and the number of squares crossed with all paws crossing was counted in a 5-min session.

Determination for Contents of Nitrites/Nitrates (NO_x) in Brain and Spinal Cord SJW (1000 mg/kg), HPE (300 mg/kg), GSPE (300 mg/kg) or vehicle was administrated to mice orally, and thereafter, brain and spinal cord were dissected 80 min after the administration. Other groups of animals received herbal extracts at 60 min before formalin injection in the same way as formalin test, and brain and spinal cord were removed 20 min after formalin injection. The content of NO_x in mouse brain and spinal cord were determined as previously described with minor modification.[14] Brain and spinal cord of mice were homogenized in 3 volume distilled water and centrifuged at 15000 ***g*** for 20 min at 4 °C. Fifty microliters of supernatant of tissue homogenate was mixed with 20 μl of 0.31 M potassium phosphate buffer (pH 7.5), 10 μl of 0.86 mM β-nicotinamide adenine dinucleotide phosphate (β-NADPH), 10 μl of 0.11 mM flavin adenine dinucleotide (FAD) and 20 mU of nitrate reductase. Samples were incubated for 60 min at room temperature in the dark. Then, 5 μl of 1 M $ZnSO_4$ was added to the sample and centrifuged at 15000 ***g*** for 10 min 4 °C. One hundred microliters of Griess reagent (1 : 1 mixture of 1% sulphanilamide in 5% H_3PO_4 and 0.1% *N*-(1-naphytyl)ethylendiamine) was added to 80 μl of supernatant and the mixture incubated for 10 min at room temperature. Absorbance was measured at 550 nm by a micro plate reader (Perkin-Elmer Life Sciences) and converted to NO_x content by using a nitrate standard curve.

Measurement for Morphine Concentration in Plasma and Brain SJW (300 mg/kg), HPE (30 mg/kg), GSPE (30 mg/kg) or vehicle was orally administered to mice 45 min before the treatment with morphine (0.3 mg/kg, s.c.). Mice were sacrificed 30 min after morphine administration, and blood and brain were collected. Plasma was separated by centrifugation. Morphine concentrations in plasma and brain were determined by HPLC with electrochemical detector as previously described.[15] Plasma sample (200 μl) was mixed with 50 μl of 2 μM naloxone (internal standard) and 800 μl of 0.5 M ammonium sulfate (pH 9.3). Brain sample was homogenized in 4 volumes of saline. The homogenate (1 ml) was mixed with 50 μl of 2 μM naloxone and 100 μl of 1 M perchloric acid and centrifuged at 2000 ***g*** for 10 min at 4 °C. The supernatant was transferred to another tube containing 2 ml of 0.5 M ammonium sulfate (pH 9.3). The mixture from plasma or brain sample was then applied to the Oasis HLB cartridge (Waters, Milford, MA, U.S.A.), which was pretreated with 1 ml methanol and 1 ml distilled water. Morphine was eluted with 1 ml methanol after the cartridge was washed with 4 ml of 15% methanol in 5 mM ammonium sulfate (pH 9.3). The elute was evaporated under a stream of nitrogen at 40 °C. The residue was dissolved in 200 μl of the mobile phase and 50 μl of this solution was injected into HPLC system. The HPLC analysis was constructed with a pump (LC-20AD, Shimadzu, Kyoto, Japan), an electrochemical-detector (Coulochem III, ESA Inc., Chelmsford, MA, U.S.A.) and a injector (SIL-20AC, Shimadzu, Kyoto, Japan). The separation was performed on an analytical column (CAPCELLPAK SCX UG80, 5 μm, 100×3 mm, Shiseido, Tokyo, Japan). The mobile phase consisted of 67% acetonitrile and 33% 20 mM potassium dihydrogen phosphate (pH 2.1) at a flow rate of 0.5 ml/min. The HPLC column was maintained at 40 °C and the electrochemical detector was set to +250 mV for detector 1, +600 mV for detector 2 and 800 mV for the guard cell.

Statistical Analysis All values are expressed mean± S.E. Date were analyzed by Student's *t*-test or one-way analysis of variance followed by Dunnett's *post hoc* test. For all comparisons, differences were considered statistically significant at $p<0.05$.

RESULTS

Effects on Nociceptive Responses in the Formalin and Tai-Flick Test The s.c. injection of 2.5% formalin into the right hind paw of mice induced a biphasic licking/biting nociceptive response. SJW at doses of 500 and 1000 mg/kg reduced significantly the licking/biting time both first phase (20.0 and 24.9%, respectively) and second phase (37.2 and 56.5%, respectively) in the dose-dependent manner (Figs. 1a, b). Similarly, HPE at doses of 100 and 300 mg/kg also reduced significantly the licking/biting time in both first phase (18.1 and 27.1%, respectively) and second phase (42.5 and 59.0%, respectively) (Figs. 1c, d). GSPE at doses of 30, 100 and 300 mg/kg reduced significantly (31.7, 38.3 and 48.1%, respectively) the licking/biting time in the second phase but not in the first phase (Figs. 1e, f).

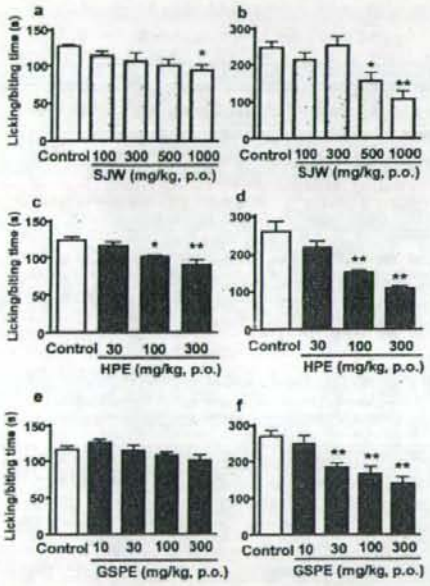

Fig. 1. Effects of SJW, HPE and GSPE on Licking and Biting Responses in First (a, c, e) and Second Phases (b, d, f) of Formalin Test in Mice

Mice received orally SJW (100, 300, 500, 1000 mg/kg) (a, b), HPE (30, 100, 300 mg/kg) (c, d) and GSPE (10, 30, 100, 300 mg/kg) (e, f) 60 min before the formalin injection to mouse paw. The licking/biting time (s) was measured. Each value represents mean±S.E. ($n=6$). Asterisks show a significant difference from control mice, $*p<0.05$, $**p<0.01$.

The effects of naloxone, yohimbine and methysergide on antinociception of SJW, HPE or GSPE in the formalin test were examined. Naloxone (5 mg/kg, s.c.) attenuated significantly (65.9%) antinociceptive effect in the second phase of formalin test by HPE (300 mg/kg, *p.o.*), but not by SJW (1000 mg/kg, *p.o.*) and GSPE (300 mg/kg, *p.o.*) (Fig. 2). None of these agents-induced antinociceptive responses in the first phase was attenuated by naloxone. In addition, naloxone at this dose effectively reversed morphine (0.3 mg/kg)-induced antinociceptive response both first and second phase. Yohimbine (3 mg/kg, s.c.) and methysergide (3 mg/kg, s.c.) did not significantly influence the antinociceptive effect of SJW (1000 mg/kg) both first and second phase (data not shown).

In the tail-flick test, there were little significant differences of tail-flick latencies between vehicle-treated group and each group treated with SJW (1000 mg/kg), HPE (1000 mg/kg) or GSPE (1000 mg/kg) (Fig. 3). Morphine increased significantly the latency at 30 and 60 min after administration.

Effects on the Locomotor Activity in Mice In open-field test, the numbers (counts/5 min) of crossing in mice 60 min after the pretreatment with vehicle, SJW (1000 mg/kg, *p.o.*), HPE (300 mg/kg, *p.o.*) and GSPE (300 mg/kg, *p.o.*) were 106±7, 118±8, 110±7 and 114±8, respectively. Thus, these herbal extracts had little significant effect on the locomotor activity.

Effects on the Contents of NO_x in Brain and Spinal Cord The formalin injection induced a significant (1.8 fold) incease of NO_x contents in mouse spinal cord but not in the brain. The formalin-induced increase of NO_x content in the spinal cord was significantly reversed by the pretreatment with SJW or HPE but not GSPE (Table 1).

Effects on the Antinociceptive Effect and Concentration in Plasma and Brain of Morphine Morphine at the dose of 0.3 mg/kg significantly reduced the licking/biting time in the first phase (24.8%) and the second phase (36.1%) of formalin test in mice. The antinociceptive effect (reduc-

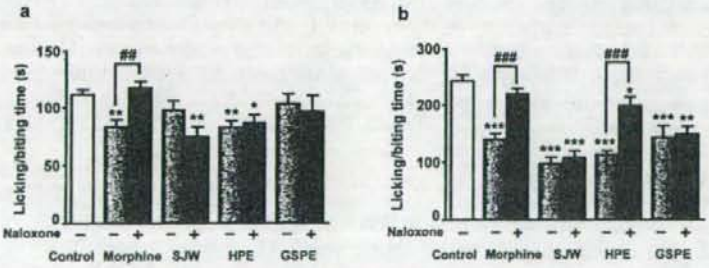

Fig. 2. Effect of Naloxone on Antinociception of SJW, HPE, GSPE and Morphine in First (a) and Second (b) Phases of Formalin Test in Mice

Mice received naloxone (5 mg/kg, s.c.) before oral administration of herbal extracts (SJW: 1000 mg/kg, HPE: 300 mg/kg, GSPE: 300 mg/kg) and then the formalin test was performed 60 min after the administration of herbal extracts. Naloxone was treated 45 min before the administration of morphine (0.3 mg/kg, s.c.), and the formalin test was performed 15 min after the administration of morphine. Each value represents mean±S.E. ($n=6$). Symbols show a significant difference from control mice (*,**,***) or from the corresponding mice without naloxone (#,##), $*p<0.05$, $**p<0.01$, $***p<0.001$, $\#p<0.01$, $\#\#p<0.001$.

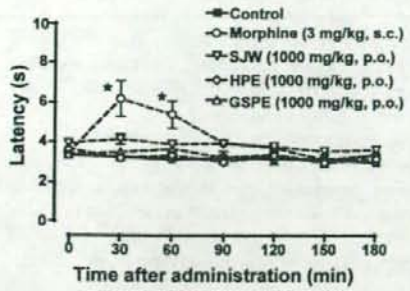

Fig. 3. Effects of SJW, HPE, GSPE and Morphine on Nociceptive Responses in Tail-Flick Test of Mice

Tail-flick latency was measured every 30 min after oral (s.c. for morphine) administration of each agent (SJW, 1000 mg/kg; HPE, 1000 mg/kg; GSPE, 1000 mg/kg; morphine, 3 mg/kg). Each value represents mean±S.E. (n=7—8). Asterisks show a significant difference from control mice at the same time, * p<0.05.

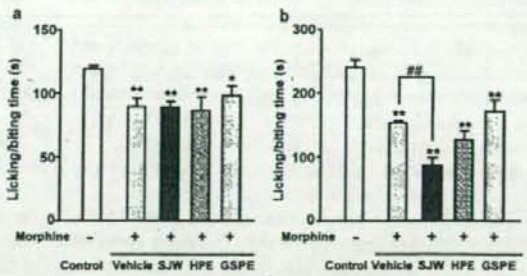

Fig. 4. Effects of SJW, HPE and GSPE on Antinociception of Morphinein First (a) and Second (b) Phases of Formalin Test in Mice

Mice received orally herbal extracts (SJW, 300 mg/kg; HPE, 30 mg/kg; GSPE, 30 mg/kg) 45 min before morphine administration (0.3 mg/kg, s.c.). The formalin test was performed at 15 min after morphine administration. Each value represents mean±S.E. (n=6—7). Symbols show a significant difference from control mice (*,**) or from mice treated with morphine (#), * p<0.05, ** p<0.01, # p<0.01.

Table 1. Effects of Oral Administration of SJW, HPE and GSPE on Contents of NO_x in Mouse Brain and Spinal Cord

Treatment	Contents of NO_x	
	Brain (nmol/g)	Spinal cord (nmol/g)
Without formalin		
Vehicle	122.6±15.4	89.2±9.7
SJW	89.3±3.3	91.7±5.0
HPE	87.3±4.2	87.4±4.3
GSPE	113.2±20.4	120.0±7.5
With formalin		
Vehicle	130.9±7.3	161.7±11.5**
SJW	112.4±5.1	119.8±13.6#
HPE	121.1±7.8	116.0±9.9#
GSPE	106.6±4.7	152.1±8.3**

Formalin (2.5%, 20 μl) was injected 60 min after oral administration of SJW (1000 mg/kg), HPE (300 mg/kg) and GSPE (300 mg/kg). Brain and spinal cord were removed 20 min after formalin injection. Each value represents mean±S.E. (n=6—8). Symbols show a significant difference from vehicle group without formalin (**) or from vehicle group with formalin (#), ** p<0.01, # p<0.05.

Table 2. Effects of Oral Administration of SJW, HPE and GSPE on Plasma and Brain Concentration of Morphine in Mice

Treatment	Concentration of morphine	
	Plasma (ng/ml)	Brain (ng/g)
Vehicle	29.4±2.9	13.0±1.2
SJW	31.1±3.8	10.5±1.1
HPE	33.3±7.0	15.3±0.8
GSPE	33.8±5.0	13.5±1.9

Morphine (0.3 mg/kg, s.c.) was administrated to mice 45 min after oral administration of SJW (300 mg/kg), HPE (30 mg/kg) and GSPE (30 mg/kg). Blood and brain samples were collected at 30 min after morphine administration. Each value represents mean±S.E. (n=3—4).

tion of licking/biting time) of morphine in the second phase of formalin test was significantly potentiated by pretreatment with low dose (300 mg/kg) of SJW (Fig. 4). On the other hand, HPE (30 mg/kg) and GSPE (30 mg/kg) had little significant effect on the antinociceptive effect of morphine. The antinociceptive effect of morphine in the first phase was unaffected by these herbals.

The concentrations of morphine in plasma and brain 30 min after s.c. injection of morphie (0.3 mg/kg) in mice pretreated with SJW, HPE and GSPE were not significantly different from those in morphine-treated mice without these herbals (Table 2).

DISCUSSION

In the present study, we investigated antinociceptive properties of SJW, HPE and GSPE in mice with mechanisms that might potentially underlie these activities. SJW and HPE attnuated significantly nociceptive (licking/biting) responses in both first and second phase of formalin test. In contrast, GSPE was significantly efficacious only against the second phase. In the formalin test, it is considered that first phase of formalin-induced behavior reflects direct activation of A-delta and C afferent fibers while the second phase reflects both ongoing peripheral sensory input and central sensitization. Therefore, it is suggested that antinociceptive effects of SJW and HPE are mediated through central or both central and peripheral antinociceptive effects while the effect of GSPE is mainly due to the peripheral effect.

Antidepressant drugs such as tricyclics have been widely used in the treatment of patients with chronic pain. The serotoninergic and adrenergic neuronal systems in CNS may be significantly involved in the descending pain-inhibitory pathways,[16,17] and the interference of tricyclic antidepressant drugs with reuptake of serotonin and noradrenaline may be responsible for their antinociceptive activity.[18] There are accumulating evidences that antidepressant activity of SJW results from the suppression of reuptake of synaptic serotonin and noradrenaline.[3—5,19] Therefore, it is plausible that antinociceptive effect of SJW is attributable partly to the activation of descending serotoninergic and adrenergic pathways. However, in the current study, the antinociceptive effect of SJW was little affected by yohimbine (α_2 adrenoceptor antagonist) and methysergide (serotonin receptor antagonist). Chatterjee *et al.*[3] showed that hyperforin inhibited the uptake of GABA and L-glutamate, with similar IC_{50} values for the inhibition of uptake of serotonin, noradrenaline and dopamine. Thus, there is a possibility that the inhibition of GABA and L-glutamate uptake may be significantly associated with the antinociceptive effect of SJW. The antinociceptive effect of SJW was unaffected by naloxone, a non-specific antagonist of opioid receptors, suggesting that this ef-

fect of SJW is not mediated by opioid receptor system. Notably, relatively low dose of SJW significantly potentiated antinociceptive effect of morphine in the second phase of formalin test. It has been reported that tricyclic antidepressant drugs potentiate antinociceptive effect of morphine both animals and human and possess clinical efficacy in the treatment of chronic pain states such as an adjuvant analgesic.[18—22] Thus, it might be rational that SJW having antidepressant effect enhances antinociceptive effect of morphine.

Many botanical dietary supplements contain pharmacologically active phytochemicals that, when consumed concomitantly with conventional medications, may result in pharmacokinetic and/or pharmacodynamic interaction. SJW is a botanical supplement recognized for interacting with prescription medications.[23—25] SJW has been shown to decrease significantly blood concentrations of drugs such as indinavir, cyclosporine and midazolam by inducing particularly cytochrome P450 3A4 activity, thereby reducing the efficacy of drugs.[23—25] In the present study, there was no significant change in plasma and brain concentrations of morphine after oral administration of SJW, in spite that antinociceptive effect of morphine was effectively enhanced by pretreatment with SJW (Fig. 4). Therefore, this potentiation by SJW of morphine effect may be attributable to the pharmacodynamic interaction rather than pharmacokinetic interaction.

The antinociceptive effect of HPE in the formalin test (second phase) was significantly antagonized by naloxone, suggesting the involvement of opioidergic mechanism. There are three subtypes of opioid receptors (μ, δ, κ), and μ-opioid receptor activation is mainly involved in the antinociceptive effect of morphine. Our preliminary radioreceptor binding study has shown that HPE binds to μ-opioid receptors in mouse brain. Therefore, these results suggest a contribution of μ-opioid receptors in the antinociceptive effect of HPE.

Free radicals are implicated with pain[26,27] and some plant antioxidants have pain alleviating properties.[28] Proanthocyanidins are major polyphenols in grape seeds and they have potent antioxidant activities *in vitro* and *in vivo* models.[29—31] Since GSPE also contain high amount (84%) of proanthocyanidins, antioxidant activities of proanthocyanidins might contribute to the antinociceptive effect of GSPE. In fact, this notion is supported also by the observation that *Croton celtidifolius* extract containing high amount of proanthocyanidins (75%) exhibited antinociceptive effect in the formalin test.[32]

SJW, HPE and GSPE did not change latencies in the tail-flick test. It is considerd that tail-flick test is spinally mediated reflex to noxious stimuli. It is widely considered that analgesic effectiveness depended on the nociceptive stimulus, and that chemical stimulus (formalin test) is more sensitive than thermal one (tail-flick test).

There are considerable evidences to indicate that NO plays an important role in the processing of nociceptive transmission.[33,34] These include an increase in the release of excitatory neurotransmitter such as glutamate in the spinal cord following peripheral inflammation and an increase in the Ca^{2+} influx by glutamate resulting in the production of NO_x due to the activation of NO synthase. It was found that inhibition of NO synthase produced antinociception in the formalin test.[35—37] In this study, it was shown that formalin injection into one hind paw increased the content of NO_x in spinal cord, in accord with previously reports using microdialysis method.[38,39] Moreover, oral administration of SJW and HPE significantly inhibited the formalin evoked increase of NO_x content in the spinal cord. Thus, this result suggests that antinociceptive activity of SJW and HPE is related partly to the suppression of spinal NO pathway.

In conclusion, the present study has shown that SJW, HPE and GSPE exert significant antinociceptive effects in the formalin test in mice. In addition, opioidergic system seems to be involved in the antinociceptive effect of HPE but not SJW and GSPE. Furthermore, SJW potentiates morphine-induced antinociception possibility by pharmacodynamic interaction.

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Invited review

Pharmacological effects of saw palmetto extract in the lower urinary tract

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Saw palmetto extract (SPE), an extract from the ripe berries of the American dwarf palm, has been widely used as a therapeutic remedy for urinary dysfunction due to benign prostatic hyperplasia (BPH) in Europe. Numerous mechanisms of action have been proposed for SPE, including the inhibition of 5α-reductase. Today, α_1-adrenoceptor antagonists and muscarinic cholinoceptor antagonists are commonly used in the treatment of men with voiding symptoms secondary to BPH. The improvement of voiding symptoms in patients taking SPE may arise from its binding to pharmacologically relevant receptors in the lower urinary tract, such as α_1-adrenoceptors, muscarinic cholinoceptors, 1,4-dihyropyridine receptors and vanilloid receptors. Furthermore, oral administration of SPE has been shown to attenuate the up-regulation of α_1-adrenoceptors in the rat prostate induced by testosterone. Thus, SPE at clinically relevant doses may exert a direct effect on the pharmacological receptors in the lower urinary tract, thereby improving urinary dysfunction in patients with BPH and an overactive bladder. SPE does not have interactions with co-administered drugs or serious adverse events in blood biochemical parameters, suggestive of its relative safety, even with long-term intake. Clinical trials (placebo-controlled and active-controlled trials) of SPE conducted in men with BPH were also reviewed. This review should contribute to the understanding of the pharmacological effects of SPE in the treatment of patients with BPH and LUTS.

Keywords: Saw palmetto extract, Pharmacological effects, Lower urinary tract receptors
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Introduction

Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are very common disorders in aging men. The prevalence of histopathologic BPH is age dependent, with initial development usually occurring after 40 years of age[1]. By 60 years of age, its prevalence is greater than 50% and by age 85, the prevalence is as high as 90%. Similar to histological evidence, the prevalence of bothersome symptoms also increases with age. The two main forms of internationally accepted medical treatment for BPH are inhibitors of 5α-reductase, such as finasteride and α_1-adrenoceptor antagonists, with the latter being more effective[2]. In addition to these medications, the ripe berries of the American dwarf palm (Serenoa repens, saw palmetto) have been traditionally used to treat genitourinary problems; to enhance sperm production, breast size, or libido; and as a mild diuretic[3]. In many European countries, phytotherapeutic agents, including saw palmetto, are very popular. Phytotherapeutic agents represent nearly half of the medications dispensed for the treatment of BPH in Italy, compared with 5% for α-blockers and 5% for 5α-reductase inhibitors[4]. In Germany and Austria, phytotherapy is the first-line treatment for mild to moderate lower urinary tract symptoms and represents more than 90% of all drugs prescribed for the treatment of BPH[4–6]. Saw palmetto is a dwarf palm tree of the family Arecaceae and is indigenous to the southeastern parts of the United States. Saw palmetto berries have traditionally been used by American Indians to cure genitourinary disturbances, relieve mucous membrane irritations, increase testicular function, or increase breast size[5, 6]. In the United States, the use of phytotherapy for LUTS has grown rapidly, and approximately 2.5 million men use saw palmetto extract

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(SPE), although a guideline panel did not recommend phytotherapy as a treatment for BPH[7, 8]. In Japan, SPE is not a prescribed medication; however, it has been receiving increasing attention recently among patients with BPH.

The mechanisms of pharmacological action of SPE are not fully understood, although numerous proposals have been made, including inhibition of 5α-reductase, anti-androgenic effects, anti-proliferative effects, anti-inflammatory effects and anti-edema effects[6]. However, most of these pharmacological effects were observed at relatively high concentrations or large doses of SPE[9, 10], and it is uncertain whether the reported modes of action of SPE are therapeutically relevant[11, 12]. As described above, α_1-adrenoceptor antagonists are commonly used in the treatment of men with voiding symptoms (urinary obstruction, pollakiuria and urinary incontinence) secondary to BPH. Goepel *et al*[13] have shown that SPE may have α_1-adrenoceptor inhibitory properties. SPE significantly affects pharmacological receptors, such as the α_1-adrenoceptor and the muscarinic receptor in the lower urinary tract, to relieve the irritative and obstructive symptoms of dysuria due to BPH and LUTS[14]. In addition to traditionally used medications, like α_1-adrenoceptor antagonists, antimuscarinics, 5α-reductase inhibitors, and phytotherapy, several new therapeutic agents, such as selective β_3-adrenoceptor agonists, are potentially useful for treating LUTS suggestive of BPH, particularly for storage symptoms secondary to outflow obstruction[15]. Thus, the effects of SPE on these receptors in the lower urinary tract might be pharmacologically relevant.

To date, more than 11 placebo-controlled trials and 4 active-controlled trials with SPE in men with BPH have been conducted. Most of these were reported in the 1980s. Patient numbers were usually limited and the evaluation periods were relatively short, so it would be difficult to evaluate the effect of SPE and ascertain the efficacy of SPE in BPH patients. However, some placebo-controlled studies and comparisons to α_1-blockers have recently been conducted with relatively long-term treatments and sufficient numbers of patients[8, 16, 17].

Herbal products, including SPE, are often used with other prescription medications, and most patients with BPH are aged men. Elderly individuals frequently take dietary supplements with prescription drugs, and such a tendency will continue to increase in the near future. In such cases, a major concern is adverse events caused by a large excess intake or interactions between dietary supplements and drugs. Thus, the safety, as well as the efficacy, of these natural products and of their active ingredients remains to be analyzed at a scientific level. This review introduces newly revealed pharmacological actions of SPE, as well as some well-known mechanisms of action of SPE, and also summarizes clinical trials of SPE in comparison with currently used medicines.

Chemical composition

SABALSELECT™, manufactured by Indena S.p.A. (Milano, Italy), was used for the animal experiments[14, 18, 19]. Indena S.p.A. explains the extraction of saw palmetto in the brochure as follows: the fruits of *S repens* are extracted with supercritical CO_2. This extractive procedure, conducted at 45 °C/220 bar, directly produces a pharmacological product (SABALSELECT™), which can be used without further purification. Table 1 shows the chemical composition of SABALSELECT™. It consists of fatty acids, alcohols and sterols (Brochure of Sabalselect™: Indena S.p.A.). Habib and Wyllie[20] reported that the contents of different brands of SPE were markedly different; for example, free fatty acids ranged from 40.7 to 80.7% (mean %), methyl and ethyl esters from 1.5 to 16.7% (mean %), and glycerides from 6.8 to 52.2% (mean %). In the United States, herbal products are regulated under the Dietary Supplement Health and Education Act (DSHEA); however, approval for launching products onto the market is not required except in cases of a new dietary ingredient. Therefore, herbal products that existed before October 15, 1994, can remain with different ingredients[21]. Levin and Das[22] issued a warning that each

Table 1. Chemical Composition of SPE (Brochure of SabalselectTM: Indena S. p. A. http://www.indena.it/pdf/sabalselect.pdf).

Fatty acids	Content (%)	Fatty alcohols and sterols	Content (%)
Total fatty acids	93.5	Fatty alcohols	0.20
		Hexacosanol	0.017
Saturated	59.8	Octacosanol	0.146
Caproic acid	1.5	Tetracosanol	0.004
Caprylic acid	2.3	Triacontanol	0.003
Capric acid	2.5		
Lauric acid	30.2	Sterols	0.32
Myristic acid	12.0	Campesterol	0.07
Palmitic acid	9.5	Stigmasterol	0.03
Stearic acid	1.8	β-Sitosterol	0.22
Unsaturated	33.7		
Oleic acid*	28.5		
Linoleic acid	4.6		
Linolenic acid	0.6		

*: Brochure of SabalselectTM: Indena S. p. A. http://www.indena.it/pdf/sabalselect.pdf

preparation must be considered individually because of differences in extraction techniques, preparation of products, composition, and biological activities.

Pharmacological properties

BPH causes dysuria and residual urine via a mechanical stoppage due to hypertrophy of prostatic tissue and via a functional stoppage caused by α_1-adrenoceptor hypertonia of prostatic smooth muscle. Previous studies have demonstrated that SPE has a number of pharmacological effects: 1) an anti-androgenic effect — inhibition of 5α-reductase I and II and inhibition of binding of dihydrotestosterone (DHT) to the cytosolic androgen receptors, 2) an anti-inflammatory effect, 3) an anti-proliferative effect, (Figure 1), and 4) significant binding of pharmacological receptors existing in the lower urinary tract.

Anti-androgenic effects

The development and growth of the prostate gland depend on androgen stimulation[23, 24]. DHT is one of several factors regulating this development and growth[24, 25] and is converted from testosterone by 5α-reductase. This enzyme has two isoforms (5α-reductase 1 and 2)[25]. The respective roles of these 5α-reductases in BPH development have not yet been elucidated[26]. SPE inhibited both isozymes in a noncompetitive manner[27-29], whereas finasteride inhibited only 5α-reductase 2 in a competitive manner[25]. Among the many components of SPE, lauric acid and linoleic acid showed inhibition of both isozymes, oleic acid was active only against 5α-reductase 1 and myristic acid was active only against 5α-reductase 2. However, palmitic acid, stearic acid, esterified fatty acids, sterols, and alcohols were inactive against both[30].

Di Silverio *et al*[26] reported a significant decrease in DHT and increase in testosterone in the periurethral region of prostate tissue from BPH patients receiving Permixon® (320 mg/day) for 3 months and thus suggested that SPE could inhibit 5α-reductase in the human prostate *in vivo*. Sultan *et al*[9] investigated the interaction of SPE with the intercellular androgen-receptor complex. SPE inhibited [^{3}H]dihydrotestosterone from binding to its receptor. The affinity of SPE was higher for cytosol receptors than for nuclear receptors. Competitive interference with the binding of [^{3}H]methyltrienolone to cytosolic androgen receptors was also shown in rat prostate cells [31].

Anti-inflammatory effects

Inflammation was frequently observed in hormonally induced hypertrophied prostates of dogs[32] and in a study of human BPH[33]. Mahapokai *et al*[32] concluded that the development of hyperplasia preceded inflammatory infiltration. An anti-inflammatory effect was indicated as one of the mechanisms of action of SPE. In fact, it is plausible that SPE affects several inflammatory mediators. SPE showed anti-inflammatory and anti-edematous effects *in vivo*[34]. The production of 5-lipoxygenase metabolites was inhibited by SPE (Permixon®) at a concentration of 5 μg/mL[35]. Breu *et al*[34] demonstrated that acid lipophilic compounds of SPE inhibited the biosynthesis of cyclooxygenase and 5-lipoxygenase metabolites with the same intensity as SPE.

Vela Navarrete *et al*[36] conducted a multicenter open pilot clinical study to make a comparison between a control group and an SPE (Permixon®) group in BPH patients. After

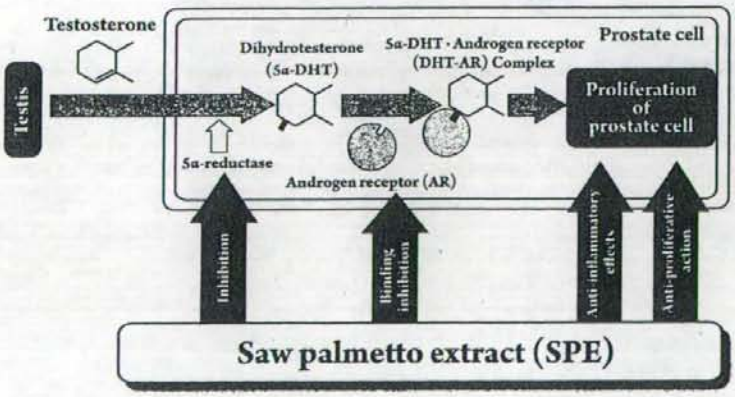

Figure 1. Mechanisms of pharmacological action of saw palmetto extract (SPE). They include antiandrogenic effects, such as inhibition of 5α-reductase I and II and inhibition of binding of dihydrotestosterone (DHT) to the cytosolic androgen receptors, anti-proliferative effects and anti-inflammatory effects.