Synergistic Effects of *Arthrospira platensis* (*Spirulina*) and *Moringa oleifera* Lam. (Malunggay) on Tumor-induced *Rattus norvegicus* (Sprague-Dawley Rat)

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Abstract

The study determined the histopathologic and hematologic effects of *Arthrospira platensis* and *Moringa oleifera* Lam., and their synergism on tumor-induced Sprague- Dawley rats. Induction of tumors was done through intraperitoneal administration of 1,2-dimethylhydrazine and 7,12-dimethylbenz[a]anthracene for four weeks. Readily available capsules and tablets of *Spirulina* and malunggay mixed with water were used to achieve the desired 50% and 75% concentration. Intraperitoneal administration of the extracts and intravenous administration of 5-fluorouracil as the positive control treatment were done for two weeks post tumor induction. After treatment, representatives of each treatment groups were drawn blood and dissected for hematologic and histopathologic examination, respectively. Results show anti-tumor activity of all extracts in the liver in contrast to their ineffectiveness in the lungs. Furthermore, all but the 75% *Spirulina* extract were effective against gastric tumor development while both concentrations of *Spirulina* and its 75% concentration successfully countered development of tumors of the small intestine and large intestine, respectively. All of the extracts caused a general negative effect on the complete blood count. In conclusion, *A. platensis* proved to be the more effective against tumor development.

Key Words
Arthrospira platensis, Hematology, Histopathology, Moringa oleifera, Lam., Tumor, Rattus norvegicus, Synergistic effects

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Introduction

Cancer is described as a wild, unrestrained growth of abnormal cells that may originate in any body organ or tissue. They grow in a disorganized and uncontrolled pattern reproducing endlessly thus causing tumor, a pile up of abnormal cells, in the process. (1) Cancer has inflicted humans with records transcribed in papyrus since 1500 B.C. in Egypt with eight cases of tumor affecting the breast until today in which it is the third leading cause of morbidity and mortality in the Philippines. (2-3) However, despite its lengthy existence and the use of modern technology in medicine, there is still no total and complete cure for cancer.

Numerous studies have been conducted to treat various types of cancer; herbal medicine of which *Arthrospira platensis* and *Moringa oleifera* Lam. are grouped, is said

to offer cures for different types of cancer. *A. platensis* commonly known as *Spirulina*, a filamentous cyanobacterium, is a widely known food supplement in the Philippines that gained recognition from several renowned institutions namely the World Health Organization (WHO) as the 21st century best health food for human beings, the United Nations Educational, Scientific and Cultural Organization (UNESCO) as the most complete ideal food for tomorrow and the American Food and Drug Administration (FDA) as one of the best protein sources. (4) *Spirulina* is said to be rich in digestible proteins and other phytochemicals, and studies have shown its role in controlling diabetes, cholesterol, coronary artery disease, wound healing and weight loss (5). In 1988, a study evaluated the chemopreventive ability of *Spirulina* by proving that β-carotene and *Spirulina-Dunaliella* extracts inhibit carcinogenesis by preventing tumor development in hamster buccal pouch. (6) Later on, it was emphasized by the first human study examining the chemopreventive potential of *Spirulina* involving the species *S. fusiformis* tested in oral cancer. (7) On the other hand, *Moringa oleifera* Lam, locally known as *malunggay*, a common backyard plant in Filipino homes, has achieved popularity in mainstream scientific journals describing its nutritional and medicinal properties Primarily, it is rich in compounds containing the simple sugar, rhamnose. (8) It is plentiful in glucosinolates and isothiocyanates which both exhibit antibacterial and antifungal activity. (9) A research conducted in Bangladeshi medicine found through MTT assay that malunggay possess moderate cytotoxic activity on leukemia (HL-60 and CEM) and melanoma cell lines along with other extracts from identified specimens. (10)

The primary goal of this paper is therefore to determine the effects of the extracts on the tumors induced in Sprague-Dawley rats and the changes in their complete blood counts.

Methods

Research Design

Eight treatment groups including two control groups were used in the experiment. Two concentrations of *Arthrospira platensis, Moringa oleifera* Lam. and their synergism namely 50% and 75% and 5-fluorouracil were given. For each treatment, two trials were performed with two replicates each. Sixteen (16) male and sixteen (16) female Sprague-Dawley rats weighing 20-35 grams (g) were purchased from the Experimental Animal House Section, Bureau of Food and Drugs, Alabang, Muntinlupa City.

Procurement and preparation of the different treatments

Fifteen (15) tablets of Spirulina Vegetabs and twenty-five (25) capsules of Malunggay Capsules were procured from Carica Herbal Health Products, Inc. About 0.5 g *Spirulina* tablets/5 ml water and 0.35 g malunggay capsules/5 ml water were considered to have a 100% concentration. Dilution formula was used to achieve the desired 50% and 75% concentrations. Synergism treatments were prepared by mixing same concentration of the two extracts. The negative control group was not given any treatment while the positive control group was administered 5-fluorouracil.

Acclimatization and maintenance of laboratory rats

The main sites for the one-week acclimatization and maintenance of the Sprague-Dawley rats extracts were in the improvised facilities of the Besa's residence. Rats caged as treatment groups were kept in good condition in a well-ventilated and well-lighted room of about 26-30°C. The cages and animal facility were sanitized regularly. Sufficient amount of food pellets and adequate amount of tap water were given to the rats twice daily. The cages were spacious enough to permit the rats to carry out normal behavior like exploring and grooming. Daily careful monitoring of the animals' health condition was conducted. Dead rats were taken out of the cages immediately and were properly discarded.

Induction and monitoring of tumors and administration of extracts and chemotherapeutic drug

Tumor induction and administration of extracts were done at the improvised laboratory facilities. Tumors were introduced to the colon by intraperitoneal injections of 30 mg 1,2-dimethylhydrazine (DMH)/1 ml saline/kg body weight after the acclimatization period every other day for three weeks. It was followed by a single intraperitoneal administration of 50 mg 7,12-dimethylbenz[a]anthracene (DMBA)/5 ml sesame oil/kg body weight at the age of 9 weeks. (11-12) After the initial induction, weekly palpation was conducted to ascertain the time of appearance, number, location and size of tumors. (12) Then, the extracts and treatment were administered through intraperitoneal injections. The 50% and 75% concentrations were given daily to treatment groups. The chemotherapeutic drug, 5- fluorouracil, was administered through intravenous injection of about 100 mg/2 ml saline/kg body weight once a week. (13) The extracts and the drug were administered in the morning for two weeks.

Histopathologic examination

One rat per treatment group manifesting the most severe signs of tumor development in terms of the behavior displayed and the morphological appearance of the organs was chosen for histopathologic examination regardless of its gender. The lungs, liver, stomach, small intestine and large intestine of each rat were initially processed observing the standard histologic technique of Bruce-Gregorios (14) at the laboratory room of Paolo Campos Hall (PCH), De La Salle University- Dasmariñas (DLSU-D), City of Dasmariñas, Cavite. The samples were forwarded to the Histopathology Laboratory, The Research Institute for Tropical Medicine (RITM), Alabang, Metro Manila for more advanced processing. Histopathologic evaluation was conducted and was documented using a digital camera (Canon Digital IXUS 860 IS) at the Biology Research Laboratory, DLSU-D, City of Dasmariñas, Cavite.

Hematologic examination

A representative of each of the different groups were subjected to Complete Blood Count (CBC). The CBC analyses included the examination of hemoglobin (Hgb) level, red blood cell (RBC) count and white blood cell (WBC) count. Analyses were performed using Medic Drabkin's reagent Cyanmethemoglobin Method, Hayem's Solution Red Blood Cells Count, White Blood Cells Count Diluting Fluid and Wright- Giemsa Stain Schilling's Differential Count. (15) The values obtained were compared to

the standard hematologic data for *Rattus norvegicus*. The samples were processed at the Wellcare Clinics & Lab, City of Dasmariñas, Cavite.

Termination of laboratory activities

Lab activities were terminated properly in compliance with the Bioethics Committee of the university on the second week of December.

Data Analysis

Developing tumors from the rats were measured using an ocular micrometer. Comparison of micrographs with images from *Wheater's Basic Histopathology* was also done to assess changes on the tissues. Validity of the histopathologic evaluations of the tissues were confirmed by a pathologist from RITM, Alabang, Metro Manila; hematologic data were analyzed through comparison with a standard reference value. (16)

Results

Histopathologic evaluation

Examination of prepared tissue slides before and after treatments indicated successful tumor induction by 1,2-DMH and 7,12-DMBA and halted development of cancer if not complete elimination, respectively. Table 1 shows the histopathologic findings in the lungs, liver, stomach, small intestine and large intestine of the treatment groups while Table 2 shows the frequency and size of the developing tumors found in the small intestine and large intestine.

All of the treatment groups exhibited signs of tumor development in the lungs (Plate 1) in contrast to the absence of any abnormalities in the liver (Plate 2). The stomach (Plate 3) was almost clear from any histopathologic manifestations in contrary to the various abnormalities seen in the small and large intestines (Plates 4 and 5).

Collapsed alveoli (**A)** along with extensive hemorrhage (**H)** were observed in the lungs of representative tissues of treatment groups except for the group that did not receive any treatment (Plate 1A-G); however, emphysema (**E)** was observed in the group wherein no treatment was applied (Plate 1H). On the other hand, the liver shows clearance from any hepatic abnormalities. However, inflammation (**In)** of some of the hepatic portal triads of the group that did not receive any treatment was observed (Plate 2H).

There is an apparent infiltration of lymphocytes (**L)** in the stomach **(**Plate 3A) in the group treated with 5-fluorouracil while the rest show no obvious abnormalities in the stomach. Remarkably, all of the treatment groups except those that were treated with 75% *Spirulina* were observed to have abnormalities in the small intestine (Plate 4A-G). Observable anomalies were inflammations (**In)** and aggregations of lymphocytes (**LA).** Fibrous tissues such as fibromuscular (**Fm)** and fibrocollagenous (**Fc)** tissue were also formed.

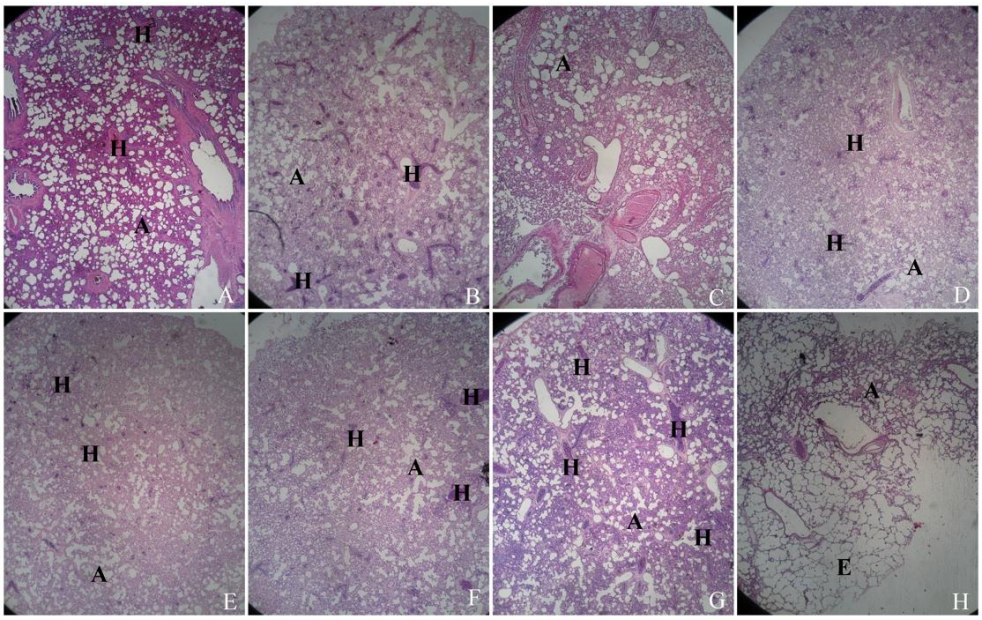

Plate 1. Effects of treatments on the histology of pulmonary tissue. Representative hematoxylin-and-eosin (H & E) stained sections (x40). Collapsed alveoli **(A),** Hemorrhage **(H)** Emphysema **(E)** A, T_0 = positive control; B, T_1= 50% *Spirulina*; C, T_2 = 75% *Spirulina*; D, T_3 = 50% malunggay; E, T_4 = 75% malunggay; F, T_5 = 50% synergism; G, T_6 = 75% synergism; H, T_7 = negative control

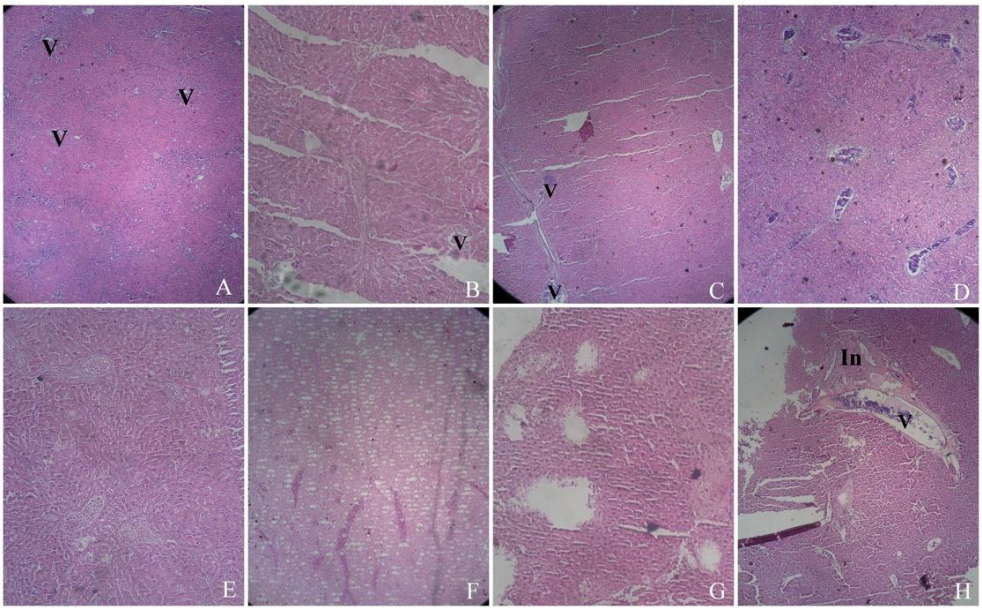

Plate 2. Effects of treatments on the histology of hepatic tissue. Representative hematoxylin-and-eosin (H & E) stained sections (A, B, C, D, F, H x40; E, G x100). Inflammation **(In)** Hepatic veins **(V)**. A, T_0 = positive control; B, T_1= 50% *Spirulina*; C, T_2 = 75% *Spirulina*; D, T_3 = 50% malunggay; E, T_4 = 75% malunggay; F, T_5 = 50% synergism; G, T_6 = 75% synergism; H, T_7 = negative control

Similar to that of the small intestine, lymphoid aggregates (**LA)** were present in the large intestine along with chronic inflammation (**In)**. Fibromuscular tissue (**Fm)** formation was also evident. The normal architecture of the colon of most of the treatment groups was maintained though infiltration of lymphocytes begins to replace normal cells especially in the mucosa (**M).** Further distinct and mass-like aggregations of lymphocytes that are tumors (**T)** on the process of development are observed in the small intestine (Plate 6A) of the group treated with 5-fluorouracil and large intestine (Plate 6B) of the group administered with 75% malunggay.

Examining the effect in the lungs, rats administered with 50% *Spirulina*, both concentrations of malunggay, both synergism concentrations of *Spirulina* and malunggay and 5-fluorouracil were observed with atelectasis and extensive hemorrhage while those given with 75% *Spirulina* were seen with atelectasis along with chronic inflammation. Rats that were not given any treatments showed signs of chronic inflammation and emphysema.

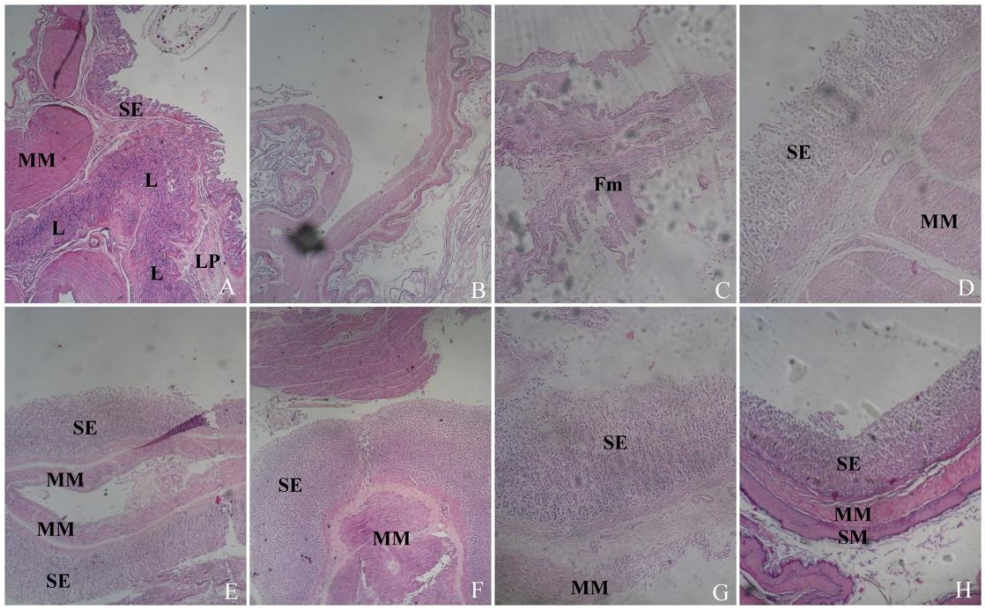

Plate 3. Effects of treatments on the histology of gastric tissue. Representative hematoxylin-and-eosin (H & E) stained sections (B, C, E, F, G, H x40; A, D x100). Surface epithelium (**SE),** Muscularis mucosae (**MM).** lymphocytes (**L),** Fibromuscular tissue (**Fm)**. A, T_0 = positive control; B, T_1= 50% *Spirulina*; C, T_2 = 75% *Spirulina*; D, T_3 = 50% malunggay; E, T_4 = 75% malunggay; F, T_5 = 50% synergism; G, T_6 = 75% synergism; H, T_7 = negative control

Only the liver of the untreated rats showed signs of chronic inflammation in some of the portal triads; others were clear from any hepatic damage regardless of treatment.

The stomach of rats that were administered with 75% *Spirulina* were observed with degenerated fibromuscular tissue while rats that were treated with 5-fluorouracil were seen to have lymphocytes infiltrating the tissue; others were clear from any gastric abnormalities.

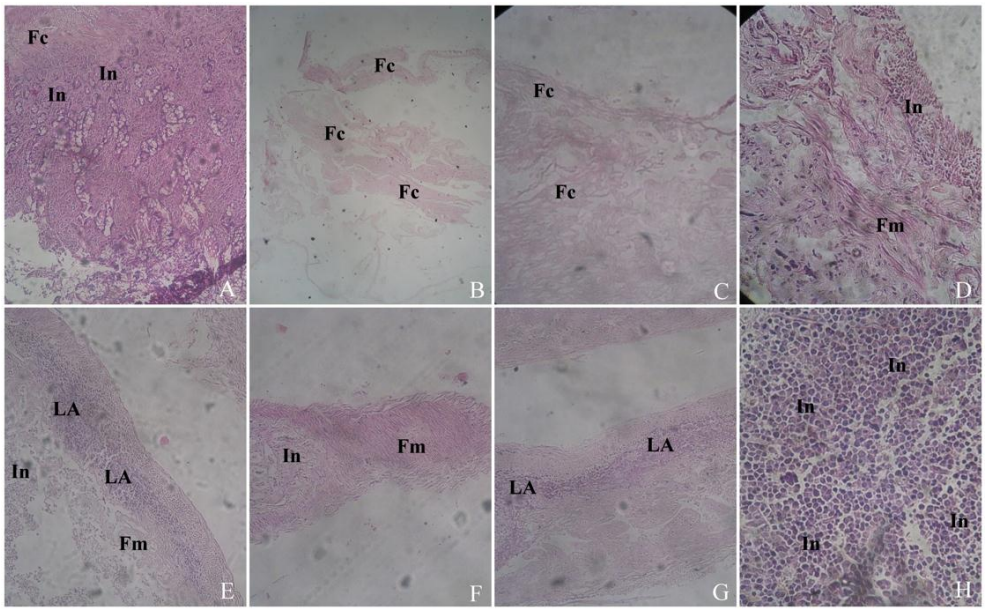

Plate 4. Effects of treatments on the histology of the tissue of the small intestine. Representative hematoxylin-and-eosin (H & E) stained sections (A, B x40; C, D, E, F, G, H x100).. A, T_0 = positive control; B, T_1= 50% *Spirulina*; C, T_2 = 75% *Spirulina*; D, T_3 = 50% malunggay; E, T_4 = 75% malunggay; F, T_5 = 50% synergism; G, T_6 = 75% synergism; H, T_7 = negative control

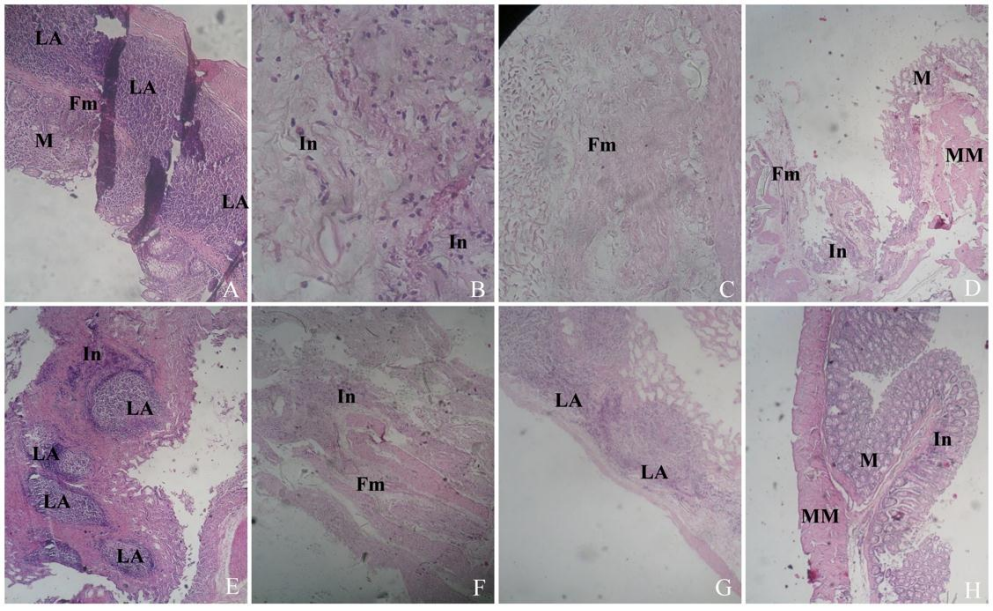

Plate 5. Effects of treatments on the histology of the colonic tissue. Representative hematoxylin-and-eosin (H & E) stained sections (D, E, F, G, H x40; A x100; B, C x400). Lymphoid aggregates (**LA),** Chronic inflammation (**In)**. Fibromuscular tissue (**Fm),** Mucosa (**M)**. A, T_0 = positive control; B, T_1= 50% *Spirulina*; C, T_2 = 75% *Spirulina*; D, T_3 = 50% malunggay; E, T_4 = 75% malunggay; F, T_5 = 50% synergism; G, T_6 = 75% synergism; H, T_7 = negative control

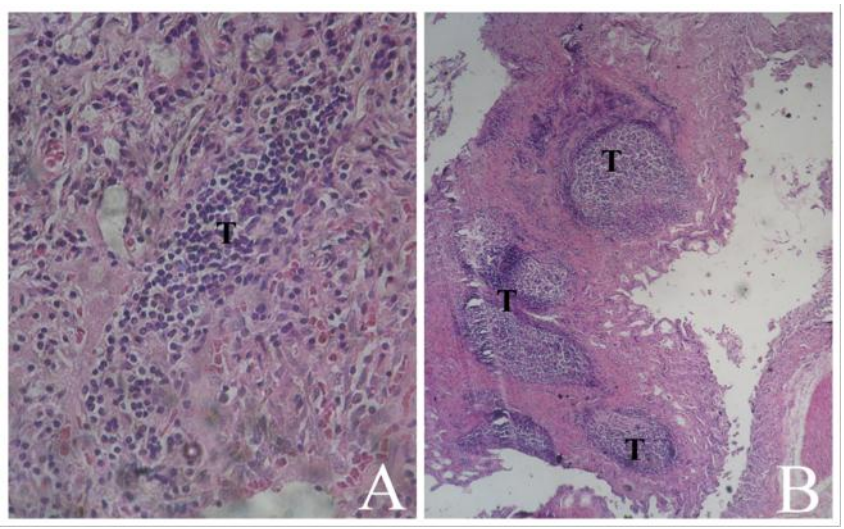

Plate 6. A. Small intestine with Tumors (**T**) in the group treated with 5-fluorouracil (400x) B. Large intestine with Tumors (**T**) in the group administered with 75% malunggay (40x). Hematoxylin-and-eosin (H & E) stained sections.

Rats that were given 50% malunggay and both synergism concentrations were observed to have chronic inflammation and degenerated fibromuscular tissue in the small intestine while those which received 75% malunggay were seen with the same abnormalities in addition to the formation of lymphoid aggregates and granulation tissue. Rats treated with 50% and 75% *Spirulina* were observed with degenerated fibrocollagenous tissue while those which were administered with 5-fluorouracil showed chronic inflammation, granulation tissue formation, lymphocyte aggregation and fibrocollagenous tissue formation. Rats that did not receive any treatment were clear from any signs of tissue damage.

Looking at the damage in the colon, rats which were treated with 75% malunggay and 5-fluorouracil showed intense signs of chronic inflammation and aggregation of lymphocytes while those which were given 50% *Spirulina* manifested the same symptoms in addition to the presence of fibrocollagenous and fibroadipose tissue. Colons of rats that were given 50% malunggay and 50% synergism were chronically inflamed and with fibromuscular tissue while those administered with 75% *Spirulina* were observed to have fibroadipose and fibromuscular tissue. Lymphoid aggregates were observed in rats that were given 75% synergism while those that did not receive any treatment were seen with chronically inflamed colon.

The largest tumors were observed in the large intestine while the largest tumor was observed in the group treated with 5-fluorouracil as seen in Table 2.

Hematologic evaluation

Analysis of CBC such as elevated monocyte levels indicated successful tumor induction by 1,2- DMH and 7,12-DMBA. Table 3 and 4 shows the complete blood count of normal and treatment groups.

CBC results from Table 3 showed that prior to the treatment period, the normal rat exhibited normal blood component count except for the RBC count; after the

treatment period, decreased WBC count and elevated monocyte count were observed. Meanwhile, prior to the treatment period, the tumor-induced rat was known to have elevated monocyte count. Platelet counts of normal rats after the treatment period and tumor-induced rats before and after the treatment phase were observed to be much higher than normal.

Comparison of the CBC of the treatment groups yielded several commonalities. A general trend of having lower than normal hematocrit, RBC count and lymphocytes and higher than normal segmenters was observed; eosinophil was also seen to be absent in most of the differential counts.

Table 1. Histopathologic findings in the lungs, liver, stomach, small intestine and large intestine.

Histopathologic characteristics*	Treatment Groups**							
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Lungs								
Atelectasis	++	+	+	+	+	+	++	-
Chronic inflammation	-	-	+	-	-	-	-	++
Hemorrhage	+	++	-	++	++	+	+	-
Emphysema	-	-	-	-	-	-	-	+
Liver								
Chronic inflammation	-	-	-	-	-	-	-	+
Stomach								
Degenerated fibromuscular tissue	-	-	+	-	-	-	-	-
Lymphocyte infiltration	+	-	-	-	-	-	-	-
Small Intestine								
Chronic inflammation	++	-	-	++	+	++	+	-
Granulation tissue formation to lymphoid aggregates	+	-	-	-	+	-	-	-
Degenerated fibrocollagenous tissue	+	+	+	-	-	-	-	-
Degenerated fibromuscular tissue	-	-	-	+	+	+	+	-
Developing tumor	++	-	-	-	+	-	-	-
Large Intestine								
Chronic inflammation	++	++	-	++	+++	++	-	+
Lymphoid aggregates	+	+++	-	-	++	-	++	-
Degenerated fibrocollagenous tissue	-	+	-	-	-	-	-	-
Degenerated fibroadipose tissue	-	+	+	-	-	-	-	-
Degenerated fibromuscular tissue	-	-	+	+	-	+	-	-
Developing tumor	+++	++	-	-	+	-	-	-

* - sign means absence; + sign means presence of the characteristic; more + signs indicate severity of damage

**T_0 = positive control, T_1= 50% *Spirulina*; T_2 = 75% *Spirulina*; T_3 = 50% malunggay; T_4 = 75% malunggay; T_5 = 50% synergism; T_6 = 75% synergism; T_7 = negative control

Physical and behavioral changes

Certain physical and behavioral changes were seen in the tumor-induced rats. All were observed to have uncoordinated body movements, loss of control of urination, periodic gnawing of cage wires and clay pots, gradual thinning and loss of fur, untidy coat and popping eyes.

Table 2. Size (μ) and frequency (%) of developing tumors in the different organs of treatment groups.

Treatment Groups*	Organ			
	Small Intestine μ	Small Intestine N (%)	Large Intestine μ	Large Intestine N (%)
T_0	500 375 250	3 (75)	3000 3000	2 (22.22)
T_1	–	0 (0)	750 1125 1000 750	4 (44.44)
T_2	–	0 (0)	–	0 (0)
T_3	–	0 (0)	–	0 (0)
T_4	–	0 (0)	312.5 500 500	3 (33.33)
T_5	–	0 (0)	–	0 (0)
T_6	300	1 (25)	–	0 (0)
T_7	–	0 (0)	–	0 (0)

* T_0 = positive control, T_1= 50% *Spirulina*; T_2 = 75% *Spirulina*; T_3 = 50% malunggay; T_4 = 75% malunggay; T_5 = 50% synergism; T_6 = 75% synergism; T_7 = negative control

Table 3. Complete blood count of normal and tumor-induced rats before and after the treatment period.

Blood Component*	Normal Rat (Pre)	Normal Rat (Post)	Tumor-Induced Rat (Pre)	Tumor-Induced Rat (Post)	Normal Values
Hemoglobin (g/dL)	14.2	14.3	14.3	12.6	11-19.2
Hematocrit (%)	42	43	43	38	36-54
WBC (cells/L)	8.4×10^9	$5.3 \times 10^{9+}$	8.7×10^9	6.8×10^9	$6.6\text{-}12.6 \times 10^9$
RBC (cells/L)	$6.52 \times 10^{12+}$	8.48×10^{12}	7.61×10^{12}	7.34×10^{12}	$6.76\text{-}9.75 \times 10^{12}$
Differential count (%)					
Segmenters	28	15	15	25	10-30
Lymphocytes	65	78	77	70	65-85
Monocytes	5	6^+	6^+	3	0-5
Eosinophils	2	1	3	2	0-6
Platelet count (cells/L)	2.07×10^{13}	$6.02 \times 10^{13+}$	$7.74 \times 10^{13+}$	$5.81 \times 10^{13+}$	$1.50\text{-}4.60 \times 10^{13}$

*Values per parameter with $^+$ indicate deviation from the normal values.

Table 4. Complete blood count of treatment groups.

Blood Component*	Treatment Groups**					
	T_0	T_1	T_2	T_3	T_4	Normal Values
Hemoglobin (g/dL)	14.3	6.0 $^+$	11.0	9.0$^+$	8.6$^+$	11-19.2
Hematocrit (%)	43	18 $^+$	33 $^+$	27 $^+$	26 $^+$	36-54
WBC (cells/L)	5.3 x 10^{9+}	1.1 x 10^{9+}	7.1 x 10^9	7.1 x 10^9	7.3 x 10^9	6.6-12.6 x10^9
RBC (cells/L)	8.48 x 10^{12}	3.42 x 10^{12+}	6.66 x 10^{12+}	5.22 x 10^{12+}	5.14 x 10^{12+}	6.76-9.75 x10^{12}
Differential count (%)						
Segmenters	15	31 $^+$	31 $^+$	39 $^+$	87 $^+$	10-30
Lymphocytes	78	61 $^+$	59 $^+$	60 $^+$	13 $^+$	65-85
Monocytes	6 $^+$	8 $^+$	10 $^+$	1	0	0-5
Eosinophils	1	0	0	0	0	0-6
Platelet count (cells/mL)	6.02 x 10^{13+}	6.5 x 10^{12+}	3.59 x 10^{13}	6.89 x 10^{13+}	2.75 x 10^{13}	1.50-4.60 x 10^{13}

*Values per parameter with $^+$ indicate deviation from the normal values;

**T_0 = positive control, T_1= 50% *Spirulina*, T_2 = 75% *Spirulina*, T_3 = 50% malunggay, T_4 = 75% malunggay,

Discussion

In the Philippines, cancer is the third leading cause of morbidity and mortality. (3) At present, there is still no complete and cost-efficient cure that poses no detrimental side effects.

Examination of tissues indicates considerable anti-cancer action of administered extracts. Observation of abnormalities in the normal rat wherein no tumor was induced is suggestive as general symptoms of many diseases, including cancer. Abnormalities seen in the group wherein 5-fluorouracil was used as treatment may suggest that rats, while of the same species, may have different levels of physiologic and immunologic responses to completely eradicate tumor and its signs of development; same explanations also account for abnormal blood counts seen on the normal rat and those treated with 5-fluorouracil.

Atelectasis of the lungs as observed in the treatment groups is a condition wherein the alveoli collapses with decreased pulmonary air capacity. (17) One possible cause of such is the obstruction of the main bronchus due to a developing tumor. (18) This was evident in all but the group that didn't receive any treatment which may suggest that the extracts did not have any effect on alleviating this condition.

However, only the untreated group showed signs of emphysema, the abnormal enlargement of the air spaces far from the terminal bronchiole. Recent studies have shown to link emphysema and cancer through activation of pleiomorphic adenoma gene-like 2 (PLAGL2). (19) On the other hand, hemorrhage that may be brought about by the developing tumor's injury to the capillary walls was observed in all but two groups that received 75% malunggay and no treatment. Such was also accompanied by thickening of the alveolar septa. (17) Then, clearance of the hepatic tissues of the various treatment groups from any abnormalities may suggest a preventive or cytotoxic activity of the extracts to the developing tumors.

Lymphoid aggregates in the gastric lamina propria are suggestive of a developing cancer such as lymphoma, the second most common malignant tumor of the said organ. (20) The same formations in the colon are indicative of an impending colorectal disease such as carcinoma, diverticular disease, Crohn's disease or ulcerative colitis. (21) However, formation of fibrous tissues such as fibroadipose, fibromuscular and fibrocollagenous tissue are signs of repair mechanism of the tissue to counter the damage of a tumor; this is exemplified by the reparative function of fibroblasts to replace the damaged tissue through deposition of collagen. (22)

A fully developed tumor was not observed in any of the tissue sample. However, lymphoid aggregates that formed distinct masses and intervened with the normal architecture of the tissue were counted as developing tumors.

Altogether, absence of a fully developed tumor may suggest therapeutic action of the extracts. Such anti-cancerous effects may be attributed to the phyocyanin and benzyl isothiocyanate found in *Spirulina* and *malunggay*, respectively. (23-24)

Tumor-induced negative control rats showed elevated monocyte levels and platelet count prior to treatment; such occurrence can be attributed to the response in the development of tumor wherein monocytes will eventually differentiate into macrophages to produce tumor necrosis factor alpha (TNF- α), a cytokine that causes disintegration of tumor cells. (25) Such production will then cause the liver to synthesize acute-phase proteins that play a role in inflammatory response, a phenomenon associated with cancer development; (26) such inflammatory conditions were observed in various organs. Meanwhile, increased platelet counts observed on tumor-induced rats may be indicative of tumor development. Various stimuli such as inflammation, tumor and cancer causes secondary or reactive thrombocytosis, a phenomenon in which platelets increase in number as a response. (27) Finally, the increased WBC of tumor-induced rat relative to that of the normal rat reflects the immunologic condition of the tumor-induced rat.

A general trend of having decreased hematocrit levels, RBC count and lymphocyte count, increase in segmenters and absence of eosinophils were observed in treatment groups administered with extracts. Decrease in hematocrit levels which is a consequence of a low RBC count can be an outcome of loss of blood such as in a bleeding colon cancer (28) or a side effect that damages the bone marrow thus impeding production of RBC and lymphocytes as well. (29) Meanwhile, increase in segmenters or neutrophils are indicative of inflammation and bone marrow disorders such as leukemia; absence or extremely low eosinophil count can be attributed to tumor growth attenuation. (30) On the contrary, the decrease in WBC observed is linked with reduced ability to ward of foreign bodies or formation which may indicate the development of tumor as seen in groups treated with 5-fluorouracil and 50% *Spirulina*. (29) Decreased hemoglobin is then related with decreased RBC count because of the physiological association of the two parameters while decreased platelet count is a causative factor of hemorrhage observed.

The physical changes such as hair loss and behavioral changes observed may be attributed to body weakness, uneasiness, stress and pain caused by the developing tumor. (17) Gnawing of non-food materials such as claypots and cage wires and squeaking are a result of acute pain and stress brought about by tumor development; (17, 31) aggressiveness towards other rats, another tumor-related manifestation, (32) was also

observed during the course of induction. Studies also associate tumor development in depressive-like behavior. (33)

Based on data interpretation and analysis, the present study concludes the effectiveness of both extracts against tumor development. However, *Arthrospira platensis* is relatively more effective in countering the manifestations of tumor development. Overall, the extracts were ineffective against tumor development in the lungs in contrast to their effectiveness in hepatic tumor development. In the stomach, all of the extracts except *A. platensis* at 75% concentration were effective while in the small intestine, both concentrations of *A. platensis* were relatively the most effective. Then, *A. platensis* at 75% was relatively the most effective against colonic tumor development. Meanwhile, a general trend of the CBC suggests tumor growth and attenuation at the same time.

It is worth recommending to use and place more replicates in a more controlled environment to lessen other influential factors; a longer induction period should be observed for more extensive tumor development. More sophisticated procedures such as methyl thiazol tetrazolium (MTT) assay and molecular examination of active components should also be used to gain deeper understanding of the extracts' cytotoxic activity.

Acknowledgments

The authors wish to thank the faculty of Biological Sciences Department for their valuable contribution to the content of the researchers' study and the technicians in the Biology Research Laboratory of De La Salle University- Dasmariñas for their support during the experimentation stage. The researchers also express their gratitude to The Research Institute for Tropical Medicine, Wellcare Clinics and Lab, Belman Laboratories and Santa Cruz Biotechnology, Inc. for their chemical supplies and technical assistance.

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