TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBAL TOOTH PASTE FORMULA #TPWI

The toxicity and tumor studies for Herbal Tooth Paste (TPWI) are presented. The Toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 1000 mm compared to the untreated controls. Both toxicity and antitumor effects are discussed.

TOXICITY AND ANTITUMOR PROTOCOL for Herbal Tooth Paste

A total of 65 balb C mice having average weights ranging from 18 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT-6). Tumor cells (2×10^6) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herb was dissolved in a minimal amount of water and administered orally once day at the specified dose regime: 500, 1000, 2000, 4000 mg/kg. Mice were also given a total of 15 Gauss of radiation during a five day period on days 7 through 11 concurrently with the administered oral doses of 500 mg/kg and 1000 mg/kg of herb. The mice were monitored until day 22 or 24. The mice were weighed accordingly on days 3, 7, 10, 14, 17, 21 and 24.

Dose regime per group of five mice

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On alternate days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On alternate days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 7 to 18 mice were given a single oral dose of 500 mg/kg.
On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On days 7 to 11 were given a total of 15 Gauss of radiation.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 7 to 11 were given a total of 15 Gauss of radiation.

Antitumor Activity for #TPWI

Some antitumor activity was seen as reflected in tumor growth delay values. The highest value of herb having a tumor growth delay of 4.09 was seen at the dosing schedule of 500 mg/kg during days 4 through 8. Other dosing regimes having antitumor activity were 1000 mg/kg, 4 to 18 days, tumor growth delay 3.43; 1000 mg/kg, alternate days 7 to 18, tumor growth delay 3.37; 1000 mg/kg, 7 to 18 days, tumor growth delay 3.19. The combination of 300 Rads with 500 mg/kg, for 5 days had the highest tumor growth delay 5.14. See Table 1. for additional values.

Toxicity

HERBAL FORMULA #TPWI

No Toxicity was observed.
No animals died from administration of #TPWI to 65 balb mice.

SUMMARY

In summary, herbal tooth paste #TPWI showed some antitumor activity as indicated by their tumor growth delay values. The highest value being 4.09 using # TPWI alone and 5.14 using the combination of radiation concurrently with #TPWI. #TPWI showed no toxicity at all dosing level the highest of which was 4000 mg/kg.

Key: Formula #TPWI Tooth Paste mixture.

TABLE 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #TPWI IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 1000 mm

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.00 ± 0.76
2	days 7 & 14	0.05 ± 0.76
4	days 7 & 14	1.34 ± 0.49
1	days 4 - 18	3.40 ± 1.80
0.5	days 4 - 18	4.09 ± 1.90
1	days 7 - 18	3.19 ± 1.60
0.5	days 7 - 18	2.16 ± 0.73
1	alternate days 4 - 18	0.46 ± 0.67
0.5	alternate days 4 - 18	0.00 ± 0.70
1	alternate days 7 - 18	3.37 ± 1.10
0.5	alternate days 7 - 18	1.41 ± 0.75
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	1.51 ± 1.20
0.5	days 4 - 18 + x-rays	5.14 ± 1.60

January 6, 1997

Sung Baek
Jiang Jing Herbs
PO Box 104
Hebron, Indiana 46341

Dear Sung,

Happy New Year to you and all at Jiang Jing Herbs. It was great to talk to you the other day. Glad to hear you are doing well.

These are the last two reports you will receive, since all of the testing from the samples that you sent are complete. As usual, tables and graph analysis are provided for easy reading. The two herbs described are the garlic tablets which we designated #95 and the tooth powder #96. Both are non-toxic and have no antitumor activity.

Just a quick comment, we had tested 14 herbal mixtures from May to December. If we take an overall view at the data in regard to its relationship to antitumor activity and dosing, we find that there does not appear to be a benefit with increased doses, such as 2000 mg or 4000 mg/kg. In fact it appears that the smaller doses given over a longer period of time have more of an effect than the larger doses. Particular, the 500 mg dose which is given on days 4 -18, a 15 day period. This seems to be the dose which has the most affect for tumor activity, in comparison to the other dose regimes. There could be several reasons for this. The first one that comes to mind may be a limitation of the mouse gut. What are your thoughts on this?

We are looking forward to future testing.

Sincerely,

Marianne Spada, Ph.D.

cc: Dr. Arthur Pardee
Dr. Beverly Teicher

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBS #95 AND #96

The toxicity and tumor studies for Herbs #95 and #96 are presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm compared to the untreated controls. Both toxicity and antitumor effects are discussed.

TOXICITY AND ANTITUMOR PROTOCOL for Herbs #95 and #96

A total of 65 C mice having average weights of 19 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT-6). Tumor cells (2×10^6) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbs was administered orally once per day at the specified dose regime: 500, 1000, 2000, 4000 mg/kg. Mice were also given a total of 15 Gauss of radiation during a five day period on days 7 through 11 concurrently with the oral doses of 500 mg/kg and 1000 mg/kg of herbs. The mice were monitored until day 22 or 24. The mice were weighed accordingly on days 4, 8, 11, 15, 18, and 22.

Dose regime per group of five mice

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On alternate days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On alternate days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 7 to 18 mice were given a single oral dose of 500 mg/kg.
On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On days 7 to 11 were given a total of 15 Gauss of radiation.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 7 to 11 were given a total of 15 Gauss of radiation.

Antitumor Activity for #95 and #96

No antitumor activity was seen as reflected in tumor growth delay values. A minimal tumor growth delay of 2.5 was seen for #96 at the dose schedule of 500 mg/kg during days 7 through 18. All other dosing schedules showed no antitumor activity. See Table 2 and 3.

Toxicity

HERBAL FORMULA #95
No toxicity was observed.
A total of 7 mice died from 65.
Control mouse died on day 22.
Two mice died with a dose schedule of 4000 mg/kg on day 22.
One mouse died with a dose schedule of 1000 mg/kg, days 4 to 18 on the 22nd day.
Two mice died with dose schedule of 1000 mg/kg, days 4 to 18 on day 18.
One mouse died with a dose schedule of 500 mg/kg, alter. days 7 to 18 on day 11.

HERBAL FORMULA #96
No toxicity was observed.
One mouse died from 65.
One mouse died with dose schedule of 500 mg/kg, days 4 to 18 on the 22nd day.

SUMMARY

In summary, the herbs showed no antitumor activity as indicated by the absence of their tumor growth delay values. Herbs #95 and #96 showed no toxicity at all dosing level the highest of which is 4000 mg/kg.

Key: Formula #95 Garlic tablets
Formula #96 Tooth powder mixture

TABLE 2.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #95 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.00 ± 0.54
2	days 7 & 14	0.44 ± 0.61
4	days 7 & 14	0.00 ± 0.25
1	days 4 - 18	0.48 ± 0.49
0.5	days 4 - 18	0.00 ± 0.35
1	days 7 - 18	0.00 ± 0.45
0.5	days 7 - 18	0.00 ± 0.51
1	alternate days 4 - 18	0.00 ± 0.39
0.5	alternate days 4 - 18	0.00 ± 0.51
1	alternate days 7 - 18	0.00 ± 0.25
0.5	alternate days 7 - 18	0.07 ± 0.33
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	0.39 ± 0.33
0.5	days 4 - 18 + x-rays	0.00 ± 0.29

	4 days	8 days	11 days	15 days	18 days	22 days
Control	**18.84**	**18.68**	**18.46**	**19.1**	**19.54**	**19.3**
2.0 g/kg, days 7+14	**19.62**	**19.52**	**18.96**	**19.28**	**20.02**	**20.46**
4.0 g/kg, days 7+14	**19.64**	**19.52**	**18.9**	**19.16**	**19.48**	**19.98**
1.0 g/kg, days 4-18	**20.54**	**20.06**	**19.6**	**20.04**	**20.52**	**20.4**
0.5 g/kg, days 4-18	**20.08**	**20.06**	**19.38**	**19.66**	**19.5**	**19.13**
1.0 g/kg, days 7-18	**19.96**	**20**	**20.04**	**20.64**	**20.88**	**21.24**
0.5 g/kg, days 7-18	**20.68**	**20.08**	**19.72**	**20**	**20.62**	**20.78**

	4 days	8 days	11 days	15 days	18 days	22 days
Control	**18.84**	**18.68**	**18.46**	**19.1**	**19.54**	**19.3**
1.0 g/kg alt days 4-18	**19.7**	**19.88**	**19.76**	**20.12**	**20.68**	**21**
0.5 g/kg alt days 4-18	**19.3**	**19.14**	**18.92**	**19.32**	**20.02**	**20.32**
1.0 g/kg alt days 7-18	**19.12**	**19.46**	**19.78**	**19.88**	**20.34**	**20.6**
0.5 g/kg alt days 7-18	**20.38**	**20.08**	**19.18**	**19.58**	**19.63**	**19.98**
1.0 g/kg 5x3GY	**20.04**	**20**	**19.56**	**19.56**	**19.86**	**20.32**
0.5 g/kg 5x3GY	**20.78**	**20.32**	**19.98**	**20.32**	**20.66**	**20.86**

	8 days	11 days	15 days	18 days	22 days
Control		**210**	**643**	**1150**	**2050**	**4208**
2.0 g/kg, days 7+14		**274**	**643**	**1268**	**1681**	**3676**
4.0 g/kg, days 7+14		**274**	**643**	**1177**	**1929**	**3676**
1.0 g/kg, days 4-18		**309**	**589**	**1081**	**1681**	**3298**
0.5 g/kg, days 4-18		**309**	**843**	**1359**	**1681**	**2809**
1.0 g/kg, days 7-18		**263**	**643**	**1159**	**2050**	**4208**
0.5 g/kg, days 7-18		**309**	**713**	**1359**	**2050**	**3676**
Control		**210**	**643**	**1159**	**2050**	**4208**
1.0 g/kg alt days 4-18		**274**	**697**	**1159**	**2050**	**4208**
0.5 g/kg alt days 4-18		**274**	**697**	**1081**	**1929**	**3676**
1.0 g/kg alt days 7-18		**274**	**697**	**1081**	**1929**	**3676**
0.5 g/kg alt days 7-18		**309**	**643**	**926**	**1681**	**3676**
1.0 g/kg 5x3GY		**274**	**643**	**908**	**1681**	**2809**
0.5 g/kg 5x3GY		**263**	**697**	**1003**	**1681**	**2809**

TABLE 3.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #96 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 = 0.4
2	days 7 & 14	0.0 ± 0.5
4	days 7 & 14	0.0 ± 0.4
1	days 4 - 18	0.6 ± 0.4
0.5	days 4 - 18	0.6 ± 0.5
1	days 7 - 18	0.0 ± 0.7
0.5	days 7 - 18	2.5 ± 1.9
1	alternate days 4 - 18	0.0 ± 0.9
0.5	alternate days 4 - 18	0.7 ± 0.5
1	alternate days 7 - 18	0.0 ± 0.2
0.5	alternate days 7 - 18	0.0 ± 0.5
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	0.0 ± 0.9
0.5	days 4 - 18 + x-rays	0.0 ± 0.5

	4 days	8 days	11 days	15 days	18 days	22 days
Control	**19.42**	**19.64**	**19.68**	**20.26**	**20.3**	**21.28**
2.0 g/kg, days 7+14	**18.82**	**18.96**	**18.96**	**19.72**	**20.06**	**20.72**
4.0 g/kg, days 7+14	**18.84**	**18.78**	**18.68**	**19.56**	**19.94**	**20.66**
1.0 g/kg, days 4-18	**19.66**	**19.54**	**19.12**	**19.04**	**19.28**	**20.26**
0.5 g/kg, days 4-18	**19.44**	**19.6**	**19.2**	**19.16**	**19.2**	**19.88**
1.0 g/kg, days 7-18	**19.6**	**19.2**	**19.28**	**19.72**	**19.98**	**20.56**
0.5 g/kg, days 7-18	**19.28**	**19.08**	**19.26**	**19.9**	**20.24**	**20.5**

	4 days	8 days	11 days	15 days	18 days	22 days
Control	**19.42**	**19.64**	**19.68**	**20.26**	**20.3**	**21.28**
1.0 g/kg alt days 4-18	**18.86**	**18.96**	**18.9**	**18.8**	**19.34**	**18.35**
0.5 g/kg alt days 4-18	**19.1**	**19.2**	**19.2**	**19.36**	**19.68**	**20.28**
1.0 g/kg alt days 7-18	**18.36**	**18.44**	**18.04**	**18.74**	**19.26**	**18.3**
0.5 g/kg alt days 7-18	**19.4**	**19.64**	**19.3**	**19.18**	**19.48**	**20.24**
1.0 g/kg 5x3GY	**19.34**	**19.08**	**18.5**	**18.74**	**19.1**	**19.68**
0.5 g/kg 5x3GY	**20.06**	**19.94**	**19.46**	**19.76**	**20.18**	**20.44**

	8 days	11 days	15 days	18 days	22 days
Control		**210**	**589**	**1081**	**2195**	**4502**
2.0 g/kg, days 7+14		**210**	**589**	**1159**	**1929**	**2809**
4.0 g/kg, days 7+14		**274**	**589**	**1159**	**1929**	**4208**
1.0 g/kg, days 4-18		**184**	**536**	**1003**	**1681**	**3676**
0.5 g/kg, days 4-18		**184**	**536**	**1003**	**1681**	**3298**
1.0 g/kg, days 7-18		**210**	**536**	**908**	**1681**	**3298**
0.5 g/kg, days 7-18		**210**	**377**	**697**	**1576**	**2506**
Control		**210**	**589**	**1081**	**2195**	**4502**
1.0 g/kg alt days 4-18		**274**	**477**	**1268**	**1929**	**3298**
0.5 g/kg alt days 4-18		**274**	**536**	**697**	**1159**	**2506**
1.0 g/kg alt days 7-18		**210**	**643**	**1081**	**1929**	**3676**
0.5 g/kg alt days 7-18		**263**	**589**	**1159**	**1929**	**3298**
1.0 g/kg 5x3GY		**263**	**536**	**1081**	**1681**	**3298**
0.5 g/kg 5x3GY		**309**	**536**	**697**	**1359**	**2809**

December 29, 1996

Sung Baek
Jiang Jing Herbs
PO Box 104
Hebron, Indiana 46341

Dear Sung,

The studies on formula 4002 are complete. These include both toxicity and antitumor data. The herbal mixture that we received did not have a number so we gave it number 4002. This is a dark brown viscous liquid which was enclosed in a small packet with Korean labeling. The mixture had to be diluted before administering to the animals. It was easily dissolved in water in a 2:1 ratio. Formula 4002 had no antitumor activity and showed no toxic effect to the mice. Tumor growth delay values and charts with data analysis are provided.

The other two formulas which you gave us, tooth powder and garlic tablets are also enclosed in this package.

Best wishes for the New Year.

Marianne Spada, Ph.D.

cc: Dr. Arthur Pardee
Dr. Beverly Teicher

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBAL FORMULA 4002

The toxicity and tumor studies for herbal formula 4002 are present in this text. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm compared to the untreated controls. Toxicity and antitumor effect of 4002 are discussed and data analysis is depicted in Table 1 and graph form.

TOXICITY AND ANTITUMOR PROTOCOL for HERBAL FORMULA 4002

A total of 65 balb C mice having average weights of 18 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT-6). Tumor cells (2×10^6) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbal formula was administered orally once per day at the specified dose regime: 0.2 ml/kg, 0.4 ml/kg, 0.1 ml/kg and 0.2 ml/kg. The mice were monitored usually until day 22. The mice were weighed accordingly on days 4, 8, 12, 15, 19 and 22.

Dose regime per group of five mice for #4002

On day 7 and 14 mice were given a single oral dose of 0.2 ml/kg.
On day 7 and 14 mice were given a single oral dose of 0.4 ml/kg.

On day 4 to 18 mice were given a single oral dose of 0.1 ml/kg.
On day 4 to 18 mice were given a single oral dose of 0.2 ml/kg.

On days 7 to 18 mice were given a single oral dose of 0.1 ml/kg.
On days 7 to 18 mice were given a single oral dose of 0.2 ml/kg.

Antitumor Effects for herbal formula 4002

No antitumor activity was observed. See Table 1 for tumor growth delay values.

Toxicity for herbal formula 4002

Graph analysis of these formulas are depicted in the text. They are presented as the mean body weight in grams as a function of time in days of post tumor implantation. The data in this study indicates no toxicity. Deaths occurring late in the study usually due to increased tumor burden or deliberate sacrifice.

SUMMARY

In summary, herbal mixture 4002 showed no antitumor activity as indicated by their low tumor growth delay values. This concentrate also did not show toxicity in EMT-6 mice at doses ranging from 0.4 ml/kg to 0.2 ml/kg.

Table 1.

TUMOR GROWTH DELAY OF HERBAL LIQUID #4002 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm

Dose milliliters/kilogram	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.34
0.2	days 7 & 14	0.71 ± 0.36
0.4	days 7 & 14	0.00 ± 0.51
0.1	days 4 - 18	0.56 ± 0.37
0.2	days 4 - 18	0.58 ± 0.30
0.1	days 7 - 18	1.34 ± 0.60
0.2	days 7 - 18	0.91 ± 0.50
0.1	alternate days 4 - 18	0.60 ± 0.42
0.2	alternate days 4 - 18	1.11 ± 0.70
0.1	alternate days 7 - 18	0.20 ± 0.46
0.2	alternate days 7 - 18	0.48 ± 027
0.1	days 4 - 18, (5 x 3 Gy on days 7 - 11)	2.32 ± 0.52
0.2	days 4 - 18, (5 x 3 Gy on days 7 - 11)	0.94 ± 0.33

	8 days	12 days	15 days	19 days	22 days
Control	**184**	**477**	**1159**	**1929**	**3676**
0.2 ml/kg, days 7+14	**135**	**477**	**1081**	**1929**	**3676**
0.4 ml/kg, days 7+14	**210**	**589**	**1159**	**2470**	**4208**
0.1 ml/kg, days 4-18	**210**	**521**	**1081**	**1929**	**3676**
0.2 ml/kg, days 4-18	**210**	**536**	**1081**	**2050**	**3676**
0.1 ml/kg, days 7-18	**184**	**477**	**778**	**1681**	**2809**
0.2 ml/kg, days 7-18	**184**	**477**	**1003**	**2050**	**3676**
0.1 ml/kg alt days 4 - 18	**184**	**477**	**1081**	**2050**	**3676**
0.2 ml/kg alt days 4-18	**210**	**434**	**843**	**1929**	**2809**
0.1 ml/kg alt days 7-18	**184**	**536**	**1268**	**2050**	**3676**
0.2 ml/kg alt days 7-18	**184**	**536**	**1003**	**1929**	**3298**
0.1 ml/kg 4 -18, (5x3Gy 7-11)	**154**	**390**	**643**	**1359**	**2506**
0.2 ml/kg 4 -18, (5x3Gy 7-11)	**184**	**536**	**1003**	**2050**	**3676**

Sheet 2

	4 days	8 days	12 days	15 days	19 days	22 days
Control	**21.1**	**20.52**	**20.92**	**21.2**	**21.66**	**22.34**
0.2 ml/kg, 7+14	**19.68**	**19.7**	**20.06**	**20.88**	**21.26**	**21.86**
0.4 ml/kg, 7+14	**21.12**	**21.3**	**21.86**	**22.26**	**22.38**	**23.12**
0.1 ml/kg, 4-18	**19.46**	**19.44**	**20.32**	**20.94**	**21.4**	**22.08**
0.2 ml/kg, 4-18	**20.04**	**20.26**	**20.76**	**21.22**	**22.08**	**22.62**
0.1 ml/kg, 7-18	**18.98**	**18.46**	**19.72**	**20.18**	**20.74**	**21.1**
0.2 ml/kg, 7-18	**20.24**	**19.98**	**20.88**	**21.24**	**21.66**	**22.32**
0.1 ml/kg alt days 4 - 18	**20.1**	**20.08**	**21.24**	**21.34**	**21.68**	**22.32**
0.2 ml/kg alt days 4-18	**19.08**	**18.86**	**19.88**	**20.56**	**20.74**	**21.16**
0.1 ml/kg alt days 7-18	**19.96**	**20.08**	**20.28**	**21.04**	**21.7**	**22.12**
0.2 ml/kg alt days 7-18	**19.9**	**20.22**	**20.84**	**21.26**	**21.5**	**22.08**
0.1 ml/kg 4 -18, (5x3Gy 7-11)	**19.12**	**18.88**	**20.02**	**20.16**	**20.76**	**21.22**
0.2 ml/kg 4 -18, (5x3Gy 7-11)	**19.32**	**19.68**	**21.54**	**21.6**	**22.04**	**22.48**

September 6, 1996

Sung Baek
Jiang Jing Herbs
PO Box 104
Hebron, Indiana 46341

Dear Sung,

Enclosed are the reports for the concentrated formulas #15.3 B, C and #14.2 B, C. No toxicity was observed for all four concentrates (see charts 1 through 6). All of these mixtures showed no antitumor activity, refer to Tables 1 and 2 for tumor growth delay values and charts for data analysis.

Please let me know if I can be of further assistance.

Cordially,

Marianne Spada, Ph.D.

cc: Dr. Arthur Pardee
Dr. Beverly Teicher

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBAL FORMULAS #15.3 B, C and #14.2 B, C

The toxicity and tumor studies for herbal formulas #15.3 B, C and #14.2 B, C are presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm^3 compared to the untreated controls. Both toxicity and antitumor effects of #15.3 B, C and #14.2 B, C are discussed and depicted in Tables 1 and 2, pages 3 and 4 and in graphic form on pages 5 through 10.

TOXICITY AND ANTITUMOR PROTOCOL for HERBAL FORMULAS #15.3 B, C and #14.2 B, C

A total of 65 balb C mice having average weights of 18 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT-6). Tumor cells (2×10^6) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbal formulas were administered orally once per day at the specified dose regime: 2000 mg/kg, 4000 mg/kg, 1000 mg/kg and 500 mg/kg.

Dose regime per group of five mice for #15.3 B, C and #14.2 B, C

On day 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On day 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 4 to 18 mice were given a single oral dose of 500 mg/kg.

On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 7 to 18 mice were given a single oral dose of 500 mg/kg.

Antitumor Effects for herbal formulas #15.3 B, C and #14.2 B, C

No antitumor activity was observed. See Tables 1 and 2 and pages 3 - 4 for tumor growth delay values.

Toxicity for herbal formulas #15.3 B, C and #14.2 B, C

Graph analysis of these formulas are depicted on pages 5 through 10. They are presented as the mean body weight in grams as a function of time in days of post implantation. The data in this study indicates no toxicity. Deaths occurring late in the study are usually due to increased tumor burden or deliberate sacrifice.

Herbal formula #15.3 B, C

One death occurred on day eleven for formula #15.3 B at a dose of 500 mg/kg, treated days 7 to 18. No deaths are reported for formula #15.3 C.

Herbal formula #14.2 B

No deaths reported.

SUMMARY

In summary, herbal mixtures 15.3 B, C and #14.2 B, C showed no antitumor activity as indicated by their low tumor growth delay values. These concentrates also do not show toxicity in EMT -6 mice at doses ranging from 4000 mg/kg to 500 mg/kg. Consumption of a single dose of 4000 mg/kg for an adult weighing 70 kg may be represented as ingesting 0.6 lbs.

Table 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #15.3 B AND C IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilogram	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.43
#15.3 B		
2	days 7 & 14	0.00 ± 0.31
4	days 7 & 14	0.19 ± 0.36
1	days 4 - 18	0.00 ± 0.61
0.5	days 4 - 18	0.85 ± 0.47
1	days 7 - 18	0.81 ± 0.38
0.5	days 7 - 18	0.76 ± 0.13
#15.3 C		
2	days 7 & 14	1.70 ± 1.00
4	days 7 & 14	0.90 ± 0.34
1	days 4 - 18	0.00 ± 0.35
0.5	days 4 - 18	0.80 ± 0.33
1	days 7 - 18	0.36 ± 0.31
0.5	days 7 - 18	0.70 ± 0.20

Table 2.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #14.2 B AND C IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilogram	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.69
#14.2 B		
2	days 7 & 14	0.00 ± 0.47
4	days 7 & 14	0.00 ± 0.41
1	days 4 - 18	0.37 ± 0.40
0.5	days 4 - 18	0.00 ± 0.36
1	days 7 - 18	0.00 ± 0.40
0.5	days 7 - 18	0.00 ± 0.27
#14.2 C		
2	days 7 & 14	0.00 ± 0.60
4	days 7 & 14	0.00 ± 0.47
1	days 4 - 18	0.00 ± 0.47
0.5	days 4 - 18	0.58 ± 0.86
1	days 7 - 18	0.00 ± 0.28
0.5	days 7 - 18	0.00 ± 0.58

	8 days	11 days	15 days	18 days	22 days
Control	**296**	**477**	**1359**	**2308**	**4502**
2.0 g/kg, days 7+14	**318**	**536**	**1268**	**2050**	**3676**
4.0 g/kg, days 7+14	**309**	**500**	**1229**	**1855**	**3458**
1.0 g/kg, days 4-18	**396**	**589**	**1081**	**1681**	**3676**
0.5 g/kg, days 4-18	**282**	**477**	**1081**	**1681**	**2809**
1.0 g/kg, days 7-18	**259**	**679**	**1075**	**1786**	**3223**
0.5 g/kg, days 7-18	**284**	**597**	**1041**	**1332**	**3503**

	8 days	11 days	15 days	18 days	22 days
Control	**296**	**761**	**1390**	**2366**	**3995**
2.0 g/kg, days 7+14	**279**	**519**	**956**	**1664**	**3017**
4.0 g/kg, days 7+14	**293**	**597**	**1102**	**1834**	**3382**
1.0 g/kg, days 4-18	**309**	**646**	**1350**	**2155**	**3609**
0.5 g/kg, days 4-18	**262**	**611**	**1229**	**1884**	**3371**
1.0 g/kg, days 7-18	**275**	**652**	**1381**	**1979**	**3366**
0.5 g/kg, days 7-18	**300**	**591**	**1102**	**1583**	**3117**

	7 days	10 days	14 days	17 days	21 days	24 days
Control	**235**	**477**	**1072**	**1687**	**2850**	**4044**
2.0 g/kg, days 7+14	**214**	**513**	**981**	**1778**	**3282**	**4219**
4.0 g/kg, days 7+14	**236**	**627**	**977**	**1872**	**3242**	**4003**
1.0 g/kg, days 4-18	**203**	**449**	**881**	**1596**	**2718**	**4384**
0.5 g/kg, days 4-18	**237**	**553**	**916**	**1766**	**3366**	**4384**
1.0 g/kg, days 7-18	**228**	**513**	**811**	**1605**	**2981**	**4278**
0.5 g/kg, days 7-18	**235**	**537**	**1077**	**1515**	**2802**	**4202**
Control	**235**	**477**	**1072**	**1687**	**2850**	**4044**
2.0 g/kg, days 7+14	**206**	**538**	**973**	**1488**	**3153**	**3983**
4.0 g/kg, days 7+14	**218**	**627**	**1209**	**1759**	**3276**	**4267**
1.0 g/kg, days 4-18	**246**	**575**	**1036**	**1515**	**2694**	**3987**
0.5 g/kg, days 4-18	**248**	**482**	**817**	**1177**	**2257**	**3397**
1.0 g/kg, days 7-18	**170**	**462**	**1087**	**1506**	**2802**	**3920**
0.5 g/kg, days 7-18	**218**	**507**	**1035**	**1518**	**2570**	**3690**

July 30, 1996

Sung Baek
Jiang Jing Herbs
PO Box 104
Hebron, Indiana 46341

Dear Sung,

Enclosed are the reports for formulas #20, #0 and #16. No toxicity was observed for all three formulas. Two of the formulas, #20 and #0 showed no antitumor activity. Mixture #16 had modest antitumor activity as reflected by its tumor growth delay, see tables. Charts are also presented representing analysis of data.

Call me if you have any questions about the data.

Cordially,

Marianne Spada, Ph.D.

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBAL FORMULAS #20, #0 AND #16

The toxicity and tumor studies for herbal formulas #20, #0 and #16 are presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm^3 compared to the untreated controls. Both toxicity and antitumor effects of #20, #0 and #16 are discussed and depicted in Tables 1-3, pages 3-5 and in graphic form pages 6-8.

TOXICITY AND ANTITUMOR PROTOCOL for HERBAL FORMULAS #20. #0 and #16

A total of 65 balb C mice having average weights of 18 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT -6). Tumor cells (2 x 106) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbal formulas were administered orally once per day at the specified dose regime: 2000 mg/kg, 4000 mg/kg, 1000 mg/kg and 500 mg/kg. Mice were also given a total of 300 rads of radiation during a five day period on days 7 through 11 concurrently with the administered oral doses of 1000 mg/dg and 500 mg/kg of herbs. The mice were monitored usually until day 25. The mice were weighed accordingly on days 8, 11, 15, 18 and 22.

Dose regime per group of five mice for #20, #0 and #16

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 4 to 18 mice were given a single oral dose of 500 mg/kg.

On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 7 to 18 mice were given a single oral dose of 500 mg/kg.

On alternate days 4 to 18 mice were given a single oral dose of 1000 mg/kg.
On alternate days 4 to 18 mice were given a single oral dose of 500 mg/kg.

On alternate days 7 to 18 mice were given a single oral dose of 1000 mg/kg.
On alternate days 7 to 18 mice were given a single oral dose of 500 mg/kg.

On days 4 and 18 mice were given a single oral dose of 1000 mg/kg and on days 7 to 11 were given a total of 300 rads of radiation, 15 Gy.

On days 4 and 18 mice were given a single oral dose of 500 mg/kg and on days 7 to 11 were given a total of 300 rads of radiation, 15 Gy.

Antitumor for herbal formulas #20, #0 and #16

No antitumor activity was observed in formula #20. Minimal to zero antitumor activity was seen in formula #0. Herbal mixture #16 showed modest antitumor activity. See Tables 1-3 and pages 3-5 for tumor growth delay values.

Toxicity for herbal formulas #20, #0 and #16

Graph analysis of these formulas are depicted on pages 6-8. They are presented as the mean body weight in grams as a function of time in days of post implantation. The data in this study indicates no toxicity. Deaths occurring late in the study are usually due to increased tumor burden or deliberate sacrifice.

Herbal formula #20

No deaths reported during treatment.

Herbal formula #0

One death with dose schedule 0.5 g/kg, alternate days 7 to 18 on day 18.

Herbal formula #16

No deaths reported.

SUMMARY

In summary, herbal mixtures #20 and #0 showed no antitumor activity as indicated by their low tumor growth delay values. Formula #16 had minimal activity. These 3 herbal mixtures also do not show toxicity in EMT -6 mice at doses ranging from 4000 mg/kg to 500 mg/kg. Consumption of a single dose of 4000 mg/kg for an adult weighing 70 kg may be represented as ingesting 0.6 lbs.

TABLE 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #20 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.3
2	days 7 & 14	0.0 ± 0.4
4	days 7 & 14	0.0 ± 0.3
1	days 4 - 18	0.0 ± 0.4
0.5	days 4 - 18	0.3 ± 0.9
1	days 7 - 18	0.0 ± 0.5
0.5	days 7 - 18	0.0 ± 0.1
1	alternate days 4 - 18	0.0 ± 0.4
0.5	alternate days 4 - 18	0.0 ± 0.4
1	alternate days 7 - 18	0.4 ± 0.6
0.5	alternate days 7 - 18	0.2 ± 0.7
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	1.0 ± 0.4
0.5	days 4 - 18 + x-rays	1.3 ± 0.5

TABLE 2.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #0 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.5
2	days 7 & 14	0.1 ± 0.5
4	days 7 & 14	0.1 ± 0.5
1	days 4 - 18	0.0 ± 0.6
0.5	days 4 - 18	0.5 ± 0.5
1	days 7 - 18	1.0 ± 0.7
0.5	days 7 - 18	0.0 ± 0.5
1	alternate days 4 - 18	0.2 ± 0.5
0.5	alternate days 4 - 18	0.0 ± 0.6
1	alternate days 7 - 18	0.2 ± 0.3
0.5	alternate days 7 - 18	0.2 ± 0.3
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	1.2 ± 0.5
0.5	days 4 - 18 + x-rays	1.1 ± 0.7

TABLE 3.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #16 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.4
2	days 7 & 14	2.4 ± 0.7
4	days 7 & 14	1.9 ± 0.3
1	days 4 - 18	1.8 ± 0.7
0.5	days 4 - 18	1.6 ± 0.4
1	days 7 - 18	2.0 ± 0.3
0.5	days 7 - 18	2.1 ± 0.4
1	alternate days 4 - 18	1.4 ± 0.4
0.5	alternate days 4 - 18	1.7 ± 0.6
1	alternate days 7 - 18	2.3 ± 0.5
0.5	alternate days 7 - 18	2.4 ± 0.5
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	2.8 ± 0.7
0.5	days 4 - 18 + x-rays	3.6 ± 0.3

Sheet 1

	8 days	11 days	15 days	18 days	22 days
Control	**149**	**471**	**1031**	**1963**	**3701**
2.0 g/kg	**149**	**478**	**960**	**1794**	**3178**
4.0 g/kg	**135**	**460**	**1087**	**2015**	**3746**
1.0 g/kg, days 4-18	**177**	**522**	**1119**	**2043**	**3601**
0.5 g/kg, days 4-18	**133**	**449**	**957**	**1581**	**2924**
1.0 g/kg, days 7-18	**102**	**356**	**952**	**1666**	**3121**
0.5 g/kg, days 7-18	**155**	**467**	**1097**	**1815**	**3366**
1.0 g/kg alt days 4-18	**138**	**471**	**1048**	**1775**	**3225**
0.5 g/kg alt days 4-18	**182**	**515**	**1165**	**1945**	**3494**
1.0 g/kg alt days 7-18	**138**	**453**	**867**	**1629**	**3019**
0.5 g/kg alt days 7-18	**109**	**435**	**1035**	**1868**	**3284**
1.0 g/kg 5x3GY	**188**	**366**	**593**	**1309**	**2318**
0.5 g/kg 5x3GY	**123**	**422**	**830**	**1350**	**2193**

	8 days	11 days	15 days	18 days	22 days
Control	**235**	**648**	**1200**	**1862**	**3597**
2.0 g/kg	**108**	**431**	**799**	**1434**	**2754**
4.0 g/kg	**112**	**435**	**1036**	**1766**	**3056**
1.0 g/kg, days 4-18	**156**	**471**	**855**	**1515**	**3329**
0.5 g/kg, days 4-18	**130**	**44**	**1055**	**1694**	**3325**
1.0 g/kg, days 7-18	**95**	**416**	**955**	**1599**	**3076**
0.5 g/kg, days 7-18	**123**	**433**	**962**	**1670**	**2927**
1.0 g/kg alt days 4-18	**140**	**455**	**973**	**1478**	**2843**
0.5 g/kg alt days 4-18	**148**	**467**	**1039**	**1470**	**2726**
1.0 g/kg alt days 7-18	**106**	**327**	**830**	**1500**	**2988**
0.5 g/kg alt days 7-18	**107**	**410**	**925**	**1522**	**2794**
1.0 g/kg 5x3GY	**152**	**335**	**720**	**1294**	**2499**
0.5 g/kg 5x3GY	**96**	**260**	**591**	**1099**	**2107**

July 25, 1996

Sung Baek
Jiang Jing Herbs
PO Box 104
Hebron, Indiana 46341

Dear Sung,

The final toxicity and antitumor reports for herbal formulas #33.3 A, B are complete. All of these formulas showed no toxicity and zero to minimal antitumor activity at the specified doses, see charts. Formulas in which ethanol was used as a solubizing agent were found to be toxic, causing animal death. When ethanol was removed from the herbal mixture no toxicity was noted.

Experiments were performed as described in text. Analysis of the data is presented in Table 1 and Charts 1-4.

Please feel free to contact me if you have any question regarding the experimentation.

Sincerely,

Marianne Spada, Ph.D.

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBAL FORMULAS #33.3 A, B AND C

The toxicity and tumor studies for herbal formulas #33.3 A, B and C are presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm^3 compared to the untreated controls. Both toxicity and antitumor effects of #33.3 A, B and C are discussed and depicted in Table 1, page 3 and in graphic form pages 4-7.

TOXICITY AND ANTITUMOR PROTOCOL for HERBAL FORMULAS #33.3 A, B and C

A total of 65 balb C mice having average weights of 18 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT -6). Tumor cells (2 x 106) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbal formulas were administered orally once per day at the specified dose regime: 2000 mg/kg, 4000 mg/kg, 1000 mg/kg and 500 mg/kg. Mice were also given a total of 300 rads of radiation during a five day period on days 7-11 concurrently with the administered oral doses of 1000 mg/kg and 500 mg/kg of herbs. The mice were monitored usually until day 25. The mice were weighed accordingly on days 8, 11, 15, 18 and 22.

Dose regime per group of five mice for #33.3 A, B and C

On days 7 and 14 were given a single oral dose of 2000 mg/kg.

On days 7 and 14 were given a single oral dose of 4000 mg/kg.

On days 4 to 18 were given a single oral dose of 1000 mg/kg.

On days 4 to 18 were given a single oral dose of 500 mg/kg.

Antitumor for herbal formulas #33.3 A, B and C

No antitumor activity was observed in formula 33.3 A. Minimal to zero antitumor activity was seen in formulas 33.3 B and C. See Table 1 for tumor growth delay values. Also see Chart 1.

Toxicity for herbal formulas #33.3 A, B and C

Graph analysis of these formulas are depicted on Charts 2-4. They are presented as the mean body weight in grams as a function of time in days of post implantation. The data in this study indicates no toxicity. Deaths occurring at an early time in this study was due to ethanol overdose caused indicates no toxicity. Deaths occurring at an early time in this study was due to ethanol overdose caused by formulation error. This was observed in herbal preparation 33.3 B at the highest dose of 4 g/kg.

Herbal formula #33.3 A

One death was reported on day 18 with a dose of 500 mg/kg, days 4 to 18.

Herbal formula #33.3 B

Two deaths with dose schedule 2.0 g/kg, days 7, 8 and 14. This was due to ethanol poisoning. All the animals died with dose 4.0 g/kg. Again this was due to ethanol overdose from formulation error.

Herbal formula #33.3 C

One death was reported on day 11 with a dose of 4.0 g/kg, days 7 and 14.

SUMMARY

In summary, herbal mixtures 33.3 A, B and C showed minimal to no antitumor activity as indicated by their low tumor growth delay values. These 3 herbal mixtures also do not show toxicity in EMT -6 mice at doses ranging from 4000 mg/kg to 500 mg/kg. Consumption of a single dose of 4000 mg/kg for an adult weighing 70 kg may be represented as ingesting 0.6 lbs.

Table 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #33.3 A, B AND C IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

	Dose grams/kilogram	Schedule	Tumor Growth Delay, Days
	CONTROL		0.0 ± 0.5
A	2	days 7 & 14	0.0 ± 0.3
	4	days 7 & 14	0.0 ± 0.3
	1	days 4 - 18	0.0 ± 0.4
	0.5	days 4 - 18	0.6 ± 0.6
B	2	days 7 & 14	0.0 ± 0.9
	4	days 7 & 14	**TOXIC**
	1	days 4 - 18	0.9 ± 0.6
	0.5	days 4 - 18	0.9 ± 0.7
C	2	days 7 & 14	0.7 ± 0.7
	4	days 7 & 14	0.7 ± 0.4
	1	days 4 - 18	0.0 ± 0.4
	0.5	days 4 - 18	1.3 ± 0.8

	8 days	11 days	15 days	18 days	22 days
Control	**210**	**477**	**1359**	**2308**	**4502**
2.0 g/kg, days 7+14	**263**	**536**	**1268**	**2050**	**3676**
4.0 g/kg, days 7+14	**250**	**500**	**1229**	**1855**	**3458**
1.0 g/kg, days 4-18	**210**	**589**	**1081**	**1681**	**3676**
0.5 g/kg, days 4-18	**154**	**477**	**1081**	**1681**	**2809**

	8 days	11 days	15 days	18 days	22 days
Control	**210**	**477**	**1359**	**2308**	**4502**
2.0 g/kg, days 7+14	**184**	**589**	**1081**	**1449**	**2809**
4.0 g/kg, days 7+14					
1.0 g/kg, days 4-18	**184**	**589**	**1081**	**1449**	**2809**
0.5 g/kg, days 4-18	**184**	**477**	**1081**	**1449**	**2809**

	8 days	11 days	15 days	18 days	22 days
Control	**210**	**477**	**1359**	**2308**	**4502**
2.0 g/kg, days 7+14	**184**	**536**	**1159**	**1929**	**3298**
4.0 g/kg, days 7+14	**154**	**521**	**1081**	**1681**	**2809**
1.0 g/kg, days 4-18	**274**	**778**	**1359**	**1681**	**3298**
0.5 g/kg, days 4-18	**184**	**337**	**843**	**1681**	**2809**

	4 days	8 days	11 days	15 days	18 days	22 days
Control	**17.46**	**18.48**	**18.74**	**19.54**	**19.88**	**20.48**
2.0 g/kg, days 7+14	**18**	**18.28**	**18.7**	**19.12**	**19.48**	**20.08**
4.0 g/kg, days 7+14	**18.16**	**18.78**	**18.42**	**19.08**	**19.32**	**19.94**
1.0 g/kg, days 4-18	**18.7**	**19.22**	**19.28**	**19.2**	**19.46**	**19.77**
0.5 g/kg, days 4-18	**17.86**	**19**	**20.35**	**20.35**	**20.35**	**20.35**

		8 days	11 days	15 days	18 days	22 days
Control	**17.46**	**18.48**	**18.74**	**19.54**	**19.88**	**20.48**
2.0 g/kg, days 7+14	**18**	**18.17**	**18.2**	**19.67**	**20.13**	**20.6**
4.0 g/kg, days 7+14						
1.0 g/kg, days 4-18	**17.94**	**17.4**	**17.8**	**18.25**	**18.85**	**19.13**
0.5 g/kg, days 4-18	**18.24**	**17.7**	**18.46**	**18.88**	**19.5**	**20.08**

		8 days	11 days	15 days	18 days	22 days
Control	**17.46**	**18.48**	**18.74**	**19.54**	**19.88**	**20.48**
2.0 g/kg, days 7+14	**17.02**	**17.26**	**17.18**	**17.46**	**17.7**	**18.08**
4.0 g/kg, days 7+14	**17.22**	**16.58**	**16.95**	**17.5**	**18.35**	**19.13**
1.0 g/kg, days 4-18	**17.52**	**18.24**	**18.24**	**18.74**	**19.12**	**19.48**
0.5 g/kg, days 4-18	**17.64**	**18.32**	**18.86**	**19.1**	**19.88**	**20.82**

July 9, 1996

FROM: Marianne Spada

TO: **Sung Baek**
Jiang Jing Herbs
P.O. Box 104
Hebron, Indiana 46341

Dear Sung,

Enclosed is the final report for herbal formulas Hua Ming #46 and Tien Dao #10. Both herbs showed no toxicity and zero to minimal antitumor activity at doses given. Tumor Growth Delay values are in Tables 1 and 2. Toxicity determined as a function of mean body weight loss over time are depicted in Charts 1-4. Antitumor activity is shown in 3-D charts 5 and 6 as tumor volume vs. dose vs. time.

As requested by you we evaporated the ethanol from the liquid mixtures and will test accordingly.

Also, as you know we submit for testing one herb per week. By the end of this month the last formula will be in the cue for testing. If you have more herbs you would like to test, please send them to us by the end of this month so we can begin testing and eliminate any delay in sending you results.

Sincerely,

Marianne Spada

cc: Teicher
Pardee

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBS #46 AND #10

The toxicity and tumor studies for Herbs #46 and #10 are presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm^3 compared to the untreated controls. Both toxicity and antitumor effects are discussed.

TOXICITY AND ANTITUMOR PROTOCOL for Herbs #46 and #10

A total of 65 balb C mice having average weights of 18 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT -6). Tumor cells (2×10^6) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbs was administered orally once per day at the specified dose regime: 500, 1000, 2000, and 4000 mg/kg. Mice were also given a total of 15 Gauss of radiation during a five day period on days 7-11 concurrently with the administered oral doses of 500 mg/kg and 1000 mg/kg of herbs. The mice were monitored until day 22 or 25. The mice were weighed accordingly on days 4, 8, 11, 15. 18 and 22 and/or 25.

Dose regime per group of five mice

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On alternate days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On alternate days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 7 to 18 mice were given a single oral dose of 500 mg/kg.
On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.

On alternate days 7 to 18 mice were given a single oral dose of 500 mg/kg.
On alternate days 7 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg and
on days 7 to 11 were given a total of 15 Gauss of radiation.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg and
on days 7 to 11 were given a total of 15 Gauss of radiation.

Antitumor Activity for #46 and #10

Antitumor activity is reflected in tumor growth delay values. Basically no antitumor effect was observed with these herbal mixtures. See Tables 1 and 2.

Toxicity

HERBAL FORMULA #46
No toxicity was observed.
A total of 3 mice died from 60.

One mouse died with a dose schedule of 500 mg/kg, days 4 to 18 on the 22nd day.
One mouse died with a dose schedule of 1000 mg/kg, alternative days 4 to 18 on day 22.
One mouse died with a dose schedule of 500 mg/kg, alternative days 7 to 18 on day 22.

HERBAL FORMULA #10
No toxicity was observed.
A total of 3 mice died from 60.

One mouse died with a dose schedule of 2000 mg/kg on day 11.
One mouse died with a dose schedule of 500 mg/kg, days 4 to 18 on the 15th day.
One mouse died with a dose schedule of 1000 mg/kg, days 7 to 18 on day 18.

SUMMARY

In summary, the herbs showed no antitumor activity as indicated by the absence of tumor growth delay. Herbs #46 and #10 showed no toxicity at all dosing level the highest of which is 4000 mg/kg giving as a single dose.

TABLE 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #10 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.6
2	days 7 & 14	0.0 ± 0.3
4	days 7 & 14	0.3 ± 0.4
1	days 4 - 18	0.0 ± 0.7
0.5	days 4 - 18	0.0 ± 0.4
1	days 7 - 18	0.0 ± 0.3
0.5	days 7 - 18	0.0 ± 0.4
1	alternate days 4 - 18	0.0 ± 0.6
0.5	alternate days 4 - 18	0.0 ± 0.3
1	alternate days 7 - 18	0.0 ± 0.4
0.5	alternate days 7 - 18	0.0 ± 0.4
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	1.4 ± 0.4
0.5	days 4 - 18 + x-rays	0.5 ± 0.5

TABLE 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #10 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 ± 0.6
2	days 7 & 14	0.2 ± 0.4
4	days 7 & 14	0.2 ± 0.3
1	days 4 - 18	0.4 ± 0.2
0.5	days 4 - 18	0.0 ± 0.3
1	days 7 - 18	0.0 ± 0.4
0.5	days 7 - 18	0.0 ± 0.5
1	alternate days 4 - 18	0.0 ± 0.6
0.5	alternate days 4 - 18	0.0 ± 0.3
1	alternate days 7 - 18	0.0 ± 0.5
0.5	alternate days 7 - 18	0.0 ± 0.5
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	2.1 ± 0.5
0.5	days 4 - 18 + x-rays	1.2 ± 0.5

June 28, 1996

FROM: Marianne Spada, Ph.D.

TO: **Dr. Sung Baek**
Jiang Jing Herbs
P.O. Box 104
Hebron, Indiana 46341

Dear Sung,

Here is the animal study data on herbal formula #35, Xue. The herb showed no toxicity and minimal antitumor effects at doses given. Tumor Growth Delay data is explained in Table 1. Toxicity determined as a function of mean body weight loss over time are depicted in Charts 1 and 2. Antitumor activity is shown in 3-D Charts 3 and 4 as tumor volume vs. dose vs. time.

Results for the herbal concentrates #33.2 a, b, c will be available shortly.

Sincerely,

Marianne Spada

cc: Teicher
Pardee

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBS #35

The toxicity and tumor studies for Herbs #35 is presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm^3 compared to the untreated controls. Both toxicity and antitumor effects are discussed.

TOXICITY AND ANTITUMOR PROTOCOL for Herbs #35

A total of 65 balb C mice having average weights of 18 to 19 grams were implanted with epithelial mouse tumor cells clone #6 (EMT -6). Tumor cells (2 x 10^6) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbs was administered orally once per day at the specified dose regime: 500, 1000, 2000 and 4000 mg/kg. Mice were also given a total of 300 rads of radiation during a five day period on days 7-11 concurrently with the administered oral doses of 500 mg/kg and 1000 mg/kg of herbs. The mice were monitored until day 22 or 25. The mice were weighed accordingly on days 4, 7, 11, 14, 18 and 22 and/or 25.

Dose regime per group of five mice

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On alternate days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On alternate days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 7 to 18 mice were given a single oral dose of 500 mg/kg.
On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.

On alternate days 7 to 18 mice were given a single oral dose of 500 mg/kg.
On alternate days 7 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg and on days 7 to 11 were given a total of 15 Gauss of radiation.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg and on days 7 to 11 were given a total of 15 Gauss of radiation.

Antitumor Activity for #35

Basically no antitumor activity was seen as reflected in tumor growth delay values. A minimal tumor growth delay of 1.4 was seen at the dose schedule of 1000 mg/kg during alternate days 7-18. All other dosing schedules showed no antitumor activity. See Table 1.

Toxicity

HERBAL FORMULA #35
No toxicity was observed.
A total of 7 mice died from 60.

One mouse died with a dose schedule of 1000 mg/kg, days 4 to 18 on the 21st and 25th day.
One mouse died with a dose schedule of 1000 mg/kg, days 7 to 18 on day 21.
One mouse died with a dose schedule of 500 mg/kg, days 4 to 18, 300 rads on day 21 and 25.
One mouse died with a dose schedule of 1000 mg/kg, days 4 to 18, 300 rads on day 21 and 25.

SUMMARY

In summary, the showed no antitumor activity as indicated by the absence of tumor growth delay. Herbs #35 showed no toxicity at all dosing level the highest of which is 4000 mg/kg.

TABLE 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #35 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 = 0.3
2	days 7 & 14	0.1 ⊕ 0.3
4	days 7 & 14	0.3 ⊕ 0.3
1	days 4 - 18	0.3 ⊕ 0.3
0.5	days 4 - 18	0.3 ⊕ 0.2
1	days 7 - 18	1.2 ⊕ 0.7
0.5	days 7 - 18	0.5 ⊕ 0.3
1	alternate days 4 - 18	0.7 ⊕ 0.3
0.5	alternate days 4 - 18	1.2 ⊕ 0.3
1	alternate days 7 - 18	1.4 ⊕ 0.4
0.5	alternate days 7 - 18	0.5 ⊕ 0.1
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	1.2 ⊕ 0.4
0.5	days 4 - 18 + x-rays	2.2 ⊕ 0.5

June 10, 1996

FROM: Marianne Spada

TO: **Sung Baek**
Jiang Jing Herbs
P.O. Box 104
Hebron, Indiana 46341

Dear Sung,

Enclosed is the final report for herbal formulas Chui Hai #13 and Gao Liu #22. Both Herb's showed no toxicity and zero to minimal antitumor activity at doses given. Tumor Growth Delay data can be found on pages 2 and 3, Tables 1 and 2. Toxicity determined as a function of mean body weight loss over time are depicted in charts 1-4 and antitumor activity in is shown in 3-D charts 5-8 as tumor volume vs. dose vs. time.

Sincerely,

Marianne Spada

cc: Teicher
Pardee
Sotiropoulou

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBS #13 AND #22

The toxicity and tumor studies for Herbs #13 and #22 are presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm^3 compared to the untreated controls. Both toxicity and antitumor effects are discussed.

TOXICITY AND ANTITUMOR PROTOCOL for Herbs #13 and #22

A total of 65 balb C mice having average weights of 19 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT -6). Tumor cells (2×10^6) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbs was administered orally once per day at the specified dose regime: 500, 1000, 2000 and 4000 mg/kg. Mice were also given a total of 15 Gauss of radiation during a five day period on days 7-11 concurrently with administered oral doses of 500 mg/kg and 1000 mg/kg of herbs. The mice were monitored until day 22 or 24. The mice were weighed accordingly on days 4, 8, 11, 15, 18, and 22 and/or 24.

Dose regime per group of five mice

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On alternate days 4 to 18 mice were given a single oral dose of 500 mg/kg.
On alternate days 4 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 7 to 18 mice were given a single oral dose of 500 mg/kg.
On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 500 mg/kg and on days 7 to 11 were given a total of 15 Gauss of radiation.
On days 4 to 18 mice were given a single oral dose of 1000 mg/kg and on days 7 to 11 were given a total of 15 Gauss of radiation.

Antitumor Activity for #13 and #22

No antitumor activity was seen as reflected in tumor growth delay values. A minimal tumor growth delay of 1.5 was seen at the dose schedule of 1000 mg/kg during days 4 through 18. All other dosing schedules showed basically no antitumor activity. See Table 1 and 2.

Toxicity

HERBAL FORMULA #13
No toxicity was observed.
A total of 5 mice died from 60.
Control mouse died on day 18.
One mouse died with a dose schedule of 1000 mg/kg on day 22.
One mouse died with a dose schedule of 500 mg/kg, days 4 to 18 on the 11th day.
One mouse died with a dose schedule of 1000 mg/kg, days 7 to 18 on day 18.
One mouse died with a dose schedule of 500 mg/kg, days 7 to 18 on day 11.
One mouse died with a dose schedule of 500 mg/kg, days 4 to 18, 300 rads on day 15.

HERBAL FORMULA #22
No toxicity was observed.
A total of 3 mice died from 60.
Control mouse died on day 24.
One mouse died with a dose schedule of 500 mg/kg on days 4 to 18 on the 24th day.
One mouse died with a dose schedule of 1000 mg/kg, alternative days 4 to 18 on day 14.
One mouse died with a dose schedule of 500 mg/kg, alternative days 7 to 18 on day 24.

SUMMARY

In summary, the herbs showed no antitumor activity as indicated by the absence of tumor growth delay. Herbs #13 and #22 showed no toxicity at all dosing level the highest of which is 4000 mg/kg.

TABLE 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #13 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 = 0.3
2	days 7 & 14	0.1 ⊕ 0.3
4	days 7 & 14	0.1 ⊕ 0.3
1	days 4 - 18	0.0 ⊕ 0.3
0.5	days 4 - 18	0.0 ⊕ 0.3
1	days 7 - 18	0.0 ⊕ 0.3
0.5	days 7 - 18	0.7 ⊕ 0.3
1	alternate days 4 - 18	0.0 ⊕ 0.3
0.5	alternate days 4 - 18	0.0 ⊕ 0.3
1	alternate days 7 - 18	0.0 ⊕ 0.3
0.5	alternate days 7 - 18	0.0 ⊕ 0.3
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	0.0 ⊕ 0.4
0.5	days 4 - 18 + x-rays	0.0 ⊕ 0.4

TABLE 1.

TUMOR GROWTH DELAY OF HERBAL MIXTURE #22 IN THE EMT-6 MURINE MAMMARY CARCINOMA AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
CONTROL		0.0 = 0.3
2	days 7 & 14	0.4 ⊕ 0.6
4	days 7 & 14	0.5 ⊕ 0.4
1	days 4 - 18	1.5 ⊕ 0.6
0.5	days 4 - 18	0.2 ⊕ 0.6
1	days 7 - 18	1.2 ⊕ 0.8
0.5	days 7 - 18	1.3 ⊕ 0.8
1	alternate days 4 - 18	0.6 ⊕ 0.6
0.5	alternate days 4 - 18	0.5 ⊕ 0.8
1	alternate days 7 - 18	1.3 ⊕ 0.2
0.5	alternate days 7 - 18	0.9 ⊕ 0.7
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	2.2 ⊕ 0.3
0.5	days 4 - 18 + x-rays	2.8 ⊕ 0.3

TRIPLE S RESEARCH ASSOCIATES, INC.

P.O. Box 428
East Boston, MA 02178
*Tel. (617) 569-6237 Fax (617) 569-6237*51*

April 25, 1995

Grace Szczepaniec, President
Jiang Jing Herbs
P.O. Box 104
Hebron, Indiana 46341

Dear Grace,

Presented are the final toxicity and antitumor reports for herbal formulas #2, #3, #4 and #5. All four of these formulas showed no toxicity or antitumor activity at the reported doses. Experimental details and analysis of the data are discussed and presented in Tables 1-4 and in graphic form.

Also, an additional protocol was designed using radiation therapy concurrently with herbal administration. It was hoped that the herbs would enhance the antitumor effect of radiation on the EMT -6 mice. Unfortunately, no change in response rate was seen with these herbs.

Please feel free to contact me if you have any questions regarding the experimentation.

Sincerely,

Marianne Spada, Ph.D.

TOXICOLOGICAL AND ANTITUMOR STUDIES FOR HERBAL FORMULAS #2, #3, #4 AND #5

The toxicity and tumor studies for herbal formulas #2, #3, #4 and #5 are presented. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. The tumor growth delay (TGD) is calculated as the difference in days it takes each individual tumor to reach a volume of 500 mm^3 compared to the untreated controls. Both toxicity and antitumor effects of #2, #3, #4 and #5 are discussed and depicted in Tables 1-4, pages 3-6 and in graphic form on pages 7-10.

TOXICITY AND ANTITUMOR PROTOCOL for HERBAL FORMULAS #2, #3, #4 AND #5

A total of 65 balb C mice having average weights of 19 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT -6). Tumor cells (2 x 106) were injected subcutaneously in the hind leg of the mice. The mice were divided into twelve groups and a control group each containing five mice. The herbal formulas were administered orally once per day at the specified dose regime: 2000 mg/kg, 4000 mg/kg, 1000 mg/kg and 500 mg/ kg. Mice were also given a total of 300 rads of radiation during a five day period on days 7-11 concurrently with the administered oral doses of 1000 mg/kg and 500 mg/kg of herbs. The mice were monitored usually until day 25. The mice were weighed accordingly on days 8, 11, 15, 18, 22 and 25 unless otherwise specified.

Dose regime per group of five mice for #2, #3, #4 and #5

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 4 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 4 to 18 mice were given a single oral dose of 500 mg/kg.

On days 7 to 18 mice were given a single oral dose of 1000 mg/kg.
On days 7 to 18 mice were given a single oral dose of 500 mg/kg.

On alternate days 4 to 18 mice were given a single oral dose of 1000 mg/kg.
On alternate days 4 to 18 mice were given a single oral dose of 500 mg/kg.

On alternate days 7 to 18 mice were given a single oral dose of 1000 mg/kg.
On alternate days 7 to 18 mice were given a single oral dose of 500 mg/kg.

On days 4 and 18 mice were given a single oral dose of 1000 mg/kg and on days 7 to 11 were given a total of 300 rads of radiation, 15 Gy.

On days 4 and 18 mice were given a single oral dose of 500 mg/kg and on days 7 to 11 were given a total of 300 rads of radiation, 15 Gy.

Antitumor for herbal formulas #2, #3, #4 and #5

No antitumor activity as reflected in low tumor growth delay values. See Tables 1-4 and pages 3-6.

Toxicity for herbal formulas #2, #3, #4 and #5

Graph analysis of these formulas are depicted on pages 7-10. They are presented as the mean body weight in grams as a function of time in days of post implantation. The data in this study indicates no toxicity. Deaths occurring late in the study are usually due to increased tumor burden or deliberate sacrifice.

Herbal formula #2

No deaths reported during treatment.

Herbal formula #3

One death with dose schedule 4 g/kg days 7 and 14 on day 22 and four deaths on day 25, two of these were sacrificed.
One death with dose schedule 1 g/kg days 4 to 18 on day 22 and one death on day 235 and two were sacrificed on day 25.
One death with dose schedule 1 g/kg, alternate days 4 to 18 on day 22.

Herbal formula #4

No deaths reported.

Herbal formula #5

No deaths reported.

SUMMARY

In summary, herbal mixtures #2, #3, #4 and #5 showed no antitumor activity as indicated by their low tumor growth delay values. These 4 herbal mixtures also do not show toxicity in EMT -6 mice at doses ranging from 4000 mg/kg to 500 mg/kg. Consumption of a single dose of 4000 mg/kg for an adult weighing 70 kg may be represented as ingesting 617 lbs.

TABLE 1.

TUMOR GROWTH DELAY OF THE EMT -6 MURINE MAMMARY CARCINOMA PRODUCED BY HERBAL MIXTURE #2 AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
2	days 7 & 14	0.3 ± 0.3
4	days 7 & 14	0.8 ± 0.3
1	days 4 - 18	0.3 ± 0.3
0.5	days 4 - 18	0.3 ± 0.3
1	days 7 - 18	0.5 ± 0.3
0.5	days 7 - 18	1.3 ± 0.3
1	alternate days 4 - 18	0.2 ± 0.3
0.5	alternate days 4 - 18	0.5 ± 0.3
1	alternate days 7 - 18	0.3 ± 0.3
0.5	alternate days 7 - 18	0.7 ± 0.3
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	4.2 ± 0.4
0.5	days 4 - 18 + x-rays	2.8 ± 0.4

TABLE 2.

TUMOR GROWTH DELAY OF THE EMT -6 MURINE MAMMARY CARCINOMA PRODUCED BY HERBAL MIXTURE #3 AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
2	days 7 & 14	0.8 ± 0.3
4	days 7 & 14	0.4 ± 0.3
1	days 4 - 18	0.6 ± 0.3
0.5	days 4 - 18	0.3 ± 0.3
1	days 7 - 18	0.3 ± 0.3
0.5	days 7 - 18	0.3 ± 0.3
1	alternate days 4 - 18	0.2 ± 0.3
0.5	alternate days 4 - 18	0.8 ± 0.3
1	alternate days 7 - 18	0.3 ± 0.3
0.5	alternate days 7 - 18	0.7 ± 0.3
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	1.3 ± 0.4
0.5	days 4 - 18 + x-rays	2.7 ± 0.4

TABLE 3.

TUMOR GROWTH DELAY OF THE EMT -6 MURINE MAMMARY CARCINOMA PRODUCED BY HERBAL MIXTURE #4 AT 500 mm^3

Dose grams/kilograms	Schedule	Tumor Growth Delay, Days
2	days 7 & 14	0.7 ± 0.3
4	days 7 & 14	0.3 ± 0.3
1	days 4 - 18	0.5 ± 0.3
0.5	days 4 - 18	1.3 ± 0.3
1	days 7 - 18	1.6 ± 0.3
0.5	days 7 - 18	1.6 ± 0.3
1	alternate days 4 - 18	1.3 ± 0.3
0.5	alternate days 4 - 18	0.7 ± 0.3
1	alternate days 7 - 18	0.6 ± 0.3
0.5	alternate days 7 - 18	0.3 ± 0.3
X-rays (5 x 3Gy) days 7-11		
1	days 4 - 18 + x-rays	0.8 ± 0.4
0.5	days 4 - 18 + x-rays	2.1 ± 0.4

TRIPLE S RESEARCH ASSOCIATES, INC.

P.O. Box 428
East Boston, MA 02178
*Tel. (617) 569-6237 Fax (617) 569-6237*51*

November 6, 1994

Grace Szczepaniec, President
Jiang Jing Herbs
P.O. Box 104
Hebron, Indiana 46341

Dear Grace,

Enclosed is the final toxicology report for herbal formulas #33 and #14.2. We apologize for the delay of these results. The delay was due to additional testing performed on formulas #33 and #14.2. As a result we present the results in triplicate at no additional cost.

Both herbal formulas showed no toxicity even at high single doses of 2000 mg/kg and 4000 mg/kg.

Both herbal formulas showed antitumor activity in tumor bearing mice at all levels of dosing. Formula #33 showed a higher antitumor effect compared to formula #14.2. Nonetheless, all had some antitumor activity and showed no toxicity.

If you have any question about the data in this report please call me.

Cordially,

Marriane Spada, Ph.D.

TOXICOLOGICAL STUDIES FOR HERBAL FORMULAS #33 AND #14.2

The toxicity and tumor studies for herbal formulas #33 and #14.2 are presented in this report. The toxicity is determined by animal weight loss or death. The antitumor effect is determined by the tumor growth delay factor. Both toxicity and antitumor effects are discussed and depicted in graphic form provided in this report.

The data given represents triplicate experiments in tumor bearing mice. Each set of experiments involves a total of 65 mice which are designated by groups A, B and C. Group A refers to the first set of experiments for herbal formulas #33 and #14.2. Group B refers to the duplicate experiments for #33 and #14.2. And group C represents the triplicate experimentation.

The mice have average weights of about twenty grams and were implanted with mammary mouse tumor clones. These primary tumors once allowed to grow have the potential to become metastatic. The tumors were allowed to grow until the fourth and seventh day before administration of formulas #33 and #14.2. The tumors burden at day seven is most comparable to the tumor model in humans. The herbs were given orally in a single dose (see protocol for details). The herbal formulas were given up to day 11 and day 18 depending on the specified protocol. The mice remained under continued observation for weight gain or loss caused by tumor burden until day 29. After day 29 animals were sacrificed.

Group A

Antitumor effect for #33

Oral administration of #33, at the doses of 500 mg/kg at alternate days 7-18 shows the highest tumor growth delay in this group of 30 mice. It has taken 19.11 days to reach a tumor volume of 500 mm^3 as compared to the control group which received no herb taking 12.94 days to reach a tumor volume of 500 mm^3. Therefore, this group showed a TGD of 6.17 days. See graph.

Group B

Antitumor effect for #33

Group B as group A also expressed the highest tumor growth delay at 7-18 alternate days. A dose of 500 mg/kg at alternate days from 7 to 18 had a TGD of 6.84 days compared to the control group. The control group reached a tumor volume of 500 mm^3 at day 13.71. The treated animals reached a tumor volume of 500 mm^3 at day 20.55. See graph.

Group C

Antitumor effect for #33

The highest TGD was seen in the group given a high single oral dose of 2000 mg/kg at days 7 and day 14. The control group took 13.12 days to reach a volume of 500 mm^3. The mice given the herb took 19.13 days to reach a tumor volume of 500 mm^3. Therefore, at this high dose, compared to the control group, the herb delayed the tumor growth rate to 6.01 days. See graph.

Group A

Antitumor effect for #14.2

Oral administration of #14.2, at a dose of 500 mg/kg at days 4 to 11 shows the highest tumor growth delay in this group of 30 mice. It has taken 18.86 days to reach a tumor volume of 500 mm^3 as compared to the control group

which received no herb taking 12.94 days to reach a tumor volume of 500 mm^3. Therefore, this group showed a TGD of 5.92 days. See graph.

Group B

Antitumor effect for #14.2

A 500 mg/kg dose at 4-18 alternate days exhibited the highest TGD at 5.17 days. Mice receiving no herb took 13.71 days to reach a tumor volume of 500 mm^3. Treated mice reached this tumor volume at day 19.42. See graph.

Group C

Antitumor effect for #14.2

Group C mice had the highest TGD factor at 4-11 days and 4-18 alternate days of treatment. It has taken 17.43 and 17.54 days to reach a tumor volume of 500 mm^3 as compared to the control group which received no herb taking 13.12 days to reach a tumor volume of 500 mm^3. Therefore, the tumor growth delays were 4.30 and 4.40 days respectively. See graph.

TOXICITY PROTOCOL for HERBAL FORMULAS #33 AND #14.2

A total of 65 balb C mice having average weights of 19 to 20 grams were implanted with epithelial mouse tumor cells clone #6 (EMT -6). Tumor cells (2×10^6) were injected subcutaneoulsy in the hind leg of the mice. The mice were divided into six groups and a control group each containing five mice. The herbal formulas #33 and #14.2 were administered orally once per day at the specified dose regime. The experiment was repeated three times, designated as Groups **A**, **B** and **C**, with the following doses: 2000 mg/kg, 4000 mg/kg and 500 mg/kg. The mice were monitored until day 29. The mice were weighed accordingly on days 3, 8, 11, 15, 18, 22, 25 and 29.

Groups A and B

Dose regime per group of five mice for #33 and #14.2

On day 7 mice were given a single oral dose of 2000 mg/kg.
On day 7 mice were given a single oral dose of 4000 mg/kg.

On days 7 to 11 mice were given a single oral dose 500 mg/kg.
On alternate days 7 to 18 mice were given a single oral dose of 500 mg/kg.

On days 4-11 mice were given a single oral dose of 500 mg/kg.
On alternate days 4-18 mice were given a single oral dose of 500 mg/kg.

Group C

Dose regime per group of five mice for #33 and #14.2

On days 7 and 14 mice were given a single oral dose of 2000 mg/kg.
On days 7 and 14 mice were given a single oral dose of 4000 mg/kg.

On days 7 to 11 mice were given a single oral dose of 500 mg/kg.
On alternate days 7 to 18 mice were given a single oral dose of 500 mg/kg.

On days 4-11 mice were given a single oral dose of 500 mg/kg.
On alternate days 4-18 mice were given a single oral dose of 500 mg/kg.

Toxicity for herbal formulas #33 and #14.2

Graph analysis of herbal formulas #33 and #14.2 are presented as mean body weight (grams) as a function of time (days post implantation). The data shows no toxicity in the triplicate experiments performed. Both herbs do not show any toxicity under these experimental conditions. Deaths occurring late in the study are usually due to increased tumor burden or deliberate sacrifice.

Group A for herbal formula #33 had five deaths four of which were at or around day 29. Deaths during this late in the experiment are most likely due to the presence of the tumor.

Group A for herbal formula #14.2 had eight deaths 87% occurring after day 25.

Group B for herbal formula #33 had nine deaths all which occurred about day 29. One animal was sacrificed at day 29 because of increased tumor burden.

Group B for herbal formula #14.2 had five deaths all at day 29.

Group C for herbal formula #33 had nine deaths at day 29. One death occurred at day 25.

Group C for herbal formula #14.2 had six deaths. Five at day 29 and one at day 25.

SUMMARY

In summary, the herbal formula #33 at dose 500 mg/kg alternate days 7-18 had the best response rate with a tumor growth delay average of 5.43 days based on triplicate experimentation. The mean tumor volume for groups A, B and C is 333 mm^3 (see graph day 17) which compared to the control is 1200 mm^3. This is a significant difference indicating a 72% tumor inhibition. The second highest response rate is for groups having doses of 500 mg/kg at 4-11 days. The average TGD is 4.78, a 66% tumor inhibition, a TGD of 4.51 days.

Formula #14.2 for groups A, B and C show the best response rate at days 4-11 and 4-18 alternate. Their average tumor growth delays are 4.74 and 4.45 days, representing a 65% tumor inhibition.

Other chemotherapeutic drugs currently used with their tumor growth delays are melphalan at 2.3 days, cyclophosphamide at 6.2 days, carmustine at 2.5 days, cisplatin at 7.8 days, radiation (1 week at 300 rads) 4.3 days and adriamycin at 3.0 days. Adriamycin is the single most effective chemotherapeutic agent in treating human breast cancer, but has a low tumor growth delay in mice mammary tumors.

COMPARISON OF TUMOR GROWTH DELAY (TGD) IN GROUPS A, B, C AT 500 mm^3

	GROUP A	GROUP B	GROUP C	AVERAGE (TGD)
TSRA-33				
2 g/kg day 7	3.27	2.84		3.06
2 g/kg days 7, 14			6.01	
4 g/kg day 7	3.88	3.16		3.52
4 g/kg days 7, 14				
500 mg/kg days 7-11	1.22	2.68	2.42	2.11
500 mg/kg alternate days 7-18	6.17	6.84	3.27	5.43
500 mg/kg days 4-11	5.34	2.99	6.00	4.78
500 mg/kg alternate days 4-18	4.75	4.09	4.70	4.51
TSRA-14.2				
2 g/kg day 7	4.66	3.78		4.22
2 g/kg days 7, 14			2.40	
4 g/kg day 7	3.83	3.08		3.46
4 g/kg days 7, 14			3.10	
500 mg/kg days 7-11	3.81	4.13	2.20	3.38
500 mg/kg alternate days 7-18	3.17	3.95	2.70	3.27

500 mg/kg days 4-11	5.92	3.99	4.30	4.74
500 mg/kg alternate days 4-18	3.25	5.71	4.40	4.45