Agricultural Research Service

Dr. Duke's Phytochemical and Ethnobotanical Databases

Ethnobotanical uses

Gymnema sylvestre (ASCLEPIADACEAE)

Bilious Woi.4; Bite(Snake) Broun; Cardiotonic Woi.4; Cough Woi.4; Diabetes Woi.4; Diuretic Woi.4; Errhine Woi.4; Eye Woi.4; Furunculosis Woi.4; Laxative Woi.4; Parageusia Woi.4; Stimulant Woi.4; Stomachic Woi.4; Tongue Uphof; Uterotonic Woi.4

---

* = Chemical(s) found in plant shown to be effective for the ailment medicated
** = Plant itself shown to be effective for the ailment medicated

---

Phytochemical Database, USDA - ARS - NGRL, Beltsville Agricultural Research Center, Beltsville, Maryland
Wed Oct 27 14:45:16 EDT 2004

Please send questions and comments to:

James A. Duke

Jim Duke
Green Farmacy Garden
8210 Murphy Road
Fulton, MD 20759

or

Mary Jo Bogenschutz *(E-Mail: bogie@hawaii.rr.com)*

Please send technical questions and comments to:

WebMaster *(E-Mail: dbmuqs@ars-grin.gov)*

The USDA does not recommend self diagnosis or self medication. Please see the disclaimer for more

Agricultural Research Service

Phytochemical and Ethnobotanical Databases

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Common names: Gymnema -- Miracle Fruit

Activities

Number of distinct activities for species = 250. [View activities]

List of chemicals

Chemical	Part	Lo ppm	Hi ppm	Reference
(-)-VIBURNITOL	Leaf			JBH
ASCORBIC-ACID	Leaf	152	800	PED98
ASH	Leaf	11400	60000	PED98
BETA-CAROTENE	Leaf	6	30	PED98
BETAINE	Leaf			12054
CALCIUM	Leaf	169	890	PED98
CHOLINE	Leaf			12054
CHROMIUM	Leaf	0.9	4.5	PED98
COBALT	Leaf			PED98
CONDURITOL-A	Leaf			13021
FAT	Leaf	3990	21000	PED98
FIBER(CRUDE)	Leaf		80000	PED98
FIBER(DIETARY)	Leaf		320000	PED98
GURMARIN	Leaf			15581 15582 15599 15618 15568
GYMNEMAGENIN	Plant			14122
GYMNEMASAPONIN-I	Leaf		60	13841
GYMNEMASAPONIN-II	Leaf	25	27	13841 15570
GYMNEMASAPONIN-III	Leaf		10	13841 15583
GYMNEMASAPONIN-IV	Leaf	12	17	13841 15570 15585
GYMNEMASAPONIN-V	Leaf	133	160	15570 13841 15583

GYMNEMASIDE-I	Leaf		20	12469
GYMNEMASIDE-II	Leaf		23	12469
GYMNEMASIDE-III	Leaf		13	12469
GYMNEMASIDE-IV	Leaf		10	12469
GYMNEMASIDE-V	Leaf		133	12469
GYMNEMASIDE-VI	Leaf		10	12469
GYMNEMASIDE-VII	Leaf		13	12469
GYMNEMASIN-A	Leaf		83	15569
GYMNEMASIN-B	Leaf		57	15569
GYMNEMASIN-C	Leaf		73	15569
GYMNEMASIN-D	Leaf		48	15569
GYMNEMIC-ACID	Leaf			15575 15576 15617 15598 15600 15622 15616
GYMNEMIC-ACID	Seed		2500	13670
GYMNEMIC-ACID	Plant			14481
GYMNEMIC-ACID-A	Leaf			12308 15565
GYMNEMIC-ACID-B	Leaf			12308 15565
GYMNEMIC-ACID-C	Leaf			12308 15565
GYMNEMIC-ACID-D	Leaf			12308 15565
GYMNEMIC-ACID-I	Leaf	67	120	15570 13689 15569 15583
GYMNEMIC-ACID-II	Leaf	86	160	13689 15570 15569 15583
GYMNEMIC-ACID-III	Leaf	27	91	15570 12855 13689 13804 15593 15569 15583
GYMNEMIC-ACID-IV	Leaf	47	73	12855 15570 13689 13804 15593 15569
GYMNEMIC-ACID-IX	Leaf	21	28	13804 12855
GYMNEMIC-ACID-V	Leaf	18	53	13773 15570 12855 13804 15583
GYMNEMIC-ACID-VI	Leaf		10	13773 15583
GYMNEMIC-ACID-VII	Leaf	60	333	13773 15570
GYMNEMIC-ACID-VIII	Leaf	23	32	13804 12855
GYMNEMIC-ACID-W	Leaf			12308
GYMNEMIC-ACID-X	Leaf		5	12490 15583 12308
GYMNEMIC-ACID-XI	Leaf		30	12490 15583
GYMNEMIC-ACID-XII	Leaf		8	12490 15583
GYMNEMIC-ACID-XIII	Leaf		13	12490 15583
GYMNEMIC-ACID-XIV	Leaf		5	12490 15583
GYMNEMIC-ACID-XV	Leaf		5	12540 15583
GYMNEMIC-ACID-XVI	Leaf		5	12540 15583
GYMNEMIC-ACID-XVII	Leaf		5	12540 15583
GYMNEMIC-ACID-XVIII	Leaf		8	12540 15583
GYMNEMIC-ACID-Y	Leaf			12308

GYMNEMIC-ACID-Z	Leaf			12308
GYMNEMOSIDE-A	Leaf		83	15570
GYMNEMOSIDE-B	Leaf		46	15570
GYNOSAPONIN-TN-2	Leaf			13773
GYPENOSIDE-II	Leaf			13773
GYPENOSIDE-LV	Leaf		10	12469
GYPENOSIDE-LXII	Leaf		27	12469
GYPENOSIDE-LXIII	Leaf		33	12469
GYPENOSIDE-LXXIV	Leaf			13773
GYPENOSIDE-V	Leaf			13773
GYPENOSIDE-XLIII	Leaf			13773
GYPENOSIDE-XLV	Leaf			13773
GYPENOSIDE-XLVII	Leaf			13773
GYPENOSIDE-XXVIII	Leaf		30	12469
GYPENOSIDE-XXXVII	Leaf		60	12469
IRON	Leaf	9	49	PED98
MAGNESIUM	Leaf	201	1060	PED98
MANGANESE	Leaf	0.4	2	PED98
NIACIN	Leaf	4.2	22	PED98
PHOSPHORUS	Leaf	561	2950	PED98
POTASSIUM	Leaf	2470	13000	PED98
PROTEIN	Leaf	20900	110000	PED98
RIBOFLAVIN	Leaf			PED98
SELENIUM	Leaf	0.4	2.3	PED98
SILICON	Leaf	0.6	3	PED98
SODIUM	Leaf	266	1400	PED98
THIAMINE	Leaf			PED98
TRIMETHYLAMINE	Leaf			12054
WATER	Leaf		810000	PED98
ZINC	Leaf			PED98

WARNING and DISCLAIMER

Please send farmacy comments to Jim Duke at:

Jim Duke
Green Farmacy Garden
8210 Murphy Road

Fulton, MD 20759

| USDA | ARS | NGRP | NPGS | FARMACY |

Cite as: USDA, ARS, National Genetic Resources Program. *Phytochemical and Ethnobotanical Databases*. [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. 27 October 2004.

[Production Version]

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Aldose-Reductase-Inhibitor Activity:

ASCORBIC-ACID
CONDURITOL-A

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Antiaging Activity:

ASCORBIC-ACID
BETA-CAROTENE
CHROMIUM

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Antidiabetic Activity:

ASCORBIC-ACID
CHOLINE
CHROMIUM
GYMNEMIC-ACID
MAGNESIUM
MANGANESE
ZINC

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Antiobesity Activity:

ASCORBIC-ACID
CHROMIUM
GYMNEMIC-ACID
ZINC

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Antisyndrome-X Activity:

ASCORBIC-ACID
CHROMIUM
MAGNESIUM
MANGANESE
SELENIUM
ZINC

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Diuretic Activity:

ASCORBIC-ACID
BETAINE
CALCIUM
MAGNESIUM
POTASSIUM

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Hypocholesterolemic Activity:

ASCORBIC-ACID
CALCIUM
CHROMIUM
MAGNESIUM

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Hypoglycemic Activity:

ASCORBIC-ACID
CHROMIUM
MANGANESE
NIACIN

Exit

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Chemicals with Vasodilator Activity:

ASCORBIC-ACID
CALCIUM
MAGNESIUM
NIACIN
POTASSIUM

Exit

Agricultural Research Service

Phytochemical and Ethnobotanical Databases

Gymnema sylvestre (RETZ.) SCHULT. - Asclepiadaceae

Activities

The number in () indicates how many separate chemicals this species has for that activity, for example, ***Analgesic (3)*** indicates this species has three separate chemicals that have Analgesic activity. Select the activity to see the chemicals.

ACE-Inhibitor (1)
AP-1-Inhibitor (1)
Abortifacient (1)
Acidulant (1)
Aldose-Reductase-Inhibitor (2)
Allergenic (2)
Amphiglycemic? (1)
Analgesic (2)
Androgenic? (1)
Angiotensin-Receptor-Blocker (2)
Anorexic (1)
AntiAGE (2)
AntiCrohn's (2)
AntiMeniere's (1)
AntiNF-kB (1)
AntiPMS (3)
Antiacne (3)
Antiacrodermatitic (1)
Antiacrodynic (1)
Antiaggregant (3)
Antiaging (3)
Antiakathisic (1)
Antialcoholic (1)
Antiallergic (2)
Antialopecic (1)
Antialzheimeran (2)
Antiamblyopic (1)
Antianemic (2)
Antianginal (2)
Antiangiogenic (1)
Antianorectic (1)
Antianorexic (1)

Antianxiety (2)
Antiarabiflavinotic (1)
Antiarrhythmic (2)
Antiarteriosclerotic (1)
Antiarthritic (2)
Antiarthritic? (1)
Antiasthmatic (3)
Antiatherosclerotic (4)
Antibacterial (1)
Anticancer (1)
Anticanker (1)
Anticarcinomic (1)
Anticariogenic (1)
Anticarpal-Tunnel (1)
Anticataract (3)
Anticheilitic (2)
Antichilblain (1)
Antichoreic (1)
Anticirrhotic (2)
Anticoeliac (1)
Anticold (2)
Anticolitic (1)
Anticonvulsant (2)
Anticorneotic (1)
Anticoronary (4)
Anticystinuric (1)
Antidandruff (2)
Antidecubitic (2)
Antidementia (1)
Antidepressant (4)
Antidermatitic (1)
Antidiabetic (7)
Antidiscotic (1)
Antidote (Aluminum) (2)
Antidote (Cadmium) (2)
Antidote (Lead) (3)
Antidote (Mercury) (1)
Antidote (Paraquat) (1)
Antidyskinetic (2)
Antidysmenorrheic (1)
Antidysphagic (1)
Antieczemic (2)
Antiedemic (1)
Antiencephalitic (1)
Antiencephalopathic (1)
Antiepileptic (4)
Antifatigue (1)
Antiflu (3)
Antifuruncular (1)
Antigastritic (1)
Antigingivitic (1)

Antiglaucomic (2)
Antiglossitic (1)
Antiglycosuric (1)
Antihemorrhagic (1)
Antihepatitic (1)
Antihepatotoxic (1)
Antiherpetic (1)
Antiherpetic? (1)
Antihistaminic (2)
Antihomocysteine (1)
Antihomocystinuric (1)
Antihyperactivity (1)
Antihyperkeratotic (1)
Antihyperkinetic (2)
Antihypertensive (4)
Antihypoglycemic (1)
Antiichythyotic (1)
Antiimpotence (1)
Antiinfective (1)
Antiinfertility (2)
Antiinflammatory (2)
Antiinsomniac (3)
Antiinsomnic (1)
Antikeratitic (1)
Antikeshan (1)
Antilepric (2)
Antileukemic (1)
Antileukonychic (1)
Antileukoplakic (1)
Antileukotriene (1)
Antilithic (1)
Antilupus (1)
Antimanic (1)
Antimastalgic (1)
Antimastitic (1)
Antimeasles (1)
Antimelanomic (1)
Antimenorrhagic (1)
Antimetastatic (1)
Antimigraine (3)
Antimutagenic (2)
Antimyalgic (1)
Antimyoatrophic (1)
Antineuralgic (1)
Antineurotic (1)
Antinitrosic (1)
Antiobesity (4)
Antiorchitic (1)
Antiosteoarthritic (2)
Antiosteoporotic (4)
Antiototic (1)

Antioxidant (4)
Antiozenic (1)
Antiparkinsonian (2)
Antiparotitic (1)
Antipellagric (2)
Antiperiodontitic (2)
Antiphotophobic (2)
Antipityriasic (1)
Antiplaque (1)
Antipneumonic (1)
Antipodriac (1)
Antipoliomyelitic (1)
Antiporphyric (1)
Antiprolactin (1)
Antiproliferant (2)
Antiprostatitic (1)
Antipsoriac (1)
Antipyretic (1)
Antiradicular (3)
Antiretinopathic (1)
Antirheumatic (1)
Antiscorbutic (1)
Antiscotomic (1)
Antiseptic (1)
Antishingles (1)
Antispare-Tire (1)
Antispasmodic (2)
Antispasmophilic (1)
Antistomatitic (1)
Antistress (1)
Antistroke (1)
Antisyndrome-X (6)
Antitic (1)
Antitinnitic (1)
Antitriglyceride (2)
Antitumor (2)
Antitumor (Breast) (1)
Antitumor (Lung) (2)
Antitumor (brain) (1)
Antiulcer (3)
Antiulcerogenic (1)
Antivertigo (1)
Antiviral (4)
Antiviral? (1)
Antixerophthalmic (1)
Apoptotic (2)
Asthma-preventive (1)
Astringent (1)
Beta-Adrenergic Receptor Blocker (1)
Beta-Blocker (1)
Beta-Glucuronidase-Inhibitor (1)

Bruchiphobe (1)
CNS-Depressant (1)
Calcium-Antagonist (2)
Calcium-Channel-Blocker (1)
Cancer-Preventive (5)
Cardiodepressant (1)
Cardiomyopathogenic (1)
Cardioprotective (1)
Cardiotoxic (1)
Cerebrotonic (1)
Cholinergic (1)
Cold-preventive (1)
Collagenic (1)
Colorant (1)
Copper-Antagonist (1)
Deodorant (1)
Depressant (1)
Detoxicant (1)
Diuretic (5)
Emmenagogue (1)
Erythrocytogenic (1)
Ethanolytic (1)
Fistula-Preventive (1)
Fungicide (1)
Hepatoprotective (3)
Hypertensive (1)
Hypocholesterolemic (4)
Hypoglycemic (4)
Hypolipidemic (1)
Hypotensive (6)
Ileorelaxant (1)
Immunostimulant (5)
Immunosuppressant (1)
Insulinogenic (3)
Interferon-Synergist (1)
Interferonogenic (1)
Leptingenic (1)
Lipotropic (2)
Lithogenic (1)
Memorigenic (1)
Mucogenic (2)
Mucolytic (1)
Myorelaxant (1)
Ornithine-Decarboxylase-Inhibitor (1)
Osteogenic (1)
Parasympathomimetic (1)
Pesticide (6)
Phagocytotic (1)
Polyamine-Synthesis-Inhibitor (1)
Prooxidant (1)
Prostaglandin-Sparer (1)

Protein-Kinase-C-Inhibitor (1)
Sedative (1)
Serotoninergic (1)
Spermigenic (1)
Testosteronigenic (1)
Thymoprotective (1)
Trichomonicide (1)
Ubiquiot (1)
Uricosuric (1)
Urinary-Acidulant (1)
Uterorelaxant (1)
VEGF-Inhibitor (1)
Vasodilator (5)
Vulnerary (2)

WARNING and DISCLAIMER

Please send farmacy comments to Jim Duke at:

Jim Duke
Green Farmacy Garden
8210 Murphy Road
Fulton, MD 20759

Cite as: USDA, ARS, National Genetic Resources Program. *Phytochemical and Ethnobotanical Databases*. [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. 27 October 2004.

[Production Version]

1: J Endocrinol. 1999 Nov;163(2):207-12.

Gymnema sylvestre stimulates insulin release in vitro by increased membrane permeability.

Persaud SJ, Al-Majed H, Raman A, Jones PM.

Physiology Division, School of Biomedical Sciences, King's College, London, UK.

To determine whether extracts of Gymnema sylvestre may have therapeutic potential for the treatment of non-insulin-dependent diabetes mellitus (NIDDM), we examined the effects of an alcoholic extract of G. sylvestre (GS4) on insulin secretion from rat islets of Langerhans and several pancreatic beta-cell lines. GS4 stimulated insulin release from HIT-T15, MIN6 and RINm5F beta-cells and from islets in the absence of any other stimulus, and GS4-stimulated insulin secretion was inhibited in the presence of 1 mM EGTA. Blockade of voltage-operated Ca(2+) channels with 10 microM isradipine did not significantly affect GS4-induced secretion, and insulin release in response to GS4 was independent of incubation temperature. Examination of islet and beta-cell integrity after exposure to GS4, by trypan blue exclusion, indicated that concentrations of GS4 that stimulated insulin secretion also caused increased uptake of dye. Two gymnemic acid-enriched fractions of GS4, obtained by size exclusion and silica gel chromatography, also caused increases in insulin secretion concomitant with increased trypan blue uptake. These results confirm the stimulatory effects of G. sylvestre on insulin release, but indicate that GS4 acts by increasing cell permeability, rather than by stimulating exocytosis by regulated pathways. Thus the suitability of GS4 as a potential novel treatment for NIDDM can not be assessed by direct measurements of beta-cell function in vitro.

PMID: 10556769 [PubMed - indexed for MEDLINE]

Gymnema sylvestre stimulates insulin release *in vitro* by increased membrane permeability

S J Persaud[1], H Al-Majed[1], A Raman[2] and P M Jones[1]

[1]Physiology Division, School of Biomedical Sciences, King's College, London, UK
[2]Pharmacy Department, School of Health and Life Sciences, King's College London, UK
(Requests for offprints should be addressed to S J Persaud)

Abstract

To determine whether extracts of *Gymnema sylvestre* may have therapeutic potential for the treatment of non-insulin-dependent diabetes mellitus (NIDDM), we examined the effects of an alcoholic extract of *G. sylvestre* (GS4) on insulin secretion from rat islets of Langerhans and several pancreatic β-cell lines. GS4 stimulated insulin release from HIT-T15, MIN6 and RINm5F β-cells and from islets in the absence of any other stimulus, and GS4-stimulated insulin secretion was inhibited in the presence of 1 mM EGTA. Blockade of voltage-operated Ca^{2+} channels with 10 μM isradipine did not significantly affect GS4-induced secretion, and insulin release in response to GS4 was independent of incubation temperature. Examination of islet and β-cell integrity after exposure to GS4, by trypan blue exclusion, indicated that concentrations of GS4 that stimulated insulin secretion also caused increased uptake of dye. Two gymnemic acid-enriched fractions of GS4, obtained by size exclusion and silica gel chromatography, also caused increases in insulin secretion concomitant with increased trypan blue uptake. These results confirm the stimulatory effects of *G. sylvestre* on insulin release, but indicate that GS4 acts by increasing cell permeability, rather than by stimulating exocytosis by regulated pathways. Thus the suitability of GS4 as a potential novel treatment for NIDDM can not be assessed by direct measurements of β-cell function *in vitro*.

Journal of Endocrinology (1999) 163, 207–212

Introduction

Diabetes mellitus is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic β-cells (insulin-dependent diabetes mellitus; IDDM), inadequate release of insulin from the pancreatic β-cells, or insensitivity of target tissues to insulin (non-insulin-dependent diabetes mellitus; NIDDM). NIDDM accounts for up to 90% of the UK diabetic population, and there is an increasing drive to develop novel methods for its treatment, to improve either the insulin output of β-cells or the sensitivity of peripheral tissues to circulating insulin. Of the currently available therapies for NIDDM, only the sulphonylureas are used to stimulate β-cells to secrete more insulin. The others, such as biguanides, α-glucosidase inhibitors and thiazolidinediones, reduce hyperglycaemia independently of increases in circulating insulin.

Sulphonylureas act at a proximal stage in the β-cell stimulus–secretion coupling cascade: they close plasma membrane ATP-sensitive K^+ channels and the consequent decrease in K^+ efflux depolarises the cells, leading to Ca^{2+} influx via voltage-operated Ca^{2+} channels (Henquin 1980, Sturgess *et al.* 1985). Increases in intracellular Ca^{2+} are sufficient to stimulate insulin release from β-cell secretory granules (Jones *et al.* 1985). Novel drugs that act at downstream sites, perhaps directly on the exocytotic release of insulin, would be useful adjuncts to sulphonylureas in the treatment of NIDDM.

The therapeutic potential of *Gymnema sylvestre* R.Br., a woody climber of the Asclepiadaceae family, has been known for many years and it has a key place in Ayurvedic medicine. There are reports that *G. sylvestre* leaf extracts reduce hyperglycaemia in diabetic rabbits (Shanmugasundaram *et al.* 1983), rats (Srivastava *et al.* 1985) and humans (Khare *et al.* 1983, Baskaran *et al.* 1990), and these glucose-decreasing effects may be mediated by increases in insulin secretion (Shanmugasundaram & Panneerselvam 1981). There has been little systematic characterisation of the identities of the insulinotropic agents within the leaf extracts, but aqueous ethanolic extractions of the leaves provide two potentially active fractions, one containing conduritol A, an acid-soluble polyol-polyhydroxy cyclic compound (Miyatake *et al.* 1993), and the other containing a mixture of acid-insoluble triterpenoid saponins (gymnemic acids), designated GS3 and GS4 (Shanmugasundaram *et al.* 1990).

0022–0795/99/0163–207
Online version via http://www.endocrinology.org

Conduritol A has been reported to have small stimulatory effects on basal insulin secretion through an undefined mechanism (Billington *et al.* 1994). GS3 and GS4, which would be devoid of conduritol, have been reported to reduce hyperglycaemia in diabetic rats, increase insulin release *in vivo* and *in vitro*, and increase β-cell number after streptozotocin-induced diabetes (Shanmugasundaram *et al.* 1990). GS3 is a fairly crude fraction prepared by acid precipitation from a hydroalcoholic extract of *G. sylvestre* leaves. Most studies have been performed using GS4, which is purified from GS3 by reprecipitation with acid of GS3 solubilised in alkali (Shanmugasundaram *et al.* 1990). GS4 has been used clinically to treat NIDDM, and it was shown to increase serum insulin concentrations, normalise blood glucose concentrations and reduce the requirement for sulphonylurea (Baskaran *et al.* 1990). The potential use of GS4 in the treatment of diabetes is intriguing, and in the current study we have examined whether GS4, prepared according to a previously described method (Shanmugasundaram *et al.* 1990), exerts insulinotropic effects on β-cell lines and on isolated islets of Langerhans. We have also investigated its mode of action *in vitro*, to assess its potential for the treatment of NIDDM.

Materials and Methods

Dried leaves of *G. sylvestre* were a gift from Cipla Ltd (Mumbai, India). They were authenticated by the Herbarium at the Royal Botanic Gardens, Kew. A voucher specimen (reference Gy11 12) is deposited in the Pharmacognosy Museum, Department of Pharmacy, King's College London. Isradipine was purchased from Research Biochemicals International (Herts, UK). Tissue culture media, antibiotics and foetal calf serum were obtained from Life Technologies (Paisley, UK). All other reagents were of analytical grade from BDH (Poole, Dorset, UK) or Sigma Chemical Co. (Poole, Dorset, UK). Rats were supplied by King's College London Animal Unit. HIT-T15 β-cells were purchased from the American Type Culture Collection (Rockville, MD, USA), MIN6 β-cells were kindly provided by Professor J-I Miyazaki (University of Tokyo, Japan) and RINm5F β-cells were from Professor A J Bone (University of Brighton, UK).

Tissue preparation

Islets of Langerhans were isolated from the pancreata of male Sprague–Dawley rats (200–250 g) by collagenase digestion as previously described (Jones *et al.* 1993). Approximately 400–500 islets were obtained from each pancreas and islets were used immediately after isolation for all experiments. Pancreatic β-cell lines were maintained in culture in DMEM (MIN6) or RPMI (HIT-T15, RINm5F) supplemented with 5–15% foetal calf serum, 100 μg/ml streptomycin and 100 U/ml penicillin, in a humidified atmosphere of 5% CO_2.

Preparation of Gymnema *fractions*

GS4 was prepared as follows: dried *Gymnema* leaves (1 kg) were ground to a coarse powder in a coffee grinder. The powder was placed in a large beaker and aqueous ethanol (50%; 6 l) was added to cover the grounds. Steam, generated separately, was bubbled through the mixture for 3–4 h. After cooling, the material was filtered and ethanol removed from the filtrate by heating over a boiling water bath to leave a thick brown viscous fluid. Sulphuric acid (98%) was then added until the mixture reached pH 3. The precipitate that formed after the mixture was left at 4 °C overnight, corresponding to the GS3 fraction, was collected by filtration and then redissolved in aqueous potassium hydroxide (0·1 M, 400 ml). GS4 was precipitated from this solution by the addition of sulphuric acid to pH 3 and overnight cooling as before. The precipitate was collected by filtration and freeze-dried. Chlorophyll was removed from the methanol-soluble components of GS4 by size exclusion chromatography on Sephadex LH-20 gel using methanol as eluent, yielding a major fraction, termed F2. A major component of F2, termed F43, was isolated in semi-pure form by silica gel column chromatography and preparative thin layer chromatography (TLC) using chloroform–methanol–water mixtures. TLC analysis (data not shown) indicated that GS4 and F2 contained a complex mixture of compounds with chromatographic characteristics typical of triterpenoid saponins. The major component of GS4 and F2 was identified as gymnemic acid VIII by comparison of spectral data with values given in the literature (Yoshikawa *et al.* 1992). This compound was not available in sufficient quantities for testing in the present studies. However, F43 contains a mixture of gymnemic acid VIII and at least one other compound, most likely to be a closely related gymnemic acid. GS4, F2 and F43 were dissolved directly in the aqueous incubation medium (Gey & Gey 1936) used for the insulin secretion and trypan blue uptake experiments.

Insulin secretion

Groups of three islets were incubated in 600 μl physiological salt solution (Gey & Gey 1936), in the absence or presence of GS4, for 1 h at 37 °C, after which time a sample of the supernatant was removed for the measurement of insulin release. Groups of 30 000 MIN6, 100 000 HIT-T15 and 200 000 RINm5F β-cells were seeded into 96-well plates, left to adhere overnight, then pre-incubated in a glucose-free medium for 2 h before a 1-h incubation in glucose-free medium in the absence or presence of GS4. The insulin content of the supernatants

Table 1 GS4-stimulated insulin release from β-cell lines. Cells were incubated in a physiological salt solution (zero glucose) either in the absence of GS4 or supplemented with 0·125–0·5 mg/ml GS4 for 1 h at 37 °C, and insulin released into the supernatant was measured by radioimmunoassay. Data are means ± S.E.M., $n=5$–9

	Insulin release (ng/10^6 cells per h)		
	MIN6	RINm5F	HIT-T15
GS4 (mg/ml)			
0	22·3 ± 1·35	0·24 ± 0·02	2·5 ± 0·58
0·125	63·9 ± 0·92***	0·79 ± 0·05*	5·11 ± 0·86*
0·25	65·6 ± 0·55***	4·07 ± 0·21***	20·71 ± 1·43***
0·5		8·06 ± 0·48***	32·33 ± 2·75***

*$P<0·05$, ***$P<0·001$, compared with secretion in the absence of GS4.

was determined by radioimmunoassay (Jones *et al.* 1988). All experiments were performed with six to nine replicates per treatment group.

Membrane integrity

After incubation in the absence or presence of GS4, islets and β-cells were exposed to the membrane-impermeant dye, trypan blue (0·1% w/v) for 15 min at 4 °C or 37 °C. The presence of dye within cells was determined by light microscopy and the numbers of stained and unstained cells in a field were counted to obtain an estimate of the percentage of cells taking up the dye. MIN6 cells that had been incubated in the presence of the semi-purified GS4 extracts, F2 and F43, were also incubated with trypan blue as described above.

Statistical analysis

Data were analysed by one-way analysis of variance or Student's *t*-tests, as appropriate. Differences between experimental and control samples were considered significant at $P<0·05$.

Results

Isolation of GS4, F2 and F43

GS4, a smooth greenish-brown powder, was obtained in 2·5% w/w yield from dried *G. sylvestre* leaves. Chromatography of 10 g GS4 yielded 5·9 g F2, and 88 mg F43 were obtained from 5·4 g F2. F2 and F43 were both obtained as light-brown powders.

Effects of GS4 on insulin secretion from β-cells and islets

Exposure of β-cells to GS4 resulted in a dose-related increase in insulin release (Table 1). In the case of MIN6 β-cells, insulin release was stimulated at concentrations as low as 31 μg/ml GS4 (252 ± 15·8% basal, $P<0·001$). GS4 (0·2 mg/ml) caused significant ($P<0·001$) increases in the release of insulin from isolated rat islets at both substimulatory (2 mM glucose: 0·18 ± 0·02 ng/islet per h; +0·2 mg/ml GS4: 0·86 ± 0·18 ng/islet per h, $n=6$) and stimulatory (10 mM glucose; 594 ± 59% increase; 20 mM glucose: 259 ± 43% increase, $n=6$) concentrations of glucose. Higher concentrations of GS4 caused progressively increased insulin release from islets such that in the absence of any other stimuli (2 mM glucose), the release rate at 2 mg/ml GS4 was 16·4 ± 1·3 ng/islet per h ($n=6$, $P<0·001$ compared with 2 mM glucose).

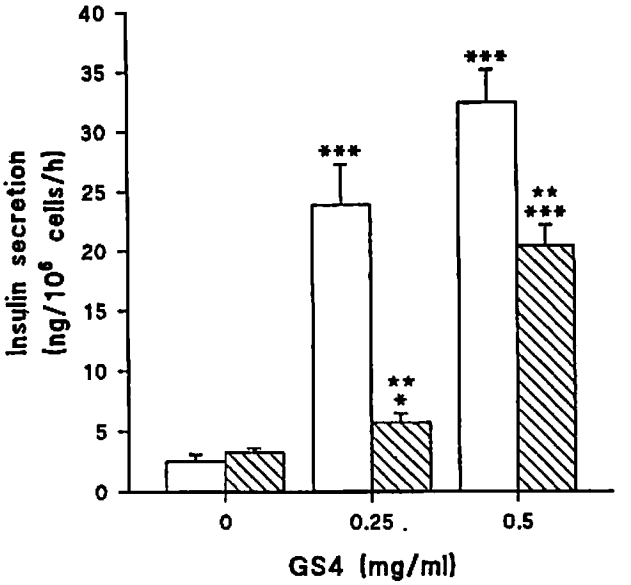

Figure 1 Effect of Ca^{2+} chelation on GS4-stimulated insulin release. HIT-T15 cells were incubated for 1 h at 37 °C in the absence (open bars) or presence (hatched bars) of 1 mM EGTA. Insulin released into the supernatant was measured by radioimmunoassay. Data are means ± S.E.M., $n=8$. *$P<0·05$, ***$P<0·001$ compared with appropriate control in the absence of GS4; **$P<0·01$ compared with secretion in the absence of EGTA.

Mode of action of GS4

As GS4 exerted stimulatory actions on all the β-cell lines tested and on whole islets, the HIT-T15 cell line was used as a representative β-cell population in which to examine the mechanisms through which GS4 increased insulin secretion. It was found that, when extracellular Ca^{2+} was chelated in the presence of 1 mM EGTA, the dose-dependent effects of GS4 were shifted to the right such that 0·25 mg/ml only had a small stimulatory effect, but 0·5 mg/ml still caused a large increase in insulin release (Fig. 1). However, EGTA significantly reduced insulin release at 0·5 mg/ml GS4 and at 0·25 mg/ml ($P<0·01$). Blockade of Ca^{2+} channels with the dihydropyridine blocker, isradipine, did not significantly inhibit the

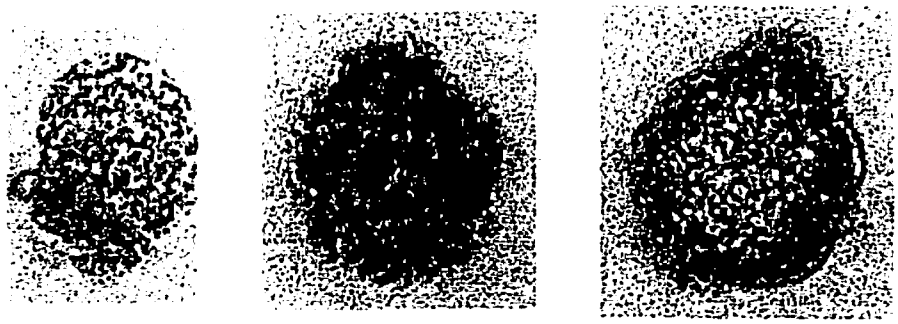

Figure 2 Effects of GS4 on trypan blue uptake by islet cells. Rat islets were incubated in a physiological salt solution supplemented with 0·1% (w/v) trypan blue at 37 °C (left), in the presence of 0·25 mg/ml GS4 and 0·1% (w/v) trypan blue at 37 °C (middle) or in the presence of 0·25 mg/ml GS4 and 0·1% (w/v) trypan blue at 4 °C (right).

stimulatory effects of 0·5 mg/ml GS4 on insulin release from HIT cells (control: 29·4 ± 2·7 ng/10^6 cells per h; +10 μM isradipine: 24·0 ± 1·9 ng/10^6 cells per h; n=8, P>0·1). Incubation of HIT-T15 β-cells at a range of temperatures indicated that GS4 (0·125 mg/ml) stimulated insulin release even at temperatures as low as 4 °C (37 °C: 6·1 ± 0·75 ng/10^6 cells per h; 30 °C: 5·1 ± 0·33 ng/10^6 cells per h; 24 °C: 4·3 ± 0·67 ng/10^6 cells per h; 4 °C: 5·7 ± 1·33 ng/10^6 cells per h; n=5–6).

Effects of GS4 on membrane integrity

Exposure of β-cells to GS4 resulted in a dose-related increase in the number of cells to which trypan blue dye gained access, with 98% of MIN6 cells, 95% of RINm5F cells and 88% of HIT-T15 cells taking up the dye at 0·25 mg/ml GS4. Islets incubated in the absence of GS4 excluded trypan blue, but those which had been incubated in the presence of 0·25 mg/ml GS4 showed substantial dye uptake, whether they were exposed to GS4 at 37 °C or at 4 °C (Fig. 2).

Effects of GS4 fractionation products on insulin release and membrane integrity

F2 and F43, fractions obtained by further purification of GS4, increased the rate of insulin release from MIN6 cells in the absence of any other stimulus (Table 2). However, F2 and F43 also caused a significant increase in the extent of trypan blue uptake by MIN6 cells (Table 2). In contrast, exposure of MIN6 cells to a maximal stimulatory combination of the nutrient secretagogue, 4α ketoisocaproate, and the protein kinase C activator, 4β phorbol myristate acetate, caused a significant increase in insulin secretion that was not accompanied by a loss of membrane integrity, as assessed by trypan blue uptake (Table 2).

Discussion

The results of the current study confirm previous observations that alcoholic extracts of the leaves of *Gymnema sylvestre* are capable of direct actions on pancreatic β-cells to increase the release of insulin (Shanmugasundaram *et al.* 1990). We found that GS4 caused a dose-related increase in insulin release from a variety of β-cell lines and rat islets in the absence of any other stimulus, and, although there were some minor differences in sensitivity to the extract, in all cases it caused a profound secretory response. In the case of islets, with which a concentration as high as 2 mg/ml was used, it was found that insulin release was in excess of 16 ng/islet per h. This high output, equivalent to ~50% of the islet insulin content, suggests that the effects of GS4 may not be physiological because, under these conditions, the insulin release would not be compensated for by sufficient insulin biosynthesis. Confirmation that the stimulatory effects of GS4 differed from those of other insulin secretagogues was provided by the observation that GS4 was able to stimulate insulin release at temperatures as low as 4 °C, whereas regulated physiological insulin secretion only occurs at temperatures in excess of 30 °C (Hedeskov 1980).

Table 2 Effect of GS4 fractionation products on insulin release and membrane integrity. MIN6 cells were incubated in a physiological salt solution for 1 h in the presence of the agents shown. A sample of the supernatant was removed for the measurment of insulin release, then the cells were incubated for a further 15 min at 37 °C in the presence of 0·1% (w/v) trypan blue. Data for insulin release are means ± S.E.M., n=6–8 and those for trypan blue uptake show the percentage range of cells to which the dye gained access, n=2

Treatment	Insulin release (ng/10^6 cells per h)	Cells taking up trypan blue (% total)
0 glucose	22·7 ± 3·3	5–10
0·5 mg/ml F2	85·4 ± 8·0***	95–100
0·5 mg/ml F43	41·4 ± 2·7***	50–60
0·5 mg/ml GS4	48·0 ± 4·7***	90–100
10 mM KIC +500 nM PMA	89·4 ± 7·3***	5–10

KIC, 4α ketoisocaproate; PMA, phorbol myristate acetate. ***P<0·001, compared with secretion at 0 glucose.

Evidence that GS4 was acting at a physical, rather than a physiological, level was provided by estimates of uptake of the membrane impermeant dye, trypan blue. Cells or islets that had been incubated in the absence of GS4 for 1 h showed low levels of trypan blue uptake, indicative of an intact plasma membrane capable of excluding dye entry. However, increasing concentrations of GS4 caused progressively more damage to the β-cells, such that, for all β-cell types, trypan blue gained access to virtually all cells when used in a concentration of 0·25 mg/ml. Similar results were obtained with whole islets, which consist of clusters of around 3–5000 cells, and dye uptake was observed at both 4 °C and at 37 °C. At 4 °C, the dye gained access to an outer mantle of cells, and at 37 °C more islet cells became permeable to trypan blue. The loss of membrane integrity after exposure to GS4 may have been mediated by gymnemic acids, a complex mixture of saponin glycosides, known to be present in the extract (Suttisri *et al.* 1995). Glycosides such as saponin and digitonin have long been used experimentally to permeabilise cellular membranes, including those of cells within islets of Langerhans (Biden *et al.* 1984, Colca *et al.* 1985), and it is known that their effects are independent of temperature (Schulz 1990) and that they cause loss of large cytosolic proteins when used in high concentrations (Ahnert-Hilger & Gratzl 1988). The likelihood that membrane damage resulted from the presence of glycosides was borne out experimentally using GS4 subfractions enriched in gymnemic acid saponins (F2 and F43), which also caused increased release of insulin from MIN6 cells concomitant with increased uptake of trypan blue dye.

Thus the ability of GS4 to stimulate insulin release at 4 °C and its effects on β-cell plasma membrane integrity are indicative of a mode of action in which GS4 causes insulin to leak from effectively permeabilised β-cells. There also appears to be a regulated component to the stimulatory effects of GS4, as the increase in insulin release was sensitive to chelation of extracellular Ca^{2+} by EGTA. However, the increase in insulin release still occurred after isradipine-induced channel blockade, indicating that it did not result from Ca^{2+} influx through voltage-operated Ca^{2+} channels. Moreover, in the presence of sufficiently high concentrations of GS4, there was still a substantial release of insulin, despite the presence of EGTA, suggesting that GS4 can affect the secretory apparatus independently of changes in Ca^{2+}.

In summary, the current data suggest that GS4 increases insulin release *in vitro* by two mechanisms: 1) the major mode of action is through permeabilisation of the β-cell plasma membranes, most likely resulting from the high saponin glycoside content of the extract, leading to unregulated loss of insulin from the cells; 2) there is also a Ca^{2+}-sensitive component, and at least part of this release of insulin may be dependent on channel-independent Ca^{2+} influx into the β-cells, perhaps through the pores formed by plasma membrane disruption. Thus, although extracts of *G. sylvestre* have been shown to reduce hyperglycaemia *in vivo*, the effects of GS4 on β-cells described herein suggest that its suitability as a potential novel treatment for NIDDM can not be assessed by direct measurements of islet and β-cell function *in vitro*. However, it is worth bearing in mind that the membrane-damaging effects of GS4 are unlikely to be observed *in vivo*, as sugars in the saponins will be hydrolysed off within the gastrointestinal tract, so islets are more likely to be exposed to the aglycone moiety. There is, as yet, no information on the effects of aglycones on insulin secretion *in vitro* or *in vivo*, but these components of *G. sylvestre* merit further investigation, particularly in light of the enhanced insulin concentrations observed *in vivo* after GS4 administration (Shanmugasundaram *et al.* 1990).

Acknowledgements

We are grateful to Elizabeth Redmond, Sudharshini Selvarajan and Anita Johansen for technical assistance, and to Dr A D Kinghorn, College of Pharmacy, University of Illinois, Chicago for providing the facilities for some of the fractionation work. We also thank the Government of Kuwait for a scholarship for H A-M.

References

Ahnert-Hilger G & Gratzl M 1988 Controlled manipulation of the cell interior by pore-forming proteins. *Trends in Pharmacological Sciences* 9 195–197.

Baskaran K, Ahamath BK, Shanmugasundaram KR & Shanmugasundaram ERB 1990 Antidiabetic effect of a leaf extract from *Gymnema sylvestre* in non-insulin-dependent diabetes mellitus patients. *Journal of Ethnopharmacology* 30 295–305.

Biden TJ, Prentki M, Irvine RF, Berridge MJ & Wollheim CB 1984 Inositol 1,4,5-trisphosphate mobilizes intracellular Ca^{2+} from permeabilized insulin-secreting cells. *Biochemical Journal* 223 467–473.

Billington DC, Perron-Sierra F, Picard I, Beaubras S, Duhault J, Espinal J & Challal S 1994 Total synthesis of novel conduritol related compounds capable of modulating insulin release. *Bioorganic and Medicinal Chemistry Letters* 4 2307–2312.

Colca JR, Wolf BA, Comens PG & McDaniel ML 1985 Protein phosphorylation in permeabilized pancreatic islet cells. *Biochemical Journal* 228 529–536.

Gey GO & Gey MK 1936 Maintenance of human normal cells in continuous culture: preliminary report; cultivation of mesoblastic tumors and normal cells and notes on methods of cultivation. *American Journal of Cancer* 27 45–76.

Hedeskov CJ 1980 Mechanism of glucose-induced insulin secretion. *Physiological Reviews* 60 442–509.

Henquin JC 1980 Tolbutamide stimulation and inhibition of insulin release: studies of the underlying ionic mechanisms in isolated rat islets. *Diabetologia* 18 151–160.

Jones PM, Stutchfield J & Howell SL 1985 Effects of Ca^{2+} and a phorbol ester on insulin secretion from islets of Langerhans permeabilised by high voltage discharge. *FEBS Letters* 191 102–106.

Jones PM, Salmon DMW & Howell SL 1988 Protein phosphorylation in electrically permeabilised islets of Langerhans. Effects of Ca^{2+}, cyclic AMP, a phorbol ester and noradrenaline. *Biochemical Journal* 254 397–403.

Jones PM, Mann FM, Persaud SJ & Wheeler-Jones 1993 Mastoparan stimulates insulin secretion from pancreatic β-cells by effects at a late stage in the secretory pathway. *Molecular and Cellular Endocrinology* 94 97–104.

Khare AK, Tondon RN & Tewari JP 1983 Hypoglycaemic activity of an indigenous drug (*Gymnema sylvestre*, 'Gurmar') in normal and diabetic persons. *Indian Journal of Physiology and Pharmacology* 27 257–258.

Miyatake K, Takenaka S, Fujimoto T, Kensho G, Upadhaya S, Kirihata M, Ichimoto I & Nakano Y 1993 Isolation of conduritol A from *Gymenma sylvestre* and its effect against intestinal glucose absorption in rats. *Bioscience, Biotechnology and Biochemistry* 57 2184–2185.

Schulz I 1990 Permeabilizing cells: some methods and applications for the study of intracellular processes. *Methods in Enzymology* 192 280–300.

Shanmugasundaram KR & Panneerselvam C 1981 The insulinotropic activity of *Gymnema sylvestre* R.Br. an Indian medical herb used in controlling diabetes mellitus. *Pharmacological Research Communications* 13 475–486.

Shanmugasundaram KR, Panneerselvam C, Samudram P & Shanmugasundaram ERB 1983 Enzyme changes and glucose utilisation in diabetic rabbits: the effects of *Gymnema sylvestre*, R.Br. *Journal of Ethnopharmacology* 7 205–234.

Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR & Rajendran VM 1990 Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given *Gymnema sylvestre* leaf extracts. *Journal of Ethnopharmacology* 30 265–279.

Srivastava Y, Bhatt HV, Prem AS, Nigam SK & Verma Y 1985 Hypoglycemic and life-prolonging properties of *Gymnema sylvestre* leaf extract in diabetic rats. *Israel Journal of Medical Sciences* 21 540–542.

Sturgess NC, Cook DL, Ashford MLJ & Hales CN 1985 The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* 2 474–475.

Suttisri R, Lee I & Kinghorn AD 1995 Plant derived triterpenoid sweetness inhibitors. *Journal of Ethnopharmacology* 47 9–26.

Yoshikawa K, Nakagawa M, Arihara S & Matssra K 1992 Antisweet natural products V. Structures of gymnemic acids VIII-XII from *Gymnema sylvestre* R. Br. *Chemical and Pharmaceutical Bulletin* 40 1779–1782.

Received 13 November 1998
Revised manuscript received 22 April 1999
Final manuscript received 22 June 1999
Accepted 22 June 1999

1: J Vet Med Sci. 1997 Apr;59(4):245-51.

Suppression of glucose absorption by some fractions extracted from Gymnema sylvestre leaves.

Shimizu K, Iino A, Nakajima J, Tanaka K, Nakajyo S, Urakawa N, Atsuchi M, Wada T, Yamashita C.

Division of Veterinary Pharmacology, Nippon Veterinary and Animal Science University, Tokyo, Japan.

Extracts containing gymnemic acids, which were extracted from the leaves of Gymnema sylvestre (GS) as nine fractions, were evaluated for their effects on a high K(+)-induced contraction of guinea-pig ileal longitudinal muscles, on glucose transport mediated by the difference of glucose-evoked transmural potential difference (delta PD) in the inverted intestine of guinea-pig and rat, and on blood glucose in rat. Among nine fractions obtained by high performance liquid chromatography from the extract, f-2 and f-4 strongly suppressed the high K(+)-induced contraction of the ileal muscle, f-3 and f-5 did so moderately, and f-8 and f-9 did so weakly, whereas the other fractions did not affect it. The degree of suppression of high K(+)-induced contraction by f-2 at 74% was almost the same as that of f-4 at 67%, at concentrations of 0.1 mg/ml. The suppressed contraction by f-2 or f-4 was recovered by adding 5.5 mM pyruvate. The delta PD increased by 5.5 mM glucose in the inverted intestines of guinea-pig and rat were equally suppressed by 0.1 mg/ml of f-2 or f-4 to 40%. In a rat sucrose tolerance test, f-2 and f-4 suppressed the elevation of blood glucose level. Both f-2 and f-4 suppressed the contraction of guinea-pig ileal longitudinal muscle, interfered with the increase in delta PD induced by glucose in the inverted intestines of guinea-pig and rat, and inhibited the elevation of blood glucose level. In conclusion, it is suggested that some of the extracts containing gymnemic acids from GS leaves suppress the elevation of blood glucose level by inhibiting glucose uptake in the intestine.

PMID: 9152931 [PubMed - indexed for MEDLINE]

1: World J Gastroenterol. 2001 Apr;7(2):270-4.

Inhibitory effect of voglibose and gymnemic acid on maltose absorption in vivo.

Luo H, Imoto T, Hiji Y.

Department of Physiology, Institute of Basic Medicine, CAMS/PUMC,5 Dongdansantiao Beijing 100005, China. hongluo1@263.net

AIM: To determine whether diabetic care can be improved by combination of voglibose and gymnemic acid (GA), we compared the combinative and individual effects of voglibose and GA on maltose absorption in small intestine. METHODS: The small intestine 30 cm long from 2 cm caudal ward Treitz's ligament of Wistar rat was used as an in situ loop, which was randomly perfused in recircular mode with maltose (10mmol/L) with or without different dosages of voglibose and/or GA for an hour. To compare the time course, perfusion of 10 mmol/L maltose was repeated four times. Each time continued for 1 hour and separated by 30 minutes rinse. In the first time, lower dosages of GA (0.5g/L) and/or voglibose (2 micromol/L) were contained except control. RESULTS: Absorptive rate of maltose was the lowest in combinative group ($P<0.05$, ANOVA), for example, the inhibition rate was about 37% during the first hour when 0.5 g/L-GA and 2 micromol/L voglibose with 10 mmol/L maltose were perfused in the loop. The onset time was shortened to 30 minutes and the effective duration was prolonged to 4 hours with the combination; therefore the total amount of maltose absorption during the effective duration was inhibited more significantly than that in the individual administration ($P < 0.05$, U test of Mann Whitney). The effect of GA on absorptive barriers of the intestine played an important role in the combinative effects. CONCLUSION: There are augmented effects of voglibose and GA. The management of diabetes mellitus can be improved by employing the combination.

PMID: 11819773 [PubMed - indexed for MEDLINE]

Effect of Long Term-Administration with *Gymnema sylvestre* R. Br on Plasma and Liver Lipid in Rats

Norihiro Shigematsu,*[,a] Ryuji Asano,[b] Makoto Shimosaka,[c] and Mitsuo Okazaki[d]

Biosci. Textile Technol., Shinshu University,[a] 3–15–1 Tokida, Ueda, Nagano 386–8567, Japan, Dept. Verterinary Pharmacol., College of Bioresource Sci., Nihon University,[b] 1866 Kameino, Fujisawa, Kanagawa 252–8510, Japan, Dept. Applied Biol., Shinshu University,[c] 3–15–1 Tokida, Ueda, Nagano 386–8567, Japan, and Gene Research Center, Shinshu University,[d] 3–15–1 Tokida, Ueda, Nagano 386–8567, Japan. Received November 16, 2000; accepted February 23, 2001

Extract of *Gymnema sylvestre* leaves was administered to rats receiving either a high fat diet or normal fat diet for 10 weeks to investigate its influence on plasma and liver lipids and on visceral fat accumulation. In addition, its effect was compared with those of chitosan and the influence of combined use of these two substances was also evaluated. Within the high fat diet groups, the extract suppressed body weight gain and accumulation of liver lipids to the same extent as chitosan and the combined use. In addition, intraperitoneal fat and fat drop vacuoles on the epithelium of renal tubules, noted in the high fat diet group, were scattered by administration of the extract with the same results as for chitosan and combined use. Within the normal fat diet groups, plasma triglyceride levels decreased by administration of the extract, with similar results as chitosan and combined use. Concerning plasma total cholesterol, there was no decreasing effects with the extract, as found with chitosan and combined use. However, the effect of chitosan on plasma total cholesterol tended to be enhanced when used in combination with the extract. In addition, long-term administration of the extract did not show any influence on hematological and blood chemical parameters.

Key words *Gymnema sylvestre*; chitosan; plasma lipid; liver lipid

Gymnema sylvestre R. Br. is a wild plant classified in the Asclepiadaceae family and is widely distributed in Southern India, tropical Africa and Australia. It has been used in Indian traditional medicine, "Ayurvedic medicine," from ancient times and is said to be effective in promoting urination and digestion, tonifying and improving diabetes.[1,2] Its suppressive effects on sweetness are well known.[3] In Japan, *G. sylvestre* has been widely used as a health food in tea bags, tablets, beverages and confectioneries in recent years. There has been a lot of research on its involvement in carbohydrate metabolism from the viewpoint of nutrition and food science or pharmacognosy.[4—6] Various effects have been reported, such as suppression of glucose absorption in the small intestine of rats, reduction of plasma glucose increment in the oral sucrose tolerance test, significantly lowered blood glucose and insulin values in dogs as well as suppression of insulin increase in glucose tolerance tests in men and the alleviation of diabetic symptoms in patients with non-insulin-dependent diabetes mellitus.[7,8] As for the active substances involved in *G. sylvestre*, the triterpenoid saponin and its derivatives have been identified. These are glycosides where gymnemagenin is formed by attachment of glucronic acid to the triterpenoid structure as aglycone. This glycoside and its derivatives are referred to as gymnemic acids.[9] Other than these glycosides, conduritol A with a tetrahydroxyhexene structure has been also confirmed to be involved in glucose absorption. Moreover, the peptide grumarin, which is a peptide consisting of 35 amino acids, has been shown to be involved in suppression of sweetness.[10] However, there are fewer reports dealing with its influence on lipid metabolism compared to those on carbohydrate metabolism. Findings concerning the lipid metabolism of *G. sylvestre* thus far reported are as follows: (1) although it slightly decreased serum cholesterol and triglyceride values in obese rats fed on high carbohydrate and low fat diets, there were no effects observed in emaciated rats; (2) intraperitoneal injection of gymnemic acid had no influence on blood cholesterol, triglyceride or free fatty acid[4]; (3) when the extract of *G. sylvestre* leaves, highly concentrated with gymnemic acid, was administered at high dose levels, fecal excretion of steroids showed a dose-related increase.[11] All these findings were obtained under limitative conditions. The aims of this current study were to investigate the long-term influence of *G. sylvestre* on lipid metabolism in rats fed a high fat diet, and to compare with the effects of chitosan,[12,13] a substance known to be effective in lowering cholesterol levels. In addition, the effects of these treatments on rats with a normal fat diet were investigated in the same way. This experiment was performed according to the Experimental Animal Regulations (IEXAS).

MATERIALS AND METHODS

Materials The feed stuff CE-2 (product of Nihon Crea Co., Ltd.) was used as normal fat diet (NF), and a modified feed stuff was used as a high fat diet (HF), in which beef tallow was added to CE-2 to the extent of 20% by weight. The nutritive constituents of the respective diets were as follows; water content (NF: 8.8%, HF: 7.0%), protein (NF: 25.2%, HF: 20.7%), fat (NF: 4.4%, HF: 23.5%), fiber (NF: 4.4%, HF: 3.5%), ash (NF: 7.0%, HF: 5.5%), nitrogen free extract (NF: 50.2%, HF: 39.8%), total calories (NF: 341 kcal/100 g, HF: 460 kcal/100 g).

The leaves of *G. sylvestre* were dried and extracted with 50% hydrous alcohol (at 60 °C for 3 h). It was then dried before being used as gymnema extract (G) (total gymnemic acid content:[14] 2.4%). Chitosan (C) employed here was adjusted to 85% deacetylation, 10^5—10^6 molecular weight, and 100 cps viscosity (estimated by a B-type viscosity meter at 0.5% and 20 °C).

Experimental Animals and Rearing Conditions Five-weeks-old male Jcl: Wistar rats were obtained from Nihon Crea Co., Ltd. Two rats were kept in each polycarbonate cage

* To whom correspondence should be addressed. e-mail: noshigematsu@fancl.co.jp

(CL-0106 from Nihon Crea Co., Ltd.) with a size of 345×403×177 mm, and were maintained at room temperature (23±2 °C) and a relative humidity of 55±5% on a light/dark cycle of 14 h/10 h (lighted up between 6:00 am and 8:00 pm). All rats were provided with NF for two weeks after initiation of preliminary rearing, and thereafter they were classified into NF-fed and HF-fed groups. The NF- and HF-fed groups were provided with NF and HF respectively until the end of the experiment. Four weeks after classification into NF- and HF-fed groups, each of the two groups (11-week-old rats) was divided into the following four groups (n: number of individuals reared in each group=10): control groups (cont-NF, cont-HF), G (G-orally-provided) groups (G-NF, G-HF), C (C-orally-provided) groups (C-NF, C-HF), and GC (provided with a mixture of G and C) groups (GC-NF, GC-HF). The treated groups were orally administered with G, C and GC once a day (10:00 a.m.—12:00 noon) for 10 weeks. The oral doses of G, C and GC were; G: 33 mg/kg (=amount required to stabilize blood glucose value in men), C: 8 mg/kg (=amount required to decrease plasma cholesterol value in men), and GC: 41 mg (33 mg of G+8 mg of C)/kg respectively. Each dose, after being suspended in 2 ml of distilled water, was given through a stomach tube. On the other hand, 2 ml of distilled water was orally given to each control group in the same way. Rats were allowed *ad libitum* to access the feed stuff and drinking water; body weight and feed intake was measured everyday during this experimental period. Rats were starved for 18 h after the end of the experiment, and blood was taken from the jugular veins under anesthesia using sodium pentobarbital. This blood was used for measurement of plasma lipid and hematological biochemical parameters. Heart, liver and kidney were extracted for measurement of their weight. In addition, the extracted livers were used for measurement of liver lipids and histopathlogical examination. Moreover, kidneys were also used for histopathological examination.

Hematological Biochemical Examination Immediately after blood samples were collected, the red blood cell (RBC) counts and white blood cell (WBC) counts were measured by the use of the Celltak (product of Nihon Koden Corp.). Thereafter, blood plasma was isolated and total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglyceride were measured by the use of Spotchem2-T-Chol, Spotchem2-HDL-C and Spotchem2-TG (Arkray Co., Ltd.), respectively. Further measurements were carried out for blood urea nitrogen (BUN), albumin (Alb), creatinine (Cre), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) and total bilirubin (T-Bil) by the use of Spotchem2-BUN, Spotchem2-Alb, Spotchem2-Cre, Spotchem2-GOT, Spotchem2-GPT and Spotchem2-T-Bil (Arkray Co., Ltd.), respectively. For quantification of these parameters, the Spotchem SP-4410[15] (Arkray Co., Ltd.) was utilized. Furthermore, the free fatty acid (FFA) content and lipoperoxide activity (Lipop) were measured by use of the Iatrolipo NFFA (Yatoron Co., Ltd.) and Determiner LPO (Kyowa Medics Co., Ltd.), respectively.

Organ Weight and Liver Lipid The extracted organs (heart, liver and kidney) were perfused by the use of cooled physiological saline for removal of remaining blood. Thereafter, the organs were weighed and the livers were frozen at −30 °C for preservation. Lipids were extracted from the livers by the method of Folch *et al.*[16] and total cholesterol and triglyceride levels were measured by use of commercial assay kits (Determiner TC555: CE-COD-POD method,[17] Determiner TG.S : LPL-GK-GPO method[18]) (Kyowa Medics Co., Ltd.).

Histopathological Examination Extracted liver and kidney were stained with hematoxylin-eosin (HE) for histopathological examination, and the tissue samples were examined microscopically.

Statistical Treatment In each diet group, after performing one-way analysis of variance, the significance of a difference was tested by Dunnet's method and $p<0.05$ *vs.* control group was considered to be statistically significant. To evaluate the effect of co-administration of G and C, the group receiving GC was compared with the control group and with the C group using Student's *t*-test. Administration of GC was considered to be significantly different from C when the group that received GC showed a greater effectiveness than both the C group and the control group at $p<0.05$.

RESULTS

Feed Intake and Body Weight In the HF-fed group, the feed intake tended to be lower by about 10% than the NF-fed group from the time feed was changed from NF to HF during the experimental period. Addtionally, the feed intake of the G-HF, C-HF and GC-HF groups almost the same as the cont-HF group during the experimental period. Similarly, in the NF-fed group, no significant difference was found in the feed intake of the G-NF, C-NF and GC-NF groups when compared to the cont-NF group (data not shown).

The changes in body weight in the NF- and HF-fed groups during the experimental period are shown in Fig. 1. The body weight in the cont-HF group tended to increase from the 5th week after the switch from NF-feeding to HF-feeding (the 7th week from the start of feeding) to the end of the experiment. Nevertheless, in the G-HF, C-HF and GC-HF groups, the increase in body weight was repressed from the beginning of oral administration of G, C, GC to the end of the experiment. At the end of the experiment, the body weights of the G-HF, C-HF and GC-HF groups were significantly depressed when compared to cont-HF. In the NF-fed group, no significant difference was found in the increase of body weights among the G-NF, C-NF and GC-NF groups compared to cont-NF during the experimental period.

Plasma Lipid Plasma total cholesterol and triglyceride were measured after starvation (for 18 h) at the end of the experiment. These values are shown in Fig. 2. In the NF-fed group, the plasma total cholesterol value in the G-NF group had no statistical significant difference compared to cont-NF. Nevertheless, the value in the C-NF group displayed a significant decrease ($p<0.05$) compared to cont-NF. Also, in the GC-NF group, its value was significant decreased ($p<0.05$) compared to cont-NF. In addition, in a comparison of the plasma total cholesterol between cont-NF and GC-NF, or between C-NF and GC-NF, a significant decrease ($p<0.05$) was seen in both cases. In the HF-fed group, there were no significant differences between the plasma total cholesterol values in cont-HF and its value in G-HF, C-HF and GC-HF. In the NF-fed group, the plasma triglyceride value in the G-NF, C-NF and GC-NF groups showed a significant decrease

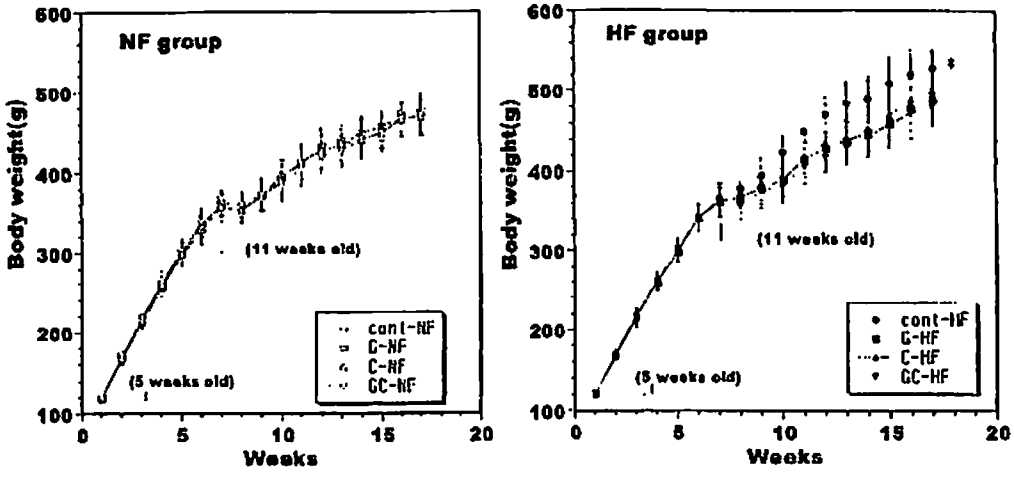

Fig. 1. Change of Body Weight in Rats Fed on NF or HF during the Experimental Period

Each point represents the mean of body weight. Vertical bars represent S.D. ($n=10$). Symbol (*) represents $p<0.05$ compared with G or C or GC-HF at the end of the experiment.

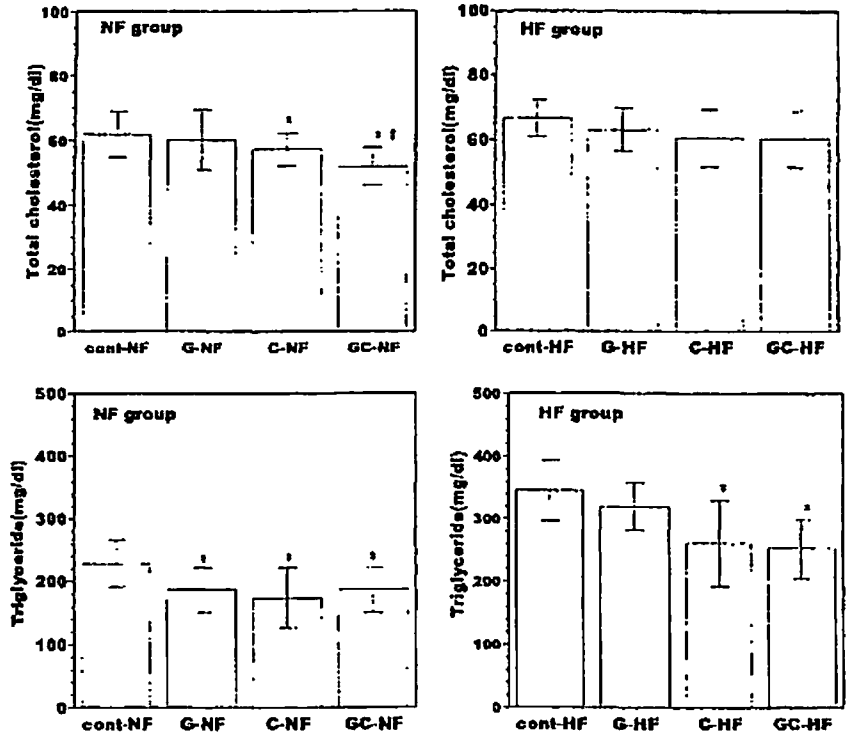

Fig. 2. Effects of Administration with G, C, GC on Plasma Lipid in Rats Fed on NF or HF at the End of the Experiment

Vertical bars represent S.D. ($n=10$). Statistical difference: * $p<0.05$ compared with cont-NF, # $p<0.05$ compared with C-NF.

($p<0.05$) compared to cont-NF. In comparisons between cont-NF and GC-NF or between C-NF and GC-NF, no significant difference was seen in both cases and no effect of co-administration with G and C on the plasma triglyceride value was identified. In the HF-fed group, plasma triglyceride values in G-HF had a decreasing tendency ($p<0.1$) compared to cont-HF, though not significant statistically. In the case of C-HF or GC-HF, a significant decrease ($p<0.05$) compared to cont-HF was noted. Moreover, no significant difference was found in a comparison of its value between GC-HF and C-HF. In addition, HDL-cholesterol levels were 26.8±4.9 mg/dl in cont-NF and 26.4±4.7 mg/dl in cont-HF. In both NF- and HF-fed groups, no influence was identified for G, C and GC by oral administration compared to cont-NF or cont-HF (data not shown).

Hematological Biochemical Parameters The results of hematological biochemical examination carried out after starvation (for 18 h) at the end of the experiment are shown in Table 1. In the NF-fed group, there was no influence of administration with G, C, GC on RBC count, WBC count, BUN, albumin, creatinine, GOT, GPT, total bilirubin, FFA and lipoperoxide compared to cont-NF. In addition, there was no effect in the HF-fed group administrated with G, C, GC compared to cont-HF.

Organ Weight and Liver Lipid The organ weights of rats are shown in Table 2. In both NF-fed and HF-fed groups,

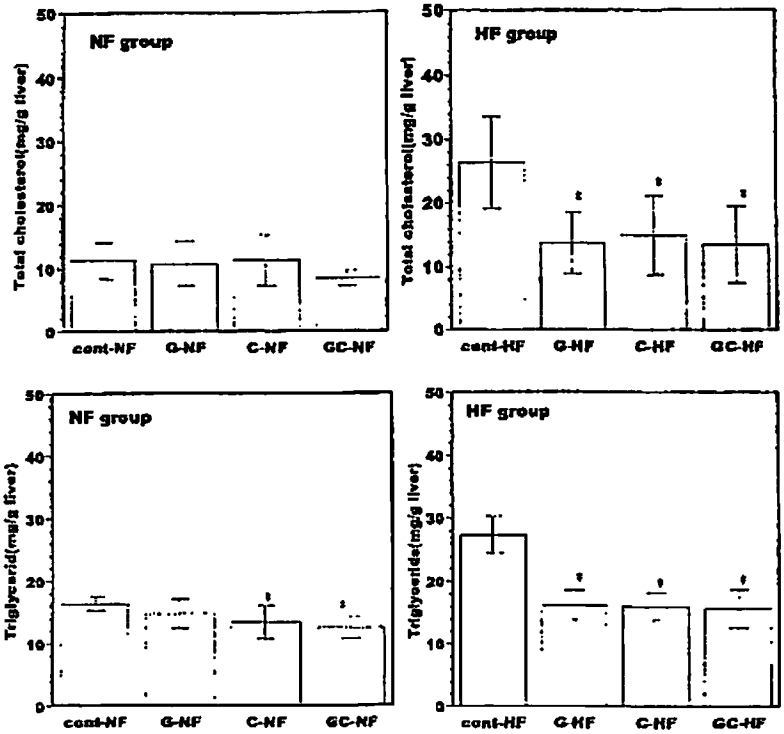

Fig. 3. Effects of Administration with G, C, GC on Liver Lipid in Rats Fed on NF or HF at the End of the Experiment
Vertical bars represent S.D. (n=8). Statistical difference: * p<0.05 compared with control group.

Table 1. Results of Hematological and Plasma Biochemical Examination at the End of the Experiment

	RBC ($\times10^4$/mm^3)	WBC ($\times10^2$/mm^3)	BUN (mg/dl)	Alb (g/dl)	Cre (mg/dl)	GOT (IU/l)	GPT (IU/l)	T-Bil (mg/dl)	FFA (μeq/l)	Lipop (nmol/ml)
Cont-NF	979±109	146±14	29±4	3.7±0.3	0.6±0.2	52.4±14.4	22.0±6.1	0.3±0.1	500±141	0.7±0.4
G-NF	973±86	150±13	29±2	3.7±0.4	0.8±0.3	39.1±19.8	20.2±8.6	0.3±0.1	490±129	0.4±0.4
C-NF	982±90	147±18	29±3	3.9±0.5	0.9±0.3	43.2±11.2	23.2±5.9	0.2±0.0	482±110	0.5±0.5
GC-NF	978±102	152±12	29±3	3.8±0.3	0.9±0.3	48.1±14.8	19.8±7.2	0.2±0.1	475±135	0.4±0.3
Cont-HF	996±89	140±20	28±4	3.8±0.2	0.8±0.2	39.1±10.5	24.3±7.0	0.3±0.2	533±148	0.5±0.3
G-HF	990±109	149±28	26±4	3.7±0.3	0.9±0.3	46.8±12.8	22.3±9.1	0.3±0.2	571±135	0.4±0.4
C-HF	965±97	144±24	27±5	3.6±0.5	0.9±0.3	48.2±15.6	20.8±5.0	0.4±0.3	560±111	0.5±0.4
GC-HF	978±80	138±21	27±4	3.7±0.3	0.9±0.4	41.4±16.0	21.4±7.6	0.3±0.2	513±110	0.5±0.5

Each value represents the mean±S.D. (n=10).

Table 2. Organ (Heart Liver Kidney) Weight of Rats in the Experimental Groups at the End of the Experiment

	Heart (g/100 g)	Liver (g/100 g)	Kidney (g/100 g)
Cont-NF	0.30±0.02	3.46±0.2	0.59±0.06
G-NF	0.29±0.02	3.39±0.2	0.59±0.04
C-NF	0.30±0.02	3.44±0.2	0.60±0.02
GC-NF	0.30±0.02	3.57±0.3	0.60±0.04
Cont-HF	0.26±0.01	3.02±0.2	0.45±0.03
G-HF	0.29±0.03	3.39±0.4	0.57±0.04
C-HF	0.28±0.02	3.28±0.2	0.56±0.04
GC-HF	0.29±0.02	3.46±0.3	0.58±0.04

Each value represent the mean±S.D. (n=8).

no influence on organ weights was identified for oral administration of G, C and GC compared to each control group. Total cholesterol and triglyceride levels in liver removed immediately after completion of the experiment are shown in Fig. 3. Total cholesterol values in the NF-fed group were not influenced by oral administration with G, C or GC compared to cont-NF. However, in the HF-fed group, the values in the G-HF, C-HF and GC-HF groups showed a significant decrease (p<0.05) compared to cont-HF. In the NF-fed group, triglyceride values in G-NF had no significant difference compared to cont-NF, however, the C-NF and GC-NF groups showed significant decrease (p<0.05) compared to cont-NF. Nevertheless, in the HF-fed group, tirglyceride values in the G-HF, C-HF and GC-HF groups had a significant decrease (p<0.05) compared to cont-HF.

Histopathological Examination Rats in cont-HF and G-HF at the end of the experiment are shown in Fig. 4. Rat in the cont-HF group (left side) grew corpulent compared to rats in the G-HF group (right side). In addition, histopathological findings revealed that intraperitoneal fat was accumulated in the cont-HF group, while it was markedly inhibited in the G-HF group (Fig. 4). Nearly the same findings as G-HF were noted in the C-HF and GC-HF groups (data not shown). The results of histopathological examination of he-

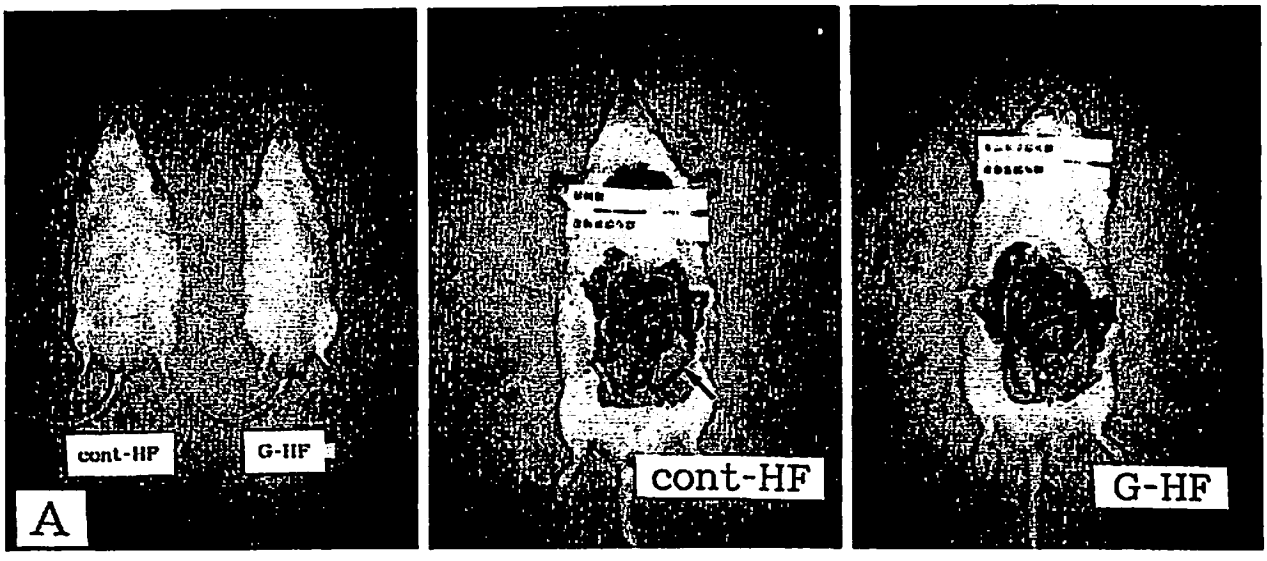

Fig. 4. Rats at the End of the Experiment
Left side, cont-HF; right side, G-HF; arrow, intraperitoneal fat.

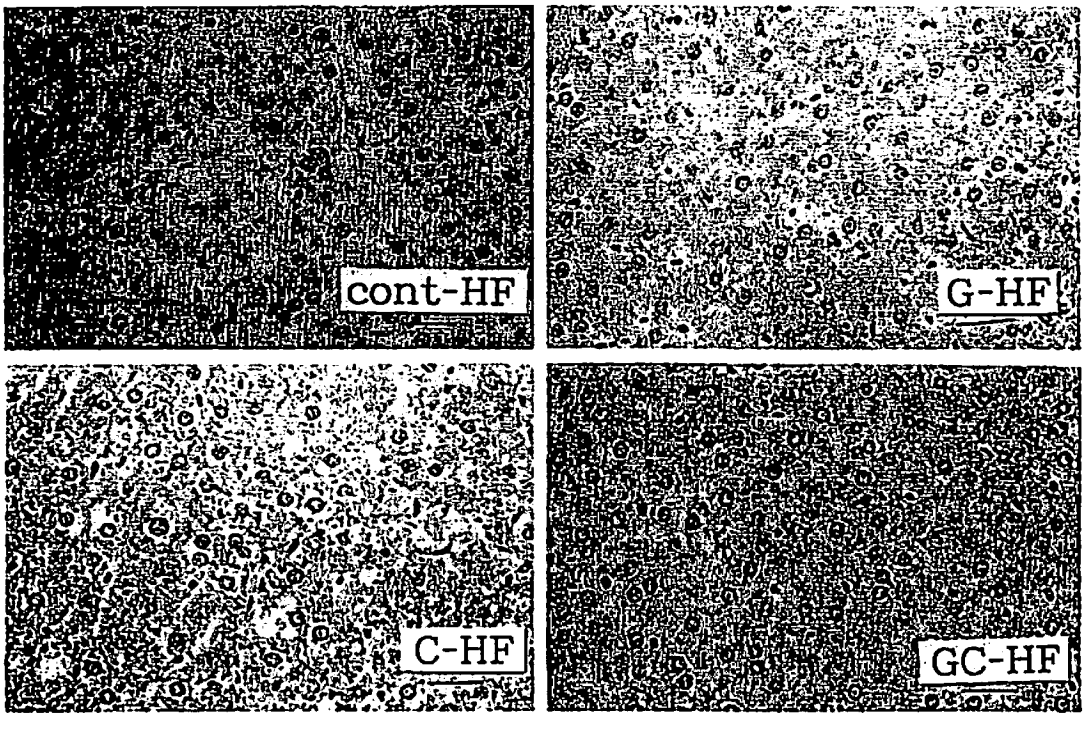

Fig. 5. Hepatocyte in Cont-HF, G-HF, C-HF and GC-HF
Hematoxylin-eosin stain (×150).

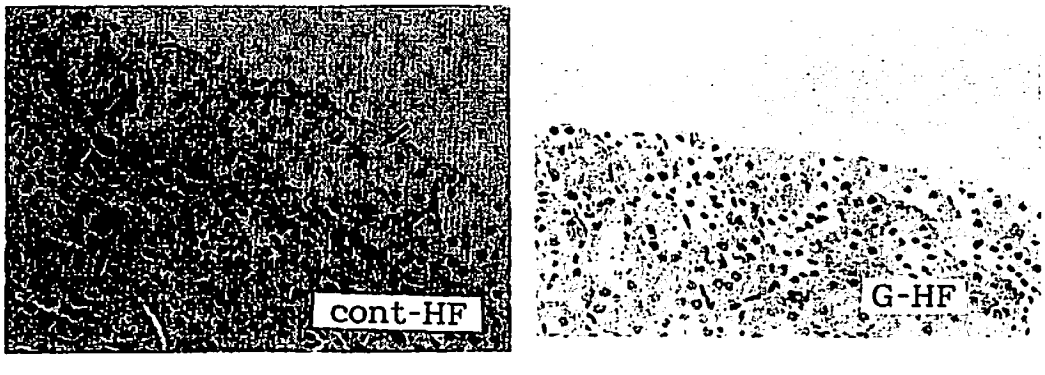

Fig. 6. Kidney in Cont-HF and G-HF
Hematoxylin-eosin stain (×300). Arrow: fat droplet vacuoles.

patocyte in the HF-fed groups are shown in Fig. 5. Fat droplets markedly accumulated in the liver of cont-HF, but fat deposition was inhibited in G-HF as a whole, though some fat vacuoles were evident. The same results were obtained with the C-HF and GC-HF groups. In the kidney, fat droplet vacuoles were noted on the epithelium of renal tubules in cont-HF, while they were noted only on the surface of cortex in G-HF (Fig. 6).

Nearly the same findings as G-HF were noted in the C-HF and GC-HF groups (data not shown). In addition, in NF fed groups, no abnormal influence was histopathologically observed (data not shown).

DISCUSSION

Since *G. sylvestre* has been widely used as dietary a food material in Japan, it has become more important to investigate the long-term effects of administration. Moreover, edible fats occupy an increasing portion of the total caloric intake in the Japanese diet of today. Therefore, the influence of *G. sylvestre*, which is effective in improving carbohydrate metabolism, on lipid metabolism in a high fat diet is very interesting from a real life point of view.

In this study, abnormal values were not observed upon hematological biochemical and histopathological examination. These results suggest the safety of *G. sylvestre* as a dietary food or functional food material. In cont-HF, the plasma cholesterol level was little affected by HF feeding, but plasma triglyceride levels and liver lipids (cholesterol and triglyceride) tended to be increased. Beef tallow has less influence on blood cholesterol level, but it increases the blood triglyceride level and accumulates liver lipid just like other edible fats.[19,20] These effects were reported by Pan *et al.* and are similar to the plasma and liver lipids level in cont-HF.

Concerning plasma total cholesterol level, administration of G alone did not have any effect, although it was found in NF-fed rats that the lowering effect of C was enhanced by co-administration of G and C. The effect of chitosan on blood cholesterol by oral administration has previously reported.[12,13] Moreover, ascorbic acid is known to intensify the inhibitory effect of chitosan on lipid absorption in the digestive tract. This potentiating effect of ascorbic acid is thought to be due to the increased solubility of chitosan in the digestive tract and to the decreased viscosity of chitosan induced by ascorbic acid.[12] Whether the triterpene saponin in G can display such an action similiar to ascorbic acid is not clear, although G may have affected the behavior of C in the digestive tract when combined with C and G may enhance the lowering effect of C on plasma total cholesterol level consequently. In both NF- or HF-fed rats, the plasma HDL-cholesterol level was not influenced by G, C or GC. Thus, the decrease of plasma total cholesterol in NF-fed rats can be considered to be due to the decrease of LDL- and VLDL-cholesterol. In NF-fed rats, G decreased the plasma triglyceride level and the same decreasing effect was also found in the C-NF and GC-NF groups. Therefore, G can be expected to be equally effective as chitosan under the conditions of a normal fat diet. In HF-fed rats, this value was little affected by G and significantly decreased by C and GC. It was considered from these findings that the decreasing effect of G on plasma triglyceride levels was weaker than that of chitosan with a high fat diet.

In this study, the lowering effects of G on cholesterol and triglyceride levels in liver were identified using a high fat diet and gave the same results as with chitosan and with combined use. The decreasing effect of G on lipid accumulation in liver was also identified by histopathological examination of hepatocytes and no abnormality was noted. Moreover, the decreasing effects of G on intraperitoneal fat and fat drop vacuoles on the epithelium of kidney noted in obese rats fed a high fat diet were identified. In addition, hematological biochemical findings also showed no abnormal data. Thus, it was suggested that *G. sylvestre* would safely suppress the accumulation of liver lipids and visceral fat at the same level as chitosan and improve obesity as a whole when administered for a long time under a high fat diet. In general, the decrease of triglyceride[21] in liver may result from (1) the suppression of lipoprotein and lipid synthesis in liver, (2) the acceleration of fatty acid oxidation in liver, (3) the acceleration of VLDL secretion from liver. In general, the decrease of cholesterol in liver may result from (1) the effects of transfer acceleration of cholesterol from liver to blood, (2) the inhibition of cholesterol synthesis in liver, (3) increased of cholesterol to bile acid, (4) the hydrolysis improvement of cholesterol ester in liver, and (5) suppression of absorption of cholesterol from intestine. Generally, the decrease of cholesterol[22] in liver may lead to a decrease of VLDL synthesized in liver and secreted to the blood. On the other hand, the decrease of cholesterol in liver may induce activation of the LDL receptor and as a result, the intake of LDL from blood to liver increases. In addition, the decrease of cholesterol in liver may lead to a shortage of blood cholesterol. In this study, the decreasing effect of C on total cholesterol and triglyceride levels in plasma tended to be linked to the decrease of these materials in liver. G decreased the cholesterol and triglyceride levels in liver more markedly than in plasma. These results suggest that G may affect lipid metabolism through a different mechanism to C.

The active ingredients contained in *G. sylvestre* should be investigated further in detail. Gymnemic acid has been investigated frequently in relation to carbohydrate metabolism. Regarding the relationship between lipid metabolism and gymnemic acid, it has been reported by Nakamura *et al.*[11] that when the extract containing 58% of gymnemic acid was administrered at doses of more than 1.0 g/kg, the excretion of acidic steroids and neutral steroid to feces were significantly increased and hepatic cholesterol was decreased, but blood cholesterol level was unaffected.[11] These results, where hepatic lipids are more liable to be affected by gymnemic acid than blood lipids, agree with the results of the present study. However, it has also been reported that feed intake was decreased and body weight gains were consequently depressed at the above doses.[11] In the present study, the total gymnemic acid content in G was 2.4% and the oral dose of G was 33 mg/kg. Thus, both gymnemic acid and the extract amount were drastically less than in the other report.[11] Though we can not directly compare our results with the results in the earlier report,[11] due differences in the experimental diet and dose of gymnemic extract, our results do not necessarily indicate that only gymnemic acid influences lipid metabolism. Other substances besides gymnemic acid may have an influ-

ence on lipid metabolism. In this study, the mechanism of the decreasing effect exerted by G is not clear and more research is required. Further studies are required to clarify the effect of *G. sylvestre* on lipid metabolism.

REFERENCES

1) Nadkarni A. K., "Indian Material Medica," Vol. 1, Popular Prakashan Pvt, Ltd., Bombay, 1982, pp. 596—599.
2) Mhaskar K. S., Caius J. F., *Ind. Med. Res. Memories.*, 16, 2—75 (1930).
3) Kurihara K., "Taste Modifiers. Handbook of Sensory Physiology," Vol. IV Chemical Sense, Part 2, Beidler L. M., Springer-Verlag, Berlin, 1971, pp. 365—370.
4) Terasawa H., Miyoshi M., Imoto T., *Yonago Acta Med.*, 37, 117—127 (1994).
5) Abe T., *J. Yonago Med. Ass.*, 44, 198—209 (1993).
6) Hirata S., Terasawa H., Katou T., Imoto T., *J. Yonago Med. Ass.*, 43, 397—404 (1992).
7) Hirai S., Abe T., Imoto T., *J. Yonago Med. Ass.*, 43, 392—396 (1992).
8) Baskaran K., Kizr B., Ahamath K. Radha., Shanmugasundaram E. R. B., *J. Ethnopharmacology*, 30, 295—305 (1990).
9) Yoshikawa M., Murakami T., Kadota M., Li Y., Murakami N., Matsuda H., *Chem. Pharm. Bull.*, 45, 1671—1676 (1997).
10) Ota M., Tonosaki K., Miwa K., Furuwatari T., Ariyoshi Y., *Biopolymers*, 39, 199—205 (1996).
11) Nakamura Y., Tsumura Y., Tonogai Y., Shibata T., *J. Nutr.*, 129, 1214—1222 (1999).
12) Kanauchi O., Deuchi K., Imasato Y., Kobayashi E., *Biosci. Biotech. Biochem.*, 58, 1617—1620 (1994).
13) Deuchi K., Kanauchi O., Imasato Y., Kobayashi E., *Biosci. Biotech. Biochem.*, 58, 1613—1616 (1994).
14) Yokota T., Mizutani K., Okada K., Tanaka O., *Nippon Shokuhin Kyogyo Gakkaishi*, 41, 202—205 (1994) (in Japanese).
15) Shibata A., *J. New Rem. & Clin.*, 42, 114—123 (1993).
16) Folch J., Lees M., Stanley S. H., *J. Biol. Chem.*, 266, 497—507 (1957).
17) Richmond W., *Clin. Chem.*, 19, 1350 (1973).
18) Uwajima T., *Methods in Enzymol.*, 89, 243 (1982).
19) Pan D. A., Storlien L. H., *J. Nutr.*, 123, 512—519 (1993).
20) Shimomura Y., Tamura T., Suzuki M., *J. Nutr.*, 120, 1291—1296 (1990).
21) Dietschy J. M., Wilson J. D., *New Eng. J. Med.*, 282, 1179—1241 (1970).
22) Venkatese S., Ward R. J., Peters T. J., *Biochem. Biophys. Acta*, 960, 61—66 (1988).

Original Paper

Comparative Effects of Chromium, Vanadium and Gymnema Sylvestre on Sugar-Induced Blood Pressure Elevations in SHR

Harry G. Preuss, MD, S. Taylor Jarrell, BS, Rich Scheckenbach, PhD, Shari Lieberman, PhD, Richard A. Anderson, PhD

Department of Medicine, Nephrology (H.G.P., S.T.J.), Georgetown University Medical Center, Washington, DC; Institute Biomolecular Nutrition (R.S.), Eugene, Oregon; New York City (S.L.); US Department of Agriculture (R.A.A.), Agriculture Research Service, Beltsville Human Nutrition Center, Beltsville, Maryland

Key words: Hypertension, sucrose-induced; chromium polynicotinate, effects on BP; bis(maltolato)oxovanadium, effects on BP; Gymnema sylvestre, effects on BP; TBARS, chromium effects

Objective: Effects on systolic blood pressure (SBP) of ingesting three agents reported to influence insulin metabolism, i.e., chromium polynicotinate, bis(maltolato)oxovanadium (BMOV), and the herb, *Gymnema sylvestre*, were assessed simultaneously in spontaneously hypertensive rats (SHR).

Methods: In the first study, SHR were fed either a starch, sugar, or sugar diet containing chromium polynicotinate, bis(maltolato)oxovanadium (BMOV), or *G. sylvestre*. Tail SBP was estimated indirectly and various blood chemistries were measured. TBARS formation was determined in hepatic and renal tissue. In a second study, tail SBP was measured in SHR ingesting diets containing different concentrations of BMOV.

Results: Compared to starch, SHR consuming sucrose showed a significant elevation of SBP within days that was maintained for the duration of study. Addition of chromium polynicotinate to the sucrose diet at the beginning of study prevented the sucrose-induced elevation of SBP for 2 weeks, but SBP rose significantly after that. BMOV at high concentrations overcame the sucrose-induced rise in SBP and even decreased SBP below values seen in SHR eating the starch diet, but marked weight loss was noted. A second study examined different concentrations of BMOV. At 0.01% w/w concentration of BMOV, SBP was still significantly decreased, even though SHR did not lose body weight (BW) early on. SHR consuming *G. sylvestre* showed no change or even elevated SBP. Hepatic thiobarbituric acid reacting substances (TBARS) formation, an estimate of lipid peroxidation, was decreased by chromium polynicotinate and BMOV, and renal TBARS by chromium polynicotinate. Circulating cholesterol concentrations were decreased in the SHR consuming *G. sylvestre*.

Conclusions: Chromium decreases the portion of SBP elevated by high sucrose intake as shown previously, but high levels of sucrose ingestion can eventually overcome this. BMOV overcame sucrose-induced elevation of SBP as well as some of the "genetic hypertension." Different from chromium, this decrease was not overcome by high levels of dietary sucrose. The significant lowering of cholesterol with *G. sylvestre* ingestion indicates some effect on metabolism, but *G. sylvestre* did not lower and even raised SBP.

INTRODUCTION

A cause-effect relationship between insulin perturbations (insulin resistance/hyperinsulinemia) and elevated blood pressure (BP) has been suggested [1–4], based largely on findings in diabetics and hypertensives [5]. Fortunately, a good laboratory model exists to examine this possibility: insulin resistance, hyperinsulinemia, and hypertension develop in certain rat strains fed diets containing high concentrations of sugars [6–9]. In support of a cause-effect relationship, a number of agents that favorably influence the insulin system by enhancing insulin sensitivity and/or reducing circulating insulin concentrations have been shown to lower BP in this frequently studied rat model, e.g., somatostatin [10], soluble fibers [11], vanadium

Mention of trade name, proprietary product, or vendor does not constitute a warranty of the product by the USDA or imply its approval to the exclusion of other products or vendors that may also be suitable.

Address reprint requests to: Harry G. Preuss, MD, FACN, Georgetown University Medical Center, Bldg D, Room 371, 4000 Reservoir Rd. NW, Washington, DC 20007.

Journal of the American College of Nutrition, Vol. 17, No. 2, 116–123 (1998)
Published by the American College of Nutrition

[12,13], chromium [14], metformin [15], and troglitazone [16]. In the present study, we simultaneously compared effects on BP of three agents with the potential to beneficially influence the insulin system in different ways, i.e., chromium polynicotinate, bis(maltolato)oxovanadium (BMOV), and *Gymnema sylvestre* (*G. sylvestre*), an herb known to affect the glucose/insulin system favorably [17], in spontaneously hypertensive rats (SHR). Chromium and vanadium influence the insulin system at the periphery, perhaps by different mechanisms [12–14], whereas *G. sylvestre* appears to work at the pancreatic level [17]. We found that effects of each agent on BP and other parameters differed.

MATERIAL AND METHODS

The first study was designed to compare the effects of chromium polynicotinate, BMOV, and the herb *G. sylvestre* on various parameters in SHR. The second study examined effects of the vanadium compound in more detail.

Male SHR of the Okamoto strain [18], weighing 150 to 200 g, were obtained from Taconic Farms, Germantown, NY. In the first study, five dietary groups were set up; each contained six SHR. After 2 to 3 weeks of acclimatization to laboratory chow, rats were provided special diets for 1 month. The five special diets were obtained from Teklad, Inc, Madison, WI (Table 1). Attempts were made to keep all diets similar with the exception of the constituent under study. The first diet derived 52% of calories from cornstarch and the last four diets replaced starch with sucrose to provide 52% of calories (Table 1). Minerals and vitamins were added at AIN (American Institute of Nutrition) levels. Additional chromium was added to the third diet as chromium polynicotinate (ChromeMate, Trade Name, InterHealth, Concord, CA) to elevate chromium content to 0.0005% w/w, and vanadium was added to the fourth diet as BMOV, (kind gift of Dr. John McNeill, University of British Columbia, Vancouver, BC, Canada) to elevate vanadium concentrations to 0.12% w/w. In the last diet, *G. sylvestre*, added at a concentration of 1.6% w/w, replaced some cellulose. The

Table 1. Basic Diets

Ingredients	% by Weight	% of Calories
Starch or sucrose	57.00	52.1%
Vegetable oil	16.44	36.0%
Casein	13.00	11.9%
Mineral mix, AIN 76A	4.00	
Vitamin mix, AIN 76A	1.20	
Cholesterol	1.10	
NaCl	0.50	
Choline bitartrate	0.50	
dl-Methionine	0.20	
Sodium cholate	0.02	
Ethoxyquin	0.04	
Cellulose	6.00	

G. sylvestre was an extract standardized to 25% gymnemic acid content.

In the second study, each of five dietary groups contained eight SHR. The same basic sucrose diet from the first study was used. The control diet contained sucrose at 52% of calories. The next four diets were variants of the basic sucrose diet. To three of the sucrose-containing diets, BMOV was added at different concentrations—0.01, 0.03, and 0.06% w/w, respectively. The last sucrose diet contained vanadyl sulfate at 0.06% w/w.

Systolic BP (SBP)

SBP was estimated by tail plethysmography in unanesthetized rats after a 5-minute warming period [6,19]. Readings were taken 30 to 60 seconds apart. To be accepted, SBP measurements had to be virtually stable for three consecutive readings. Measurements were made 2 to 3 times per week.

Blood Chemistries

Blood was obtained at the end of the experiment (1 month consuming special diets) after the food had been removed for 4 hours. Following blood drawing, rats were sacrificed by inhalation of CO_2. Chemical analyses were performed by routine clinical procedures. Glucose was determined by the hexokinase method (Serono-Baker Diagnostics, Allentown, PA) using a Centrifichem System 600. Immunoreactive insulin was determined by radioimmunoassay (Linco Research Laboratories, St Louis, MO) and glycosylated hemoglobin (HBA1C) by column chromatography (Isolab Inc, Akron, OH). Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for determining the extent of lipid peroxidation. Lipid peroxidation products are quantified by their reaction with thiobarbituric acid 9 [20]. A 1.0 ml aliquot of hepatic and renal homogenates, precipitated with 0.15 ml of 76% trichloroacetic acid (TCA), is added to 0.35 ml of 1.07% thiobarbituric acid and incubated at 80°C for 30 minutes. A 0.5 ml volume of cold 90% TCA is added and the absorbance read at 532 nm. 1,1,3,3 tetramethoxypropane serves as the standard. Formation of thiobarbituric acid reacting substances (TBARS) estimates the amount of lipid peroxidation products.

Statistical Analyses

Statistics on chemistry values were performed by a one way analysis of variance (ANOVA) using repeated measures. SBP and body weight (BW) were examined by two-way analyses of variance (one factor being diet and the second factor being time of examination). Where a significant effect of diet was detected by ANOVA ($p<0.05$), the Dunnett t-test was used to establish which differences between means reached statistical significance ($p<0.05$) [21].

RESULTS

First Study

BW (Fig. 1). SHR consuming the basic starch (Starch) and basic sucrose (Sucrose) diets, and those consuming diets containing chromium polynicotinate (Chromium) and *G. sylvestre* with sucrose as the sole carbohydrate source showed virtually the same steady increase in BW (Fig. 1). In contrast, SHR consuming vanadium as BMOV (0.12% w/w) showed decreased BW from the beginning of study. Because of this, the BMOV diet was replaced with the basic sucrose diet (no BMOV present) at various intervals. This occurred at days 7 to 10, 14 to 17, and 21 to 27. Changing to the basic sucrose diet resulted in reversal of BW loss during these brief time intervals. SHR had been consuming BMOV for 5 days at termination of study.

SBP (Fig. 2). Although the five arms of the study were examined simultaneously, data in Fig. 2 are divided into three separate graphs for easier discernment, i.e., the chromium, vanadium, and *G. sylvestre* arms are compared individually with the starch and sucrose arms. Compared to the starch diet, SHR consuming the sucrose diet showed a significant elevation of SBP by the fifth day. This significant difference was maintained throughout the entire month of study. Addition of chromium polynicotinate to the basic sucrose diet prevented the sucrose-induced increase in SBP for the initial 2 weeks (Fig. 2 upper). However, SBP eventually increased to that of SHR consuming sucrose alone.

Rats ingesting diets containing vanadium as BMOV with sucrose initially showed the same elevation of SBP above starch control as SHR consuming sucrose alone (Fig. 2 middle). However, after 2 weeks, SBP decreased significantly below that of SHR consuming the basic starch diet. This decreased SBP was maintained at a steady level despite the occasionally brief intervals when the SHR were not consuming BMOV-containing food.

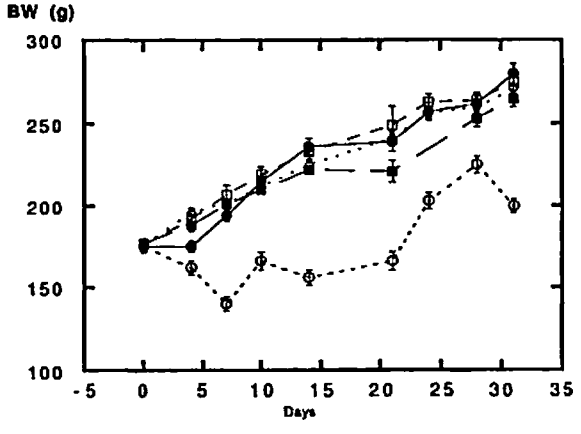

Fig. 1. Study #1. Mean±SEM of BW of 6 SHR in five groups. Symbols for each are as follows: closed circle-Starch, closed squares-Sucrose, open squares-Sucrose+chromium, open circles-Sucrose+BMOV, and open diamonds-Sucrose+*G. sylvestre*. Only values for BMOV are significantly different from other groups.

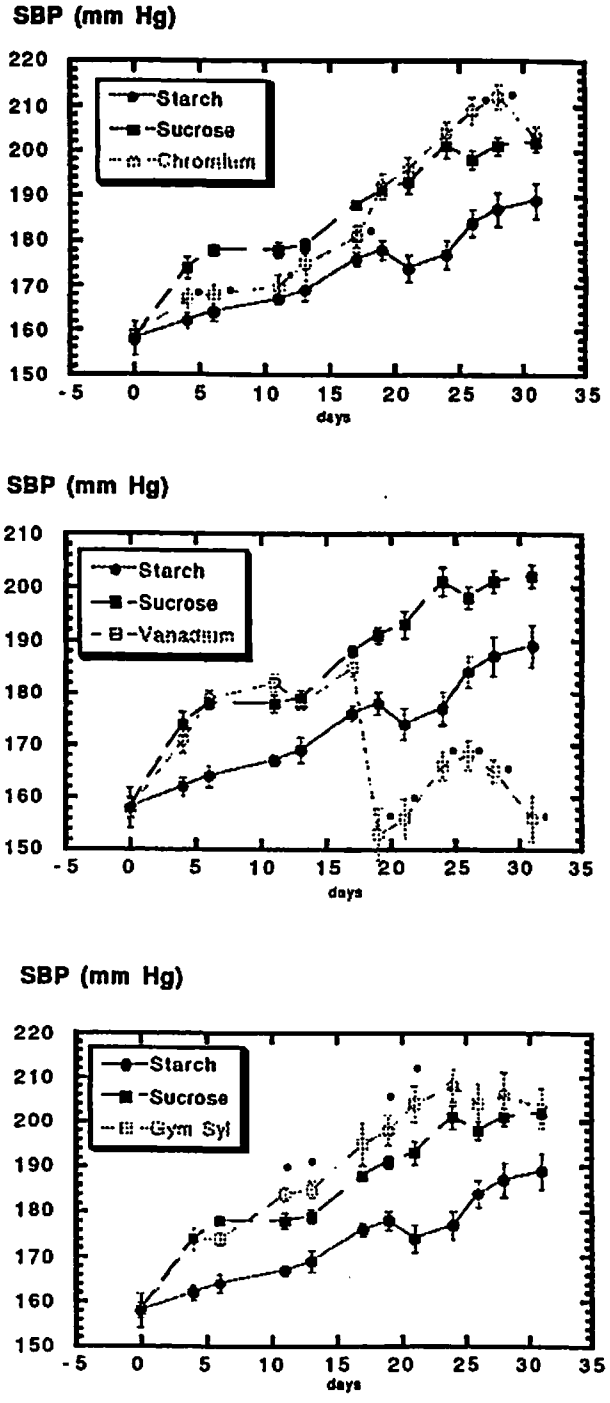

Fig. 2. Study #1. Mean±SEM of SBP of six SHR. Each variation of the sucrose diet is shown individually along with data from SHR consuming the starch and sucrose diets. Chromium data are depicted in upper figure, vanadium data in middle figure, and *G. sylvestre* data in lower figure. Open circles beside points indicate a significant difference from sucrose group.

In SHR consuming *G. sylvestre* (Fig. 2 lower), the tendency was for SBP to be higher than SHR consuming sucrose alone. Blood pressure was significantly higher in the group consuming *G. sylvestre* compared to the Sucrose group at four points indicated in Fig. 2 over the duration of study.

Blood Chemistries (Table 2). Among the five dietary groups, HbA1C was not significantly different. Glucose concentration was highest in the Starch group and lowest in the BMOV group. Compared to the starch and sucrose groups, insulin values were significantly lower in the BMOV and chromium polynicotinate groups. Eating sucrose resulted in a significant increase in cholesterol and triglyceride concentrations. Compared to pure sucrose-eaters, cholesterol was lowered significantly by *G. sylvestre* and triglycerides by BMOV. BUN, creatinine, and uric acid concentrations were significantly elevated in SHR consuming BMOV.

TBARS (Fig. 3). TBARS were measured in hepatic and renal tissues. TBARS were significantly higher in hepatic tissue of SHR consuming the Sucrose diet compared to the Starch diet. The addition of chromium polynicotinate and BMOV prevented this elevation in hepatic TBARS caused by sucrose replacement of starch. Significant differences in mean values did not occur in renal tissue between the sucrose and starch eaters, although addition of chromium polynicotinate caused statistically significantly lower mean values when compared to the basic sucrose group.

Second Study

BW (Fig. 4). The trend was for lower weights in SHR consuming BMOV and vanadyl sulfate. More BMOV present in the diet resulted in a lesser weight gain. For 2 weeks, the young SHR consuming 0.06% w/w BMOV actually lost weight. These SHR were returned to basal sucrose diet and began regaining weight rapidly. The loss of weight at equal concentrations of BMOV and vanadyl sulfate (0.06%) was significantly greater in the former. The BW changes brought on by 0.03% BMOV and 0.06% vanadyl sulfate were similar. Although mean BW was slightly lower in SHR consuming the diet containing 0.01% BMOV compared to the basic sucrose diet, this difference was not statistically significant.

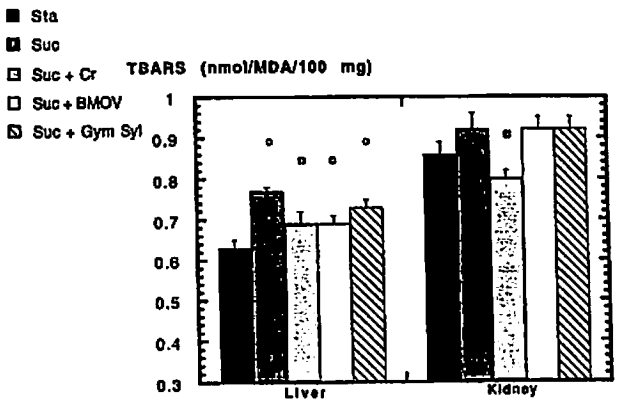

Fig. 3. Study #1. Mean±SEM for TBARS formation in liver and kidney tissue of five different dietary groups. Open circle indicates significant difference from starch group, and open square indicates significant difference from sucrose group.

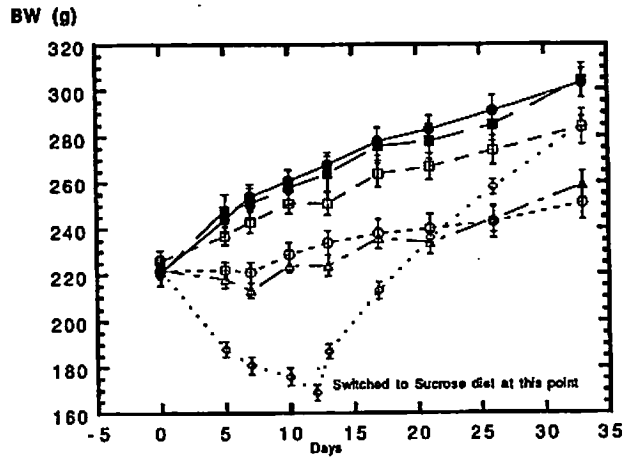

Fig. 4. Study #2. Mean±SEM for BW of eight SHR in six dietary groups is depicted. Symbols representing different dietary groups are as follows: closed circles-Starch, closed squares-Sucrose, open squares-Sucrose+BMOV 0.01, open circles-Sucrose+BMOV 0.03, open diamonds-Sucrose+BMOV 0.06, and open triangles-Sucrose+Vanadyl Sulfate 0.06.

SBP (Fig. 5). Addition of BMOV at various concentrations or vanadyl sulfate resulted in a lowering of SBP in these

Table 2. Blood Chemistries

Parameter	Sta	Suc	CP	BMOV	GS
HbA1C	5.0±.07	4.8±.03	4.8±.08	4.2±.08	5.0±.13
Glu	162±3.4	111±17(1)	136±14.1	56±3.5(1,2,3)	114±9.7(1,4)
Insulin	2.6±0.2	2.7±0.2	1.9±0.2(1,2)	0.7±0.1(1,2,3)	2.4±0.4(4)
Cholesterol	80±3.2	109±5.7(1)	101±2.7(1)	112±3.1(1)	92±4.4(1,2,4)
Triglycerides	63±9.9	139±12.1(1)	223±24	70±7.2(2,3)	165±11.4(1,3)
BUN	17±0.5	18±0.5	20±1.4	26±1.1(1,2,3)	17±0.8(4)
Creatinine	.66±.03	.60±.03	.77±.04(2)	.77±.06(2)	.67±.04
Uric Acid	3.5±0.8	4.0±0.9	3.9±0.3	4.8±0.1(1,2,3)	3.4±0.1(4)

Mean±SEM of six SHR.
Numbers in parentheses indicate significant difference from that column.

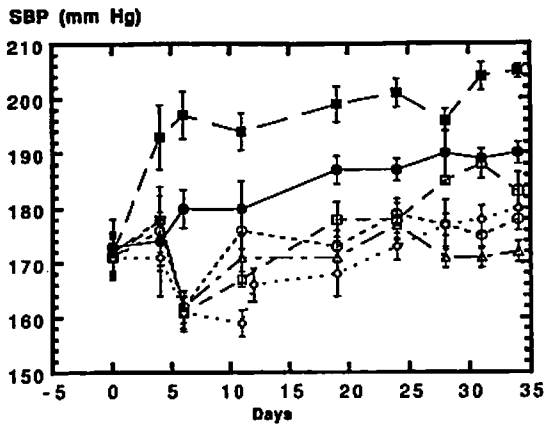

Fig. 5. Study #2. Mean±SEM of SBP of eight SHR is depicted. Symbols representing different dietary groups are as follows: closed circles-Starch, closed squares-Sucrose, open squares-Sucrose+BMOV 0.01, open circles-Sucrose+BMOV 0.03, open diamond-Sucrose+ BMOV 0.06, and open triangle-Sucrose+Vanadyl Sulfate 0.06.

sucrose eaters. Despite the switch to a basal sucrose diet after 2 weeks in the group originally consuming the diet containing 0.06% BMOV, SBP remained lower than the starch control group over the duration of study.

Blood Chemistries (Table 3). When consuming BMOV and vanadyl sulfate, circulating insulin and glucose levels tended to be lower. However, differences were statistically significant only in the group receiving the mid range dose of BMOV (0.03) for both. Blood glucose was statistically significantly lower in the group ingesting the lowest dose of BMOV ($p<0.01$). In explaining results, it is important to reemphasize that the BMOV group receiving the highest challenge ($p<0.06$) had consumed regular diet for 2 weeks, i.e., prior to blood drawing. Glucose and insulin levels were not significantly different in the vanadyl sulfate group compared to the basal sucrose eaters.

DISCUSSION

Since the discovery that increasing dietary concentrations of sugars (sucrose, fructose, and glucose) increase SBP of various rat strains significantly, many have studied sugar-induction in an attempt to understand the pathogenesis behind this model [7–9]. Perturbations in catecholamine metabolism [22–24], volume status [23], insulin regulation [6,9,10], a circulating digitalis-like factor [26], magnesium homeostasis [25,27], and the renin-angiotensin system [28] have all been associated with the rise in BP. None of these possibilities is mutually exclusive, because numerous examples exist in the literature to suggest multiple interactions among all these factors.

The role of the insulin system in regulating BP is receiving increased attention [1–5]. In a series of studies, Reaven's group implicated the insulin system in sugar-induced hypertension of rats based upon many accepted methods to evaluate glucose/ insulin homeostasis [3,4,6], and the ability of exercise [9] and somatostatin [10] to overcome sugar-induced SBP elevations. More recently, the ability of soluble fiber [11], vanadium [12,13], chromium [14], metformin [15], and troglitazone [16] to overcome or, at least, ameliorate sugar-induced hypertension strengthens the "insulin theory" because each is known to influence the insulin system.

As a first approximation, we simultaneously compared the effects of three nutrients known to influence the insulin system on sugar-induced SBP elevations. Corroborating previous findings, chromium initially overcame the sucrose-induced elevation of SBP induced by dietary sucrose [14]. Although chromium polynicotinate overcame sucrose-induced SBP elevations for only 2 weeks, a previously published study showed that the same compound prevented sucrose induction for the duration of the studies [14]. Data from previous studies were examined to explain these differences (Fig. 6). The reason for the results in the present study most likely resides in the interplay between the negative effects of sucrose and the positive effects of chromium. Up to now, studies examining sucrose at concentrations providing 18% and 52% of calories and chromium at 5 ppm and 25 ppm have been carried out. Fortunately, we have examined all four possible variations as depicted in Fig. 6. Only the combination using the lowest chromium concentration (5 ppm) and the highest sucrose challenge (52% of calories) showed an inability to restrain sugar-induced SBP elevations during the course of study. Accordingly, both intake of sucrose and chromium must both be considered in planning future therapeutic approaches.

Table 3. Blood Chemistries

Parameter	Sta	Suc	BMOV.01	BMOV.03	BMOV.06	VdSO4
HbA1C	3.88±.07	3.63±.14	3.95±.21	4.05±.24	4.08±.16	4.21±.19
Glu	134±8.1	130±4.7	107±5.0*	111±3.8*	134±6.6	119±6.9
Insulin	2.8±.3	2.4±.3	1.9±.4	1.3±.3*	2.9±.2	2.0±.3
Cholesterol	104±3.9*	86±2.5	84±2.0	113±2.5*	59±1.6*	64±2.1*
Triglycerides	184±17*	272±16	358±23*	274±28	155±6*	276±26
Creatinine	.55±.01	.60±.05	.53±.07	.66±.04	.50±.01	.58±.04
Uric Acid	2.1±.12	1.8±.05	1.9±.11	2.1±.18	2.1±.13	1.9±.09

Means±SEM for eight SHR are shown.
* Significant difference from column 2.

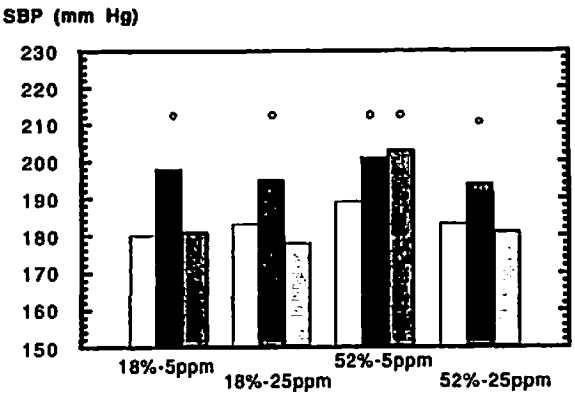

Fig. 6. Data were used from four separate studies performed over 2 to 3 months. The third set of bars depict data from present study. First white bar depicts SBP of rats receiving all carbohydrate calories from starch (52%). Second dark gray bar depicts diets containing either sucrose (52%) or sucrose (18%)—starch (34%). Third light gray bar depicts same second diet plus chromium polynicotinate at the concentration indicated below figure. Percent under bars signifies percent of calories derived from carbohydrate in second and third diets, either 18% from sucrose with rest made up by starch or sucrose 52%. PPM under bars indicates concentration of chromium polynicotinate added to sucrose diets signified by last gray bar. Circle above bar indicates statistical significance vs. first bar (starch) in each set.

The exact mechanisms behind their effects on the insulin system are uncertain; but it is likely that chromium, vanadium, and *G. sylvestre* influence insulin metabolism differently. While the exact mechanism of action for chromium is not known, it has been shown to have an effect on insulin receptor number [29]. Although having multiple effects on electrolyte transport [30], vanadium has been described as an insulin mimic with the ability to bypass the insulin receptor [31]. *G. sylvestre* may rejuvenate beta cells in the pancreas and increase insulin output [17].

How do these three agents influence the glucose/insulin system and/or intermediary metabolism in general in the present study? Examining various blood parameters, circulating insulin decreased with chromium polynicotinate, but glucose concentrations did not. BMOV decreased both glucose and insulin levels, while *G. sylvestre* did not change these levels compared to the basic sugar eaters. Evidence of effect can also be inferred from examination of oxidative metabolism. Insulin resistance is associated with augmented lipid peroxidation and free radical formation which may have some role in elevating BP [32]. We know this through our examination of TBARS. Formation of TBARS is an indirect estimate of lipid peroxidation and free radical formation, and increased formation of TBARS is associated with insulin perturbations [33,34]. Compared to starch eaters, sugar eaters showed increased TBARS formation in hepatic tissue but not renal tissue (Fig. 3). Chromium and BMOV supplementation prevented this increase in the liver, and chromium caused a significantly lower level of renal TBARS formation when compared to sucrose eaters. *G. sylvestre* produced no significant differences in these parameters but did lower cholesterol suggesting some effects on metabolism. Thus, the effects of chromium and vanadium compounds on glucose and insulin values and free radicals is also consistent with an effect of these agents on glucose/insulin metabolism and offers yet another explanation for the lowering of SBP in SHR.

Certain antioxidants appear to decrease blood pressure [32]. Many papers have shown antihypertensive effects with vitamin C [37–40], selenium [41], coenzyme Q10 [42], and nicotineamide adenine dinucleotide (NADH) [43]. Oxygen derived free radical are responsible for faster degradation of nitric oxide, an endothelial derived vasodilator. Accordingly, it has been proposed that and an imbalance in nitric oxide (NO) contributes to the development of arterial hypertension [44]. The antihypertensive effects of antioxidants may occur through protection of NO, a powerful vasodilator [45,46].

BMOV and vanadyl sulfate added to the basic diet at higher concentrations caused a loss of BW. By observation, it was apparent that rats consuming high amounts of BMOV ate less food and drank less water. This state was reversed when the rats were switched to diets devoid of BMOV. Could this decreased weight be responsible for the lowering of BP? We believe that weight loss is not a major factor for many reasons. First, BW changes have never been shown to play a significant role in the sugar-induction model of hypertension [6–11]. Second, when vanadium diets were changed to regular diets, rats on the latter gained weight; but the SBP still remained lower. Third, Bhanot and McNeill [47] noted that vanadium decreased BW while lowering SBP; but pair feeding showed that SBP was reduced by vanadium compared to SHR at the same BW. Finally, the second study was specifically designed to determine if the effects of vanadium on SBP were secondary to weight loss. At the lower concentrations of BMOV (0.1%), the significant decrease in SBP occurred, even though there was no significant loss of BW compared to control, at least in the early phases of study.

Of interest is the finding that *G. sylvestre* actually caused a significant elevation of SBP at certain times. We believe that the different response from chromium polynicotinate and BMOV may relate to mechanism of action, i.e., that *G. sylvestre* stimulates insulin release rather than improving insulin sensitivity [48–50]. Standardized extract of the herb has been reported to have hypoglycemic activity equivalent to tolbutamide.

In summary, the present studies show that some agents affecting the glucose/insulin system can favorably lower BP. Chromium and vanadium have additional benefits on TBARS formation suggesting they may lessen free radical formation. No direct evidence was obtained to indicate that *G. sylvestre* lowers SBP or influences glucose/insulin metabolism. Accordingly, agents that decrease circulating insulin levels, perhaps by augmenting insulin sensitivity, may prove useful in the treatment of high blood pressure.

ACKNOWLEDGMENTS

This work was supported by grants from the USDA (NRICGP/USDA 9201419) and InterHealth Co. of Concord CA. The authors gratefully acknowledge the excellent technical assistance of Noella Bryden, Marilyn Polansky, and Javed Gondal.

REFERENCES

1. Swislocki AL, Hoffman BB, Reaven GM: Insulin resistance, glucose intolerance and hyperinsulinemia in patients with hypertension. Am J Hyper 2:419–423, 1989.
2. Reaven GM: Role of insulin resistance in human disease (Banting Lecture 1988). Diabetes 37:1595–1607, 1988.
3. Ferrannini E, Haffner SM, Stern MP: Essential hypertension: an insulin-resistant state. J Cardiovasc Pharmacol 15(Suppl 5):518–525, 1990.
4. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194, 1991.
5. Sowers JR, Levy J, Zemel MB: Hypertension and diabetes. Med Clin North Am 72:1399–1414, 1988.
6. Preuss MB, Preuss HG: Effects of sucrose on the blood pressure of various strains of Wistar rats. Lab Invest 43:101–107, 1980.
7. Michaelis OE, Martin RE, Gardener LB, Ellwood KC: Effect of dietary carbohydrates on systolic blood pressure of normotensive and hypertensive rats. Nutr Rep Int 23:261–266, 1981.
8. Hwang IS, Ho H, Hoffman BB, Reaven GM: Fructose-induced insulin resistance and hypertension in rats. Hypertension 10:512–516, 1987.
9. Reaven GM, Ho H, Hoffman BB: Attenuation of fructose-induced hypertension in rats by exercise training. Hypertension 12:129–132, 1988.
10. Reaven GM, Ho H, Hoffman BB: Somatostatin inhibition of fructose-induced hypertension. Hypertension 14:117–120, 1989.
11. Zein M, Areas J, Knapka J, Gleim G, DiPette D, Holland B, Preuss HG: Influence of oat bran on sucrose-induced blood pressure elevations in SHR. Life Sci 47:1121–1128, 1990.
12. Bhanot S, McNeill JH, Bryer-Ash M: Vanadyl sulfate prevents fructose-induced hyperinsulinemia and hypertension in rats. Hypertension 23:308–312, 1994.
13. Bhanot S, Bryer-Ash M, Cheung A, McNeill JH: Bis(maltolato) oxovanadium (IV) attenuates hyperinsulinemia and hypertension in spontaneously hypertensive rats. Diabetes 43:857–861, 1994.
14. Preuss HG, Gondal JA, Bustos E, Bushehri N, Lieberman S, Bryden NA, Polansky MM, Anderson RA: Effect of chromium and guar on sugar-induced hypertension in rats. Clin Neph 44:170–177, 1995.
15. Verma S, Bhanot S, McNeill JH: Antihypertensive effects of metformin in fructose-fed hyperinsulinemic, hypertensive rats. J Pharmacol Exper Therap 271:1334–1337, 1994.
16. Lee M-K, Miles PDG, Khoursheed M, Gao K-M, Moossa AR, Olefsky JM: Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. Diabetes 43:1435–1439, 1994.
17. Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR, Rajendran VM: Possible regeneration of the islet of langerhans in streptozotocin-diabetic rats given Gymnema sylvestre leaf extracts. J Ethnopharmacol 30:265–279, 1990.
18. Okamoto K, Aoki K: Development of a strain of spontaneously hypertensive rat. Jap Circ J 27:282–293, 1963.
19. Bunag RD: Validation in awake rats of a tail-cuff method measuring systolic pressure. J Appl Physiol 34:279–282, 1973.
20. Buege JA, Hunt SD: Microsomal lipid peroxidation. Meth Enzymol LII:302–306, 1978.
21. Dunnett C: A multiple comparison procedure for comparing several treatments with control. J Am Statis Assoc 50:1096–1121, 1955.
22. Bunag RD, Tomita T, Sasaki S: Chronic sucrose ingestion induces mild hypertension and tachycardia in rats. Hypertension 5:218–225, 1983.
23. Fournier RD, Chiueh CC, Kopin IJ, Knapka JJ, DiPetti D, Preuss HG: The interrelationship between excess CHO ingestion, blood pressure and catecholamine excretion in SHR and WKY. Am J Physiol 250:E381–385, 1986.
24. Gradin K, Nissbrand H, Ehrenstom F, Henning M, Persson B: Adrenergic mechanisms during hypertension induced by sucrose and/or salt in the spontaneously hypertensive rat. Arch Pharmacol 337:47–52, 1988.
25. Preuss HG, Memon S, Dadgar A, Jiang G: Effects of diets high in sugar on renal fluid, electrolyte, and mineral handling in SHR: Relationship to blood pressure. J Am Coll Nutr 13:73–82, 1994.
26. Gharib N, Gao CY, Areas J, Zein M, Preuss HG: Correlation of organic anion and cation transport with blood pressure. Clin Nephrol 36:87–92, 1991.
27. Balon TW, Jasman A, Scott S, Meehan WP, Rude RK, Nadler JL: Dietary magnesium prevents fructose-induced insulin sensitivity in rats. Hypertension 23(part 2):1036–1039, 1994.
28. Iyer SN, Katovich MJ: Effects of chronic Losarten Potassium treatment on fructose-induced hypertension. Life Sci 55:PL 139–144, 1994.
29. Anderson RA, Polansky MM, Bryden NAS, Bhathena SJ, Canary J: Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. Metabolism 36:351–355, 1987.
30. Dafnis E, Sabatini S: Biochemistry and pathophysiology of vanadium. Nephron 67:133–143, 1994.
31. McNeill JH, Yuen VG, Haveyda HR, Orvig C: Bis(maltolato)oxovanadium(IV) is a potent insulin mimic. J Medicinal Chem 35: 1489-1491, 1992.
32. Diplock AT: Antioxidant nutrients and disease prevention: An overview. Am J Clin Nutr 53:189S–193S, 1991.
33. Sukalski KA, Pinto KA, Berntson JL: Decreased susceptibility of liver mitochondria from diabetic rats to oxidative damage and associated increase in a tocopherol. Free Rad Biol Med 44:57–65, 1993.
34. Jasin SK, McVie R, Duett J, Herbst JJ: Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 38:1539–1543, 1989.
35. Solonen JT, Solonen R, Ihanainen M, Parvviainen M, Seppanen R, Kantola M, Seppanen K, Rauramaa R: Blood pressure, dietary fats, and antioxidants. Am J Clin Nutr 48:1226–1232, 1988.
36. Salonen JT: Dietary fats, antioxidants, and blood pressure. Ann Med 23:295–298, 1991.

37. Jacques PF: Effects of vitamin C on high density lipoprotein cholesterol and blood pressure. Int J Vit Nutr Res 11:139–144, 1992.
38. Yoshioka M, Matsushita T, Churman Y: Inverse association of serum ascorbic acid level and blood pressure or rate of hypertension in male adults aged 30–39 years. Int J Vitam Nutr Res 54:343–347, 1984.
39. Choi ESK, Jacques PF, Dallal GE, Jacobs RA: Correlation of blood pressure with plasma ascorbic acid. Nutr Res 11:1377–1382, 1991.
40. Jacques PF: A cross-sectional study of vitamin C intake and blood pressure in the elderly. Int J Vit Nutr Res 62:252–255, 1992.
41. Oster O, Prellwitz W: Selenium and cardiovascular disease. Biol Trace Elem Res 24:91–103, 1990.
42. Greenburg S, Frishman WH: Co-enzyme Q10: a new drug for cardiovascular disease. J Clin Pharmacol 30:596–608, 1990.
43. Bushehri N, Jarrell ST, Onyejiaka V, Lieberman S, Birkmayer G, Preuss HG: Antioxidant NADH lowers BP, cholesterol, and lipid peroxidation in rats (Abstract). J Am Coll Nutr 15:532, 1996.
44. Vanhokutte PM: Endothelium and control of vascular function: State of the Art Lecture. Hypertension 13:658–667, 1989.
45. Ceriello A, Giuglan D, Quatrato A, Lefebri PJ: Anti-oxidants show an anti-hypertensive effect in diabetic and hypertensive subjects. Clin Sci 81:739–742, 1991.
46. Efstratopoulos AD, Voyaki SM: Effects of antioxidants on acute blood pressure response to smoking in normotensives and hypertensives. J Hypertens 11(suppl 5):S112–S113, 1993.
47. Bhanot S, McNeill JH: Vanadyl sulfate lowers plasma insulin and blood pressure in spontaneously hypertensive rats. Hypertension 24:625–632, 1994.
48. Baskaran K, Ahmath BK, Shanmugasundaram KR, Shanmugasundaram ERB: Antidiabetic effect of a leaf extract from Gymnema sylvestre in non-insulin-dependent diabetes mellitus patients. J Ethnopharmacol 30:295–305, 1990.
49. Bishayee A, Chatterjee M: Hypolipidaemic and antiatherosclerotic effects of oral Gymnema sylvestre R Br. leaf extract in albino rats fed a high fat diet. Phytotherapy Res 8:188–120, 1994.
50. Shanmugasundaram ERB, Rajeswari G, Baskaran K, Rajesh Kumar KK, Ahmath BK: Use of Gymnema sylvestre leaf extract in control of blood glucose in insulin-dependent diabetes mellitus. J Ethnopharmacol 30:281–294, 1990.

Received October 1996; revision accepted July 1997.