Composition useful for the prevention or reduction of the progression of prostate cancer

The present invention relates to a composition useful for the prevention or reduction of the progression of prostate cancer.

In particular, the present invention relates to a composition, which comprises as active ingredients green tea and pomegranate extract, useful for the prevention or reduction of the progression of prostate cancer.

Prostate cancer is the most common cancer in mammals, especially among human males in the western countries (Cancer Statistics 1997, CA Cancer J. Clin. 1997; 47; 5-27).

Various factors such as an unknown aetiology, variable pathology, an intricate relationship to endocrine factors, and anaplastic progression contribute to the complexity of this disease.

A team of researchers from the University of Wisconsin, Madison, Wis., and Case Western Reserve University, Cleveland, Ohio, documented the role of green tea polyphenols (GTP) in modulating the insulin-like growth factor-1 (IGF-1)-driven molecular pathway in prostate tumor cells in a mouse model for human prostate cancer. These observations bear significance in light of studies that indicate how increased levels of IGF-1 are associated with increased risk of several cancers, such as prostate, breast, lung and colon. The green tea polyphenols contributed to minimizing tumor development by governing the amount of vascular endothelial growth factor (VEGF) in the serum of the prostate cancer mouse model. The reduction of VEGF may result from GTP-induced suppression of IGF-1 levels. VEGF functions to recruit and develop

new blood vessels that carry nutrients to developing tumors. By reducing the amount of VEGF, GTP works to minimize nutrients flowing to and supporting tumor growth.

In Clinical Cancer Research, July 1, 2006; pp 4018-4026 and Clinical Cancer Research Vol. 12, 4018-4026, July 1, 2006, is reported that pomegranate extract may be useful for the prevention or reduction of the progression of prostate cancer.

In Prostate Cancer and Prostatic Diseases (2002) 5, 6-12 is reported that lycopene may be useful for the prevention or reduction of the progression of prostate cancer.

In WO0057892 is reported that serenoa is useful for the treatment of prostate cancer.

In WO 03035635 is reported that isoflavonoid derivatives are useful for the treatment of prostate cancer;

In WO04091602 is reported that L-carnitine is useful the treatment of cardiovascular diseases.

Selenium in humans is a trace element nutrient which functions as cofactor for reduction of antioxidant enzymes such as glutathione peroxidases. Dietary selenium comes from nuts, cereals, meat, fish and eggs. The "Nutritional Prevention of Cancer Project" (NPC) was a controlled, randomized cancer prevention trial in which 1,312 patients received a daily 200 µg dose of selenium or a placebo for up to 10 years. In this study a statistically significant reduction

in the incidence of prostate cancer and prostate cancer progression was obtained.

Although the anti-carcinogenic effects of green tea and pomegranate extract have already been known, none of the prior art documents cited above mention nor suggest the use of these two active ingredients in combination for the prevention or reduction of the progression of prostate cancer.

Furthermore, while there are other agents available for chemotherapy of tumors, and other invasive treatment options exist for prostate cancer such as removal of the cancerous prostate or placement of a radioactive seed designed to shrink the tumor, it would be more desirable to provide a composition useful as an adjunct or complement to traditional therapies of low toxicity to a patient which will serve as an anti-carcinogenic agent for prostate cancer.

It is therefore one object of the present invention a synergistic composition comprising as active ingredients green tea extract and pomegranate extract.

The composition mentioned above may further comprise lycopene, selenium, zinc, serenoa, isoflavonoid derivatives and L-carnitine.

It is a further object of the present invention a composition comprising:

(a) green tea extract, as active ingredient, in a dose of from 25 to 800 mg, preferred doses are 125 and 250 mg; and

(b) pomegranate extract, as active ingredient, in a dose of from 25 to 800 mg, the preferred doses are 40, 125 and 250 mg; and

(c) lycopene in a dose of from 0.03 to 30.0 mg, the preferred dose are 1.25 and 5 mg;

(d) selenium in a dose of from 8.2 to 500 μg, the preferred doses are 55 and 82.5 μg;

(d) zinc in a dose of from 1 to 200 mg, the preferred dose is 20 mg; and

(e) serenoa in a dose of from 10 to 400 mg, the preferred doses are 160 and 320 mg;

(f) isoflavonoid derivatives (soya isoflavon) in a dose of from 10 to 500 mg, the preferred dose is 100 mg; and

(g) L-carnitine in a dose of from 50 to 500 mg, the preferred dose is 200 mg.

It is a further object of the present invention the use of the composition mentioned above, for the prevention or reduction of the progression of prostate cancer.

It is a further object of the present invention the use of the composition mentioned above, for preparing a medicament for the prevention or reduction of the progression of prostate cancer.

It is a further object of the present invention the use of the composition mentioned above, for preparing a dietary supplement for the prevention or reduction of the progression of prostate cancer.

The composition of the invention may further comprise co-enzymes, mineral substances, antioxidants, vitamins and agents useful for treating prostate cancer.

The following non limiting examples further illustrate the invention.

EXAMPLE 1

For the experiments reported in the following established human prostate carcinoma cell lines LNCaP and PC3 (obtained from the American Type Culture Collection Rockville, MD) were used.

The LNCaP cells were cultured in RPMI 1640 medium (Life Technologies, Rockville, MD), supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin and Streptomycin. The cells were incubated at 37degree C in a humidified atmosphere of 5% CO2 in air. Both sets of cell cultures were grown to 80% confluence in 10 cm tissue culture flasks and split 1:8. The cells were plated in 96 cell plates and allowed to attach and reach 60-70% confluence before being used for experimentation.

Effect on Cell Growth in Vitro

The cultured set of LNCaP and PC3 cells were treated with either green tea extract (40 µg/ml in PBS), pomegranate extract (40 µg/ml in PBS) or in combination (both green tea extract and pomegranate extract) in 10 wells each containing complete cell

medium. Cells that were used as control were incubated with same amount of PBS in complete cell medium. The effects of lycopene (5 mmol/L], selenium (30 ng/ml), zinc (10 µg/ml), serenoa (10 µg/ml), and soya isoflavon (10 µg/ml) were also tested by adding them to the cell culture alone or in combination with the green tea extract and pomegranate extract. The cellular proliferation of the cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) colorimetric assay following 24 hours of incubation at 37 °C with or without the test agents. The MTT (5 ng/ml) was added to all the wells and incubated for a further 2 hr. The plate was than centrifuged at 500 x g for 5 min at 4 degree C. The MTT solution was removed from the wells by careful aspiration and buffered DMSO (0.1 ml) was added to each of the wells and plates shaken for 15 mins. The absorbance was measured on a microplate reader at the wavelength of 540nm. The effect of each compound alone and in combination on cell growth was assessed as the percentage of cell viability in which the untreated control cells were taken as those being completely viable and allowed to grow freely.

The results obtained are reported in the following Tables 1-4.

TABLE 1

Effects of test compounds on LNCaP cell proliferation

	ABSORBANCE 540 NM							
N°	**Control**	**A**	**B**	**C**	**D**	**E**	**A+B**	**A+B+C+D+E**
1	346	319	349	353	328	316	298	266
2	345	320	344	341	322	334	272	245
3	311	324	313	301	303	332	283	287
4	358	365	322	312	302	308	286	255
5	364	303	308	317	309	342	247	243
6	350	322	302	301	323	324	263	277
7	334	315	289	344	297	311	286	273
8	367	322	324	329	301	313	259	271
9	342	291	299	315	322	325	282	314
10	344	323	311	327	307	344	256	311
Mean	346	320	316	324	311	325	273	274
SE	5	6	6	6	4	4	5	8
P<vs control		0.01	0.01	0.05	0.001	0.01	0.001	0.001
P< vs A							0.001	0.001
P< vs B							0.001	0.001
P< vs C							0.001	0.001
P< vs D							0.001	0.001
P< vs E							0.001	0.001
% inhib		7.5	8.7	6.4	9.8	5.7	20.9	20.5

A=green tea extract; B=pomegranate extract; C=lycopene; D=selenium; E=zinc.

TABLE 2

Effects of test compounds on PC3 cell proliferation

	ABSORBANCE 540 NM							
N°	**Control**	**A**	**B**	**C**	**D**	**E**	**A+B**	**A+B+C+D+E**
1	394	329	319	316	313	321	258	305
2	366	345	322	382	321	339	260	234
3	365	301	314	308	374	344	283	224
4	340	337	377	377	309	355	242	266
5	371	333	365	314	341	322	248	287
6	337	324	279	322	345	395	270	263
7	401	378	281	309	286	344	278	288
8	388	304	310	299	292	312	261	289
Mean	370	331	321	328	323	342	263	270
SE	8	9	12	11	10	9	5	10
P<vs control		0.01	0.01	0.01	0.01	0.05	0.001	0.001
P< vs A							0.001	0.001
P< vs B							0.001	0.001
P< vs C							0.001	0.001
P< vs D							0.001	0.001
P< vs E							0.001	0.001
% inhib		10.5	13.2	11.4	12.7	7.8	28.9	27.0

TABLE 3

Effects of test compounds on LNCaP cell proliferation

N°	Control	A	B	F	G	H	A+B	A+B+F+G+H
1	345	334	352	345	301	345	277	288
2	365	342	312	351	389	368	289	246
3	371	311	302	314	311	366	274	299
4	366	348	343	396	364	354	252	244
5	326	308	318	304	345	312	265	234
6	380	313	341	322	328	344	255	291
7	387	317	288	315	298	325	274	264
8	352	326	320	333	311	345	261	276
9	367	298	304	312	301	355	284	311
10	332	344	342	325	319	352	266	302
Mean	359	324	322	332	327	347	270	276
SE	6	5	7	9	10	5	4	9
P< vs Control	-	0.01	0.01	0.05	0.05	ns	0.001	0.001
P< vs A							0.001	0.001
P< vs B							0.001	0.001
P< vs F							0.001	0.001
P< vs G							0.001	0.001
P< vs H							0.001	0.001
% inhib		9.7	10.3	7.5	8.9	3.3	24.8	23.1

A=green tea extract; B=pomegranate extract; F=serenoa;
G=soya isoflavon; H=L-carnitine

TABLE 4

Effects of test compounds on PC3 cell proliferation

Effects of test compounds on PC3 cell proliferation

N°	Control	A	B	F	G	H	A+B	A+B+F+G+H
1	399	354	324	309	322	392	264	226
2	373	325	352	386	359	385	254	239
3	381	331	331	314	345	385	286	241
4	365	323	381	389	352	366	234	264
5	384	339	366	312	344	341	261	281
6	345	368	312	355	385	387	277	261
7	395	342	265	341	377	320	246	228
8	388	367	356	332	365	378	251	277
Mean	379	344	336	342	356	369	259	252
SE	6	6	13	11	7	9	6	8
P< vs Control	-	0.01	0.01	0.01	0.05	ns	0.001	0.001
P< vs A							0.001	0.001
P< vs B							0.001	0.001
P< vs F							0.001	0.001
P< vs G							0.001	0.001
P< vs H							0.001	0.001
% inhib		9.2	11.3	9.8	6.1	2.6	31.7	33.5

A=green tea extract; B=pomegranate extract; F=serenoa; G=soya isoflavon; H=L-carnitine

The results reported in Tables 1, 2, 3 and 4 show that the composition of the invention is statistically more active respect to the single constituents.

Moreover, the presence of lycopene, selenium and zinc, or serenoa, soya isoflavon and L-carnitine did not increase the inhibitory activity of the composition of the invention.

EXAMPLE 2

Experiment 2 Effect on Cell Division in Vitro

The LNCaP and PC3 cells in 25 ml culture flasks were treated either with: green tea extract (40 µg/ml in PBS), pomegranate extract (40 µg/ml in PBS) serenoa (10 µg/ml), soya isoflavon (10 µg/ml) or the combination of green tea extract plus pomegranate extract.

Controls received PBS alone. Treatment was started 48 hrs after attachment. The cells were allowed to grow in culture for up to 72 hrs before being analysed by flow cytometry. The cells were pulse labelled with 10 mM bromodeoxyuridine (BrdU) for 2 hours with or without prior treatment with green tea extract or pomegranate extract or both to asynchronously growing cells. Cells were then harvested, fixed with 70% ethanol, treated with 0.1% HCl and heated for 10 min at 90 degree C to expose the labelled DNA. Cells were stained with anti-BrdU conjugated FITC (Becton Dickinson) and counterstained with propidium iodide. Cell cycle analysis was carried out on a Becton-Dickinson FACScan, using Lysis II software.

The results of the flow cytometric analysis obtained using the composition of the invention respect to the single components are reported in the following Tables.

TABLE 5

Green Tea Extract, LNCaP Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	73	80	82	77
S	21	18	16	15
G2/M	6	2	2	8
% inhibition S phase vs 0 hr		14.3	23.8	28.6

TABLE 6

Pomegranate Extract, LNCaP Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	74	69	68	64
S	20	19	16	16
G2/M	6	12	16	20
% inhibition S phase vs 0 hr		5.0	20.0	20.0

TABLE 7

Serenoa Extract, LNCaP Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	70	68	71	62
S	18	18	18	14
G2/M	12	14	11	24
% inhibition S phase vs 0 hr		0	0	22.2

TABLE 8

Soya isoflavon Extract, LNCaP Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	72	71	69	63
S	22	21	18	17
G2/M	6	8	13	20
% inhibition S phase vs 0 hr		4.5	18.2	22.7

TABLE 9

Green Tea plus Pomegranate Extract, LNCaP Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	72	83	83	79
S	23	11	8	9
G2/M	5	6	9	12
% inhibition S phase vs 0 hr		52.2	65.2	60.9

TABLE 10

Green Tea Extract PC3 Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	64	69	65	72
S	30	26	23	22
G2/M	6	5	12	6
% inhibition S phase vs 0 hr		13.3	23.3	26.7

TABLE 11

Pomegranate Extract PC3 Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	66	71	68	69
S	28	25	23	22
G2/M	6	4	9	9
% inhibition S phase vs 0 hr		10.7	17.9	21.4

TABLE 12

Serenoa Extract PC3 Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	68	70	69	67
S	29	26	21	20
G2/M	3	4	10	13
% inhibition S phase vs 0 hr		10.3	27.6	31.0

TABLE 13

Soya isoflavon Extract PC3 Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	63	68	66	68
S	25	26	23	25
G2/M	12	6	11	7
% inhibition S phase vs 0 hr		4.0	8.0	0

TABLE 14

Green Tea plus Pomegranate Extract PC3 Cell Division BrdU

	Cells Cycle %			
Time hr	**0**	**24**	**48**	**72**
G1	63	72	78	79
S	31	21	17	14
G2/M	6	7	5	7
% inhibition S phase vs 0 hr		32.3	45.2	54.8

The use of the single compounds shows an inhibitory action on the proliferation of both LNCaP and PC3 cells (see tables 5-8 and 10-13). The results reported in Tables 9 and 14 show that the combination composition of the invention is endowed with an

unexpected synergistic effect for the inhibition of the proliferation of both LNCaP and PC3 cells. The cell lines arrest in the S phase persisted for 72 hours by a maximum of 60.9% and 54.8% in LNCaP and PC3 lines, respectively.

No effects on cell division or growth arrest was observed in the controls that were treated with the vehicle alone.

EXAMPLE 3

Anti-tumoral Activity in Vivo

To evaluate the antitumoral activity of the combination of the invention a solid tumor strain LNCaP (ATCC) was used.

The cells were cultured for five to six times in nude mice. A solid cancer fragment of this strain (3 mm square) was transplanted beneath the skin of the axillary region of nu-nu nude male 8 week old mice (NxGen Biosciences, San Diego, CA). The mice in which the tumor was reliably taken (after about 20 days) were randomly assigned to 4 groups of 10 animals each. The animal groups were treated with vehicle, green tea extract, pomegranate or combination of green tea and pomegranate. The animals in control group received only drinking water whereas the green tea and pomegranate extract or their combination were administered at 1% in the drinking water (w/v, ad libitum) once a day in the morning for 28 consecutive days. Tumor diameter was measured twice a week, and tumor volume was calculated using formula 0.5238L1L2H where L1 is the long diameter, L2 the short diameter and H the height of the tumor. All

animals were euthanized once the tumors reached around 1,300 mm3 in the control animals, which was after about 28 days.

The results (mean and SE) showed that the tumor volumes at day 28 were 1305±87 in the control group, whereas in the green tea and pomegranate extract treated groups the values were 1026±98 ($p<0.05$) and 1011±91 ($p<0.05$), respectively. Whereas in the combination treatment (green tea plus pomegranate extract) there was a definite synergistic effect on tumor reduction, 623±112 ($p<0.001$). Thus the inhibition was 52.3 % by the combination compared to green tea (21.4%) or pomegranate (22.5%) alone.

The results obtained, reported in Table 15, show that the composition of the invention is statistically more active respect to the single constituents.

TABLE 15

	CONTROL	**(A) GREEN TEA**	**(B) POMEGRANATE**	**(A+B) GREEN TEA + POMEGRANATE**
Tumor Volume mm^3 ± SE	1305±87	1026±98	1011±91	623±112
P< vs control		0.05	0.05	0.001
P< vs A				0.05
P< vs B				0.05
% inhibition vs control		21.4%	22.5%	52.3 %

The dietary supplement or medicament according to the present invention is composed of active ingredients which are

familiar to operators in the medical field and already in use in clinical practice, and their pharmacotoxicological profiles are known.

Their procurement therefore is very easy, inasmuch as these are products which have been on the market now for a long time and are of a grade suitable for human or animal administration.

In the following are reported non limiting examples of compositions according to the present invention.

Composition 1

Green tea extract 250 mg;

pomegranate extract 250 mg.

Composition 2

Green tea extract 250 mg;

pomegranate extract 250 mg;

lycopene 1.25 mg;

selenium 82.5 µg;

zinc 20 mg.

Composition 3

Green tea extract 125 mg;

pomegranate extract 125 mg;

lycopene 5 mg;

selenium 82.5 µg;

zinc 20 mg;

serenoa 160 mg;

soya isoflavon 100 mg.

Composition 4

Green tea extract 125 mg;

pomegranate extract 40 mg;

lycopene 5 mg;

selenium 55 µg;

zinc 20 mg;

serenoa 320 mg;

soya isoflavon 100 mg.

Composition 5

Green tea extract 125 mg;

pomegranate extract 125 mg;

lycopene 5 mg;

selenium 82.5 μg;

zinc 20 mg;

serenoa 160 mg;

soya isoflavon 100 mg;

L-carnitine 200 mg.

CLAIMS

1. Composition comprising as active ingredients green tea extract and pomegranate extract.

2. Composition of claim 1 further comprising lycopene, selenium, zinc, serenoa, soya isoflavon and L-carnitine.

3. Composition of claims 1 or 2, in which green tea extract is present in a dose of from 25 to 800 mg; pomegranate extract is present in a dose of from 25 to 800 mg.

4. Composition of claims 1 or 2, in which lycopene is present in a dose of from 0.03 to 30.0 mg; selenium is present in a dose of from 8.2 to 500 µg; zinc is present in a dose of from 1 to 200 mg; serenoa is present in a dose of from 10 to 400 mg; soya isoflavon is present in a dose of from 10 to 500 mg; and L-carnitine is present in a dose of from 50 to 500 mg.

5. Composition of claim 2, in which green tea extract is present in a dose of 250 mg; pomegranate extract is present in a dose of 250 mg; lycopene is present in a dose of 1.25 mg; selenium is present in a dose of 82.5 µg; zinc is present in a dose of 20 mg.

6. Composition of claim 2, in which green tea extract is present in a dose of 125 mg; pomegranate extract is present in a dose of 125 mg; lycopene is present in a dose of 5 mg; selenium is present in a dose of 82.5 µg; zinc is present in a dose of 20 mg; serenoa is present in a dose of 160 mg; soya isoflavon is present in a dose of 100 mg.

7. Composition of claim 2, in which Green tea extract is present in a dose of 125 mg; pomegranate extract is present in a dose of 40 mg; lycopene is present in a dose of 5 mg; selenium is present in a dose of 55 µg; zinc is present in a dose of 20 mg; serenoa is present in a dose of 320 mg; soya isoflavon is present in a dose of 100 mg.

8. Composition of claim 2, in which green tea extract is present in a dose of 125 mg; pomegranate extract is present in a dose of 125 mg; lycopene is present in a dose of 5 mg; selenium is present in a dose of 82.5 µg; zinc is present in a dose of 20 mg; serenoa is present in a dose of 160 mg; soya isoflavon is present in a dose of 100 mg; L-carnitine is present in a dose of 200 mg.

9. Composition of claim 1 or 2, for the prevention or reduction of the progression of prostate cancer.

10. Use of the composition of claim 1 or 2, for preparing a medicament for the prevention or reduction of the progression of prostate cancer.

11. Use of the composition of claim 1 or 2, for preparing a dietary supplement for the prevention or reduction of the progression of prostate cancer.

12. Use according to claim 10 or 11, further comprising co-enzymes, mineral substances, antioxidants, vitamins and agents useful for treating prostate cancer.