Evidence and Safety Summary Report

For:

"A4+"

Sabell Corporation

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Calgary, Alberta

T2P 2T5

Date of Preparation: December 2011

1.0 Evidence Summary Report

1.1 Recommended use or purpose (health claim):

- **Claim: "Used in herbal medicine as a Hepatoprotector/Liver Protectant"**
- Non-Traditional Use claim
- Following published scientific references as well as scientific studies conducted by Sabell corporation that support this claim;
  - Bussmann R.W., Sharon D., Garcia M., "From Chamomile to Aspirin? Medicinal Plant Use Among Clients at Laboratorios Beal in Trujillo", Peru, Ethnobotany Research and Applications, 2009, Vol 7, 399-407.
  - Health Canada, NHPD monograph for Turmeric (*Curcuma longa)* USDA 2008; Date: 2010-02-25.
  - Raintree Nutrition, Tropical Plant Database, Monograph (Database File) for Graviola (*Annona muricata*).
  - Natural Medicines Comprehensive Database, Monograph for Graviola (*Annona muricata*).
  - Samanta A., "Quality of life following the use of A4 plus in a limited number of subjects with chronic hepatitis C: Open-label, non placebo-controlled, preliminary observations", Independent assessment report, 2004.
  - Cabanillas Jose, Nystrom Joseph W, Zambrano Luis, Chicolote Gerardo., "Chronic Hepatitis C Treatment with A4+, Controlled Clinical Test", Lima Peru, May-June 2004.
  - Cabanillas Jose, Joseph Nystrom, Hugo Marquez, S., "Short term effects of A4+ on clinical and biochemical markers in chronic hepatitis C", September 2001.
  - Kosinski, M., "Revised Summary Report on Study Findings of 10 Patients Receiving Nutraceutical Product A4+L for the Treatment for Chronic Hepatitis C Virus (CO-1031)", Quality Metric Inc., July 2004.
  - Coral Jose Gonzalo Cabanillas., "Herbal Compositions and Methods for Treating Hepatic Disorders", Patent Pending (PCT/CA2008/001764), International Filing Date: 03 October 2008.
  - Swain MG., "Evaluation of the Hepatoprotective and Behavioral Effects of the Herbal Compound A4+ in Acute Liver Injury Models", (University of Calgary Report - 2011).

- Cabanillas J., Fong F., "A4+ Interviews with Peruvian Medicine Experts", Peru, January 2009. Translated to English by Mr. Jorge Alvarado, Certified Translator from the Society of Translators and Interpreters of British Columbia, March 2009.
- Tyrrell L., Part 1: "Anti-viral effects of plant extracts on HCV infected cells" and Part 2: "Effect of Short-term exposure to A4+ plant extract on Natural Killer Cell Activity" (Li Ka Shing, Institute of Virology, University of Alberta Report - 2011).
- Wallace J., "Evaluation of the Anti-oxidant, Anti-inflammatory and mucosal protective Actions of A4+ and its Constituents" (Penumbra Associates Ltd, 2010).
- Adewole SO, Ojewole JA., "Protective effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats". Afr J Tradit Complement Altern Med. 2008; 25; 6(1):30-41.
- Orlando Vieira de Sousa, Glauciemar Del-Vechio Vieira, José de Jesus R. G. de Pinho, Célia Hitomi Yamamoto and Maria Silvana Alves., "Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract of *Annona muricata* L. Leaves in Animal Models". Int. J. Mol. Sci. 2010, 11, 2067-2078.
- Baskar R, Rajeswari V, Kumar TS., "In vitro antioxidant studies in leaves of *Annona* species". Indian J Exp Biol. 2007; 45(5):480-485.
- A Saravana Kumar, R Gandhimathi, KK Senthil Kumar, Kusuma Praveen Kumar., "Hepatoprotective potential of *Cordia subcordata* Lam. against carbon tetra chloride (CCl4)-induced hepatotoxicity in Wistar albino rats". J Biomed Sci and Res., 2009, Vol 1 (1), 19-26.
- M. Afzala, C. Obuekweb, A. R. Khanc and H. Barakata., "Antioxidant activity of *Cordia myxa* L. and its hepatoprotective potential". EJEAFChe 2007, 6 (6), 2109-2118.
- Thirupathi K., Sathesh Kumar S., Goverdhan P., Ravikumar B., Krishna D. R. and Krishna Mohan G., "Hepatoprotective action of *Cordia dichotoma* against Carbon tetrachloride induced liver injury in rats". Nig. J. Nat. Prod. and Med. 2007, Vol. 11, 37-40.
- D Sobiya Raj, J Jannet Vennila, C Aiyavu, K Panneerselvam., "The hepatoprotective effect of alcoholic extract of *Annona squamosa* leaves on experimentally induced liver injury in swiss albino mice". JIB, 2009, Vol. 5(3), 182-186.
- Mohamed Saleem TS, Christina AJM, Chidambaranathan N, Ravi V, Gauthaman K., "Hepatoprotective activity of *Annona squamosa* Linn. on experimental animal

model". International Journal of Applied Research in Natural Products, 2008, Vol. 1(3), 1-7.

1.2 Critical Overview:

Individually these plants and/or part of these plants (*Cordia lutea, Curcuma longa and Annona muricata*), has been used traditionally for many years. The flower component of *Cordia lutea* has been used as a traditional medicine for centuries, *Curcuma longa* (Turmeric) has a long history of use as a food ingredient as well as an herbal medicine in many cultures and *Annona muricata* has a long, rich history of use in herbal medicine as well as a lengthy recorded indigenous use. In anecdotal reports, this compound has been used for many years to treat individuals with a variety of different liver diseases. A leaf tea form of these three compounds is commonly used by both medical doctors and natural healers in the Peruvian Amazon. Two complete monographs have been published supporting the use of *Curcuma longa* (Turmeric) and *Annona muricata* (Graviola). Both monographs review the effectiveness and long history of use of these ingredients for treating patients with liver disorders.

Sabell Corporation has developed A4+ a Natural Health Product that consists of these three herbal or natural ingredients; *Cordia lutea, Curcuma longa and Annona muricata.* Sabell Corporation has conducted many scientific studies that provide sufficient evidence that A4+ components are pharmacologically active with indications in liver disease.

In-vitro/in-vivo studies conducted by Sabell Corporation show the hepatoprotective, anti-oxidant and anti-inflammatory effects of A4+ on liver tissue. The antiviral effect of A4+ also shows that the A4+ component has significant levels of antiviral activity against HCV in cell culture. Toxicology studies conducted in mice and rat shows no overt evidence of toxicity related to the A4+ at any dosage up to the highest administered to female Sprague rats (2000 mg/kg). Toxicity studies indicate no toxic effects, no mortality effects at up to 2,000 mg A4+/kg mice body weight and therefore concluded that, there is no toxicity associated with A4+ consumption.

Two clinical trials have demonstrated that A4+ is well tolerated across a wide range of doses. In these uncontrolled studies, it was found that the addition of daily A4+ either in conjunction with HCV therapy, or alone, showed a significant improvement in the following parameters: depression, health-related QOL as measured by Medical Outcomes Short Form, 36 item survey (SF-36), Hepatitis Quality of Life Quotient (HQLQ); and an improvement in clinical symptoms which included fatigue; dyspepsia; nausea and vomiting; indigestion; right upper quadrant abdominal pain and tenderness; headache; muscle, joint and bone pain. Both studies concluded that A4+ demonstrated a clinical benefit. A4+ is considered a safe product to use with only a minimum of reported mild side effects. The most common side effect of A4+ is mild headache, reported within the first 7 days of taking A4+. This side effect usually abates after the first week.

Cordia lutea: *Cordia* is a genus of flowering plants in the borage family, Boraginaceae. It contains about 300 species of shrubs and trees. Many studies have shown the hepatoprotective potential of different species of *Cordia* and have been used for many years. The flower component of *Cordia lutea* has been used as a traditional medicine for centuries in different cultural paradigms including ancient Peruvian culture.

- Bussmann RW, Sharon D, Garcia M., "From Chamomile to Aspirin? Medicinal Plant Use Among Clients at Laboratorios Beal in Trujillo", Peru, Ethnobotany Research and Applications, 2009, Vol 7, 399-407.
- Saravana Kumar, R Gandhimathi, KK Senthil Kumar, Kusuma Praveen Kumar., "Hepatoprotective potential of *Cordia subcordata* Lam. against carbon tetra chloride (CCl4)-induced hepatotoxicity in Wistar albino rats". J Biomed Sci and Res., 2009, Vol 1 (1), 19-26.
- M Afzala, C Obuekweb, A R Khanc and H Barakata., "Antioxidant activity of *Cordia myxa* L. and its hepatoprotective potential". EJEAFChe 2007, 6 (6), 2109-2118.
- Thirupathi K., Sathesh Kumar S., Goverdhan P., Ravikumar B., Krishna DR and Krishna Mohan G., "Hepatoprotective action of *Cordia dichotoma* against Carbon tetrachloride induced liver injury in rats". Nig. J. Nat. Prod. and Med. 2007, Vol. 11, 37-40.

Curcuma longa (USDA 2008): **Based on Health Canada Monograph, the use or purpose of Turmeric: Oral**

Statement(s) to the effect of:

- Provides antioxidants for the maintenance of good health (ESCOP 2003, Blumenthal et al. 2000, Mills and Bone 2000).
- Used in Herbal Medicine to aid digestion (ESCOP 2003, Williamson 2003, Blumenthal et al. 2000, Mills and Bone 2000).
- (Traditionally) used in Herbal Medicine to help relieve flatulent dyspepsia (carminative) (Mills and Bone 2005, Blumenthal et al. 2000, Wren 1907).
- **Used in Herbal Medicine as a hepatoprotectant/liver protectant** (Boon and Smith 2004, Williamson 2003**).**
- Used in Herbal Medicine to increase bile excretion by the liver (choleretic) and stimulate contraction of the gallbladder (cholagogue) (Mills and Bone 2005, Boon and Smith 2004, Wichtl 2004, Williamson 2002, Blumenthal et al. 2000, Mills and Bone 2000).
- (Traditionally) used in Herbal Medicine as an anti-inflammatory to help relieve joint pain (Winston and Kuhn 2008, Blumenthal et al. 2000).

- Used in Traditional Chinese Medicine (TCM) to eliminate blood stasis, promote the flow of qi and relieve pain of menstruation due to blood stasis (PPRC 2005).
- Traditionally used in Ayurveda to relieve pain and inflammation, and assist healing of minor wounds such as cuts and burns, and minor skin irritations (Paranjpe 2005, Murthy 2004, API 2001 [1990], Kapoor 2001).

Annona Muricata: *Annona* is a genus of flowering plants in the pawpaw/sugar apple family, *Annonaceae*. It is the second largest genus in the family containing approximately 110 species of mostly neotropical and afrotropical trees and shrubs. Many studies have shown the hepatoprotective potential of different species of *Annona* and have been used for many years. *Annona muricata* has a long, rich history of use in herbal medicine as well as a lengthy recorded indigenous use. Raintree Nutrition Monograph for *Annona muricata* (Guanabana/Graviola) also indicates that "*Annona muricata* has a long, rich history of use in herbal medicine as well as a lengthy recorded indigenous use." "In the Brazilian Amazon, a leaf tea is used for liver problems".

- Raintree Nutrition, Tropical Plant Database, Monograph (Database File) for Graviola (*Annona muricata*).
- Adewole SO, Ojewole JA., "Protective effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats". Afr J Tradit Complement Altern Med. 2008; 25; 6(1):30-41.
- Orlando Vieira de Sousa, Glauciemar Del-Vechio Vieira, José de Jesus R. G. de Pinho, Célia Hitomi Yamamoto and Maria Silvana Alves., "Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract of *Annona muricata* L. Leaves in Animal Models". Int J Mol Sci, 2010, 11, 2067-2078.
- Baskar R, Rajeswari V, Kumar TS., "In vitro antioxidant studies in leaves of *Annona* species". Indian J Exp Biol. 2007; 45(5):480-5.
- D Sobiya Raj, J Jannet Vennila, C Aiyavu, K Panneerselvam., "The hepatoprotective effect of alcoholic extract of *Annona squamosa* leaves on experimentally induced liver injury in swiss albino mice". JIB, 2009, Vol. 5(3), 182-186.
- Mohamed Saleem TS, Christina AJM, Chidambaranathan N, Ravi V, Gauthaman K., "Hepatoprotective activity of *Annona squamosa* Linn. on experimental animal model". International Journal of Applied Research in Natural Products, 2008, Vol. 1(3), 1-7.
- Natural Medicines Comprehensive Database, Monograph for Graviola (*Annona muricata*).

In addition to the above references, following studies were conducted by Sabell Corporation that summarizes the efficacy and safety of A4+:

A) **Cabanillas Jose, Joseph Nystrom, Hugo Marquez, S., "Short term effects of A4+ on clinical and biochemical markers in chronic hepatitis C", September 2001.**

This document summarizes the results of the clinical study entitled "Prospective Study of the Course of Chronic Hepatitis C after the Incorporation to the Daily Diet of A4+ in 6 Patients During 4 Weeks". The primary objective of this study was to evaluate the value of a natural nutraceutical A4+ used in 6 patients with chronic Hepatitis C. Patients with varying severity of Chronic Hepatitis C Genotype 1 were administered 20 grams per day of the product A4+ in the form of a tea infusion steeped in boiling water prior to each of three meals per day for 4 weeks. All patients were provided a balanced diet and exposed to natural sunlight on a daily basis and all patients demonstrated clinical improvement and were uniformly free of adverse reactions to the product.

The majority of patients entering the study had baseline abnormal liver functions. There were no statistical changes in the liver functions in these first 4 weeks of treatment. Likewise, there were no changes in the markers for other organ systems. While being a study of very short time length, we have noted no adverse trend on serum GTT, SGOT, SGPT, bilirubin, alkaline phosphatase, blood counts, and renal function of lipid indices. The study is too small to assign significance to results regarding viral loads or alphafetoprotein.

However, this study did conclude that the natural product A4+ demonstrated a clinical benefit and merits further study as an alternative treatment for those suffering from Chronic Hepatitis C.

B) **Samanta A., "Quality of life following the use of A4+ in a limited number of subjects with chronic hepatitis C: Open-label, non placebo-controlled, preliminary observations", Independent assessment report, 2004.**

The primary objective of this clinical study was to examine whether A4+ might have a beneficial effect in patients with chronic hepatitis C. The study was an open-label, non-randomized, non-placebo controlled preliminary assessment of the effect of oral ingestion of A4+ on symptoms of chronic hepatitis C. Clinical parameters noted below were evaluated a day prior to the administration of A4+ and then again at 28 days after the use of A4+. The impact of use of A4+ was assessed by comparing the values of these parameters at the start of the study against values at day 28 of the study.

This study included 10 Caucasian volunteer patients. All ten patients were treated with the A4+ formula taken orally 3 times a day for 28 days. Nine out of ten of the patients were long

term sufferers of Hepatitis C Genotype 1. Prior to the start of this study, the tenth patient had successfully treated Genotype 2 Hepatitis C with Interferon, but was diagnosed with severe liver cirrhosis. This patient clearly presented different results compared to the other 9 Chronic Hepatitis C Genotype 1 patients.

The parameters studied for the effect of A4+ on Hepatitis C patients before and after treatment are included below;

Depression: The severity of depression at the start of the study ranged from borderline in 10% of the subjects, mild to moderate depression in 80%, and severe depression in 10%. By the end of study period significant improvement in depression was noted and 90% of the subjects had become free of depression and 10% exhibited mild depression. Noteworthy is the reported change in the Beck Depression Inventory score. Mean score before the use of A4+ was 20.9 (range 11-35) and decreased markedly to 2.7 (range 0-3) 28 days after the use of A4+. Sixty percent of the subjects reported total Beck score of 2 or less, including score of 0-1 in 40% after 28 days of the use of A4+.

Health-Related Quality of life Burden (SF-36 HQLQ): In general, use of A4+ was accompanied by significantly improved health-related quality of life in study subjects by Day 14. By Day 28, the functional status and well-being of nine out of the ten study subjects were restored completely to normal levels. The score improvements observed in this study by Day 28 were on average larger than two standard deviations for nearly all health-related quality of life scales.

Clinical symptoms: Study subjects showed a significant improvement in most of their symptoms. This included improvement in fatigue, right upper quadrant pain and tenderness, dyspepsia, nausea-vomiting, indigestion, headache, muscle and joint/bone pain.

Fatigue: *Before the use of A4+:* Severe fatigue was present in 80%, moderate fatigue in 10% and mild fatigue in 10% of the subjects. *After 28 days of the use of A4+*: Severe fatigue was seen in 20% and 40% each having mild and moderate fatigue.

Dyspepsia: Utilizing the maximum score of 15 for dyspepsia as described above (GSRS), it was rated as mild (score 1-5), Moderate (score of 6-10) and severe (score of 11-15). Before the use of A4+: None of the subjects were free of dyspepsia. Most of the subjects had mild dyspepsia (70%), and 30% had moderate dyspepsia. After 28 days of the use of A4+: Dyspepsia was absent in 90% with 10% had moderate dyspepsia.

Nausea and Vomiting: Was assessed as one of the elements of dyspeptic syndrome domain of GSRS scoring system noted above. Before the use of A4+: Was experienced occasionally by 30%, frequently by 30% and was absent in 40% of the subjects. After 28 days of the use of A4+: Was resolved in 90% of the subjects, and 10 % had only occasional nausea and vomiting.

Indigestion: Utilizing the maximum score of 12 for indigestion as described above (GSRS), indigestion was rated as mild (score 1-5), Moderate (score 6-9) and severe (score 10-12). Before the use of A4+: Most subjects (70%) had mild indigestion, 20% experienced moderate indigestion, and 10% had severe indigestion (Figure 5). After 28 days of the use of A4+: Symptom of indigestion was absent in 70%, while 30% had moderate indigestion.

Nutritional status: In order to ascertain whether the observed changes in the health-related quality of life and symptoms of the study subjects could have been influenced by improved nutritional status, the nutritional status of the study subjects was evaluated both at the beginning and at the end of the study period. Nutritional parameters, including anthropometry and serum transferrin and pre-albumin remained unchanged during the short study period indicating that improvement in symptoms and health-related quality of life were independent of nutritional status.

Routine Liver Chemistry: Serum bilirubin, AST, ALT and albumin did not show any change at the end of the study as compared to pre-study values.

Prothrombin activity and serum cholinesterase: There was a significant increase in Prothrombin activity and serum cholinesterase which suggests possible increased protein synthesis by liver or a decrease in their degradation.

At baseline, prothrombin activity expressed as percent of control was 68.9 + 22.7% and serum cholinesterase was 5194.1 + 1590.1 U/L. After 28 days these increased to 81.2 + 28.0% and 7792.7 + 2218.8 U/L respectively.

TNF-α: Serum TNF-α was increased at the end of 28 days (10.0 + 3.0 pgm/ml) of the use of A4+ as compared to the Day 1 value (7.2 + 2.1 pgm/ml).

Liver size: There was no change in the liver size or echo-texture of the liver during the use of A4+.

Results of this study was summarised by **Kosinski M., "Revised Summary Report on Study Findings of 10 Patients Receiving Nutraceutical Product A4+L for the Treatment for Chronic Hepatitis C Virus (CO-1031)", Quality Metric Inc., July 2004**. Quality Metric Incorporated (QMI) is a health survey provider for the healthcare and life sciences industries. QMI's products and services are designed to measure patient-reported outcomes from clinical studies and provide scientifically valid assessments of physical and mental health.

The conclusions from the HQLQ report from QMI were that on average, treatment with the Nutraceutical Product A4+ was observed to significantly improve the health related quality of life of study patients. By day 14 and by day 28 of treatment, the functional status and well-being of study patients was completely restored to normal levels. The score improvements

observed in this study by day 28 were on average larger than two standard deviations for nearly all health related quality of life scales, which has rarely been observed in the thousands of treatment studies of other chronic diseases involving the SF-36 Health Survey. In addition, the evaluation of individual patient scores over time showed that 9 out of the 10 patients in this study improved by day 28 by a clinically meaningful amount on each of the health related quality of life scales, so the average results were not driven by a couple of outlier patients. The conclusions address the small patient population size of the study (n=10) but also states that despite the lack of statistical power, statistically significant changes were observed across all health related quality of life scale scores.

C) Swain MG., "Evaluation of the Hepatoprotective and Behavioral Effects of the Herbal Compound A4+ in Acute Liver Injury Models" (University of Calgary, Alberta, 2011).

The purpose of this study was to investigate whether A4+ could attenuate the detrimental behavioural (i.e., sickness behaviours) and biochemical effects associated with liver injury in two well-characterized mouse models of liver injury.

The first model was bile duct ligation and resection (BDR). For this the mice were randomly divided into two groups: BDR surgery only (control) and BDR plus A4+ (160 mg/kg/day) administered by oral gavage. Sickness behaviour was evaluated using two well established methods: (1) A social investigation paradigm and (2) open field locomotor activity measurement to assess overall mobility. Result shows that levels of plasma ALT and total bilirubin levels were similar in control and A4+ treated BDR mice at 9 days post surgery, as was time spent in social investigation behaviour. By contrast, BDR animals administered A4+ were significantly more active in overall mobility for both ambulatory movements ($p=0.03$) and enhancement of the number of horizontal movements ($p=0.04$) respectively.

The second model was Concanavalin A (Con A)-induced hepatitis. For this study, one group of mice were pre-treated with vehicle (control) and another group with A4+ (640 mg/kg). As in the first study, biochemical measurements were measured. In addition, livers were dissected and processed for flow cytometry analysis 9FACS) after staining of different cell surface markers to identify immune cell subsets and to identify cytokine production profiles of these cells using intracellular staining. Result shows that plasma ALT levels in the A4+ treated Con A mice were significantly ($p=0.04$) reduced compared to the control Con A mice. Pre-treatment with A4+ had no significant effect on IFN© cell recruitment and activation. In contrast, hepatic recruitment of IFN© expressing NK cells to the liver were significantly ($p<0.05$) increased in the A4+ Con A treated animals compared to Con A controls.

In conclusion, the first study indicated that A4+ has beneficial behavioural modifying effects in a test which examined sickness-related immobility. These improvements occurred in the absence of significant changes in biochemical indices of liver damage.

In the second study A4+ treatment attenuated Con A hepatitis as reflected by a reduction in plasma ALT levels compared to vehicle-treated controls. Pre-treatment with A4+ had no significant effect on IFN© cell recruitment and activation. However, more hepatic NK cells expressed IFN© in mice pre-treated with A4+ which received ConA than in vehicle-treated mice.

Overall, the finding of positive changes in behaviour, liver enzymes and some aspects of immune function in these animal models of hepatic injury indicate a potential clinical relevance for the treatment of patients with hepatic injury.

D) Wallace J., "Evaluation of the Anti-oxidant, Anti-inflammatory and mucosal protective Actions of A4+ and its Constituents" (Penumbra Associates Ltd, 2010).

The purpose of this study was to investigate the anti-oxidant, anti-inflammatory and mucosal protective effects of A4+. The anti-oxidant activity and its constituents were evaluated using an *in vitro* assay in which a stable free radical was allowed to interact with the test substance. The result of this study shows that A4+ exhibited potent anti-oxidant activity. The results suggest that the anti-oxidant effects of A4+ can mainly be attributed to the *Cordia* and *Annona*, but not with *Curcuma*.

Anti-inflammatory action of A4+ was evaluated by one of the most widely used *in vivo* "air pouch" model. This model allows one to determine the effects of a drug on many different aspects of the inflammatory process, thereby allowing for determination of mechanism(s) of action. The results showed that administration of A4+ to the site of inflammation results in reduced levels of inflammatory mediators, or reduced inflammation.

A4+ administration reduced the severity of gastric damage induced by subsequent administration of a potent nonsteroidal anti-inflammatory drug (indomethacin), but only at quite a high dose (≥300 mg/kg) and only when given at least 4 hours prior to the indomethacin. The result suggests that sufficient blood levels of A4+ much be achieved to observe the gastroprotective effect.

These studies demonstrated the anti-oxidant and anti-inflammatory properties of A4+ and provided insight into the mechanisms of action of A4+ for its beneficial effects on hepatitis.

E) Tyrrell L., Part 1: "Anti-viral effects of plant extracts on HCV infected cells" (Li Ka Shing, Institute of Virology, University of Alberta, 2011).

The purpose of this study was to determine the anti-viral effect of A4+ on Hepatitis C virus (HCV) infected cells. The studies were carried out using Huh7.5 cells and a tissue-culture adapted strain of HCV, JFH. Cells were seeded, allowed to adhere and establish, followed by 4hr infection incubation with HCV strain. After infection, cells were washed with fresh media followed by treatment with A4+. Cells were exposed to diluted A4+ powder (0.1, 0.5, 1, 5 and 10 μg/mL in 45% ethanol) for 4 days. This was followed by collection of supernatant and cells for viral titering. The studies were repeated three times and in each, results showed that A4+L component of the A4+ powder indicated significant levels of antiviral activity. Intracellular and extracellular fractions were measured after 4 days of drug treatment, and both fractions showed a significant drop of HCV titres at A4+L concentrations greater than 1 μg/mL. The A4+L showed activity at all concentrations used – but was most active at 1 μg/ml or greater with approximately 90% inhibition of HCV in cell cultures at 10 μg/ml. To prove beyond a doubt that the A4+L drug is antiviral, viral protein levels were visualized by western blot. Cells were plated, infected, and drug treatment was conducted as previously described. After treatment, cells were lysed with RIPA buffer to release cell contents and prevent protein degradation. Protein levels were quantified with a BioRad Protein assay so the same amount of protein could be added to each well. Two HCV antibodies were used to determine viral quantity: NS3 and core. A drop in NS3 is seen in lanes for A4+, A4+L and A4+R, with the greatest being in A4+L. This observation indicates that viral load is decreased as a result of A4+ exposure. In Part 1, it was demonstrated that the A4+ plant extract had significant antiviral activity against HCV in cell culture.

F) Tyrrell L., Part 2: "Effect of Short-term exposure to A4+ plant extract on Natural Killer Cell Activity" (Li Ka Shing, Institute of Virology, University of Alberta, 2011).

The purpose of this study was to determine if the antiviral benefits of the A4+ plant extract attributed to enhancement of Natural Killer (NK) activity. C57/B6 mice were treated with A4+ daily for 14 days. After 14 days, mice were euthanized and their spleens were removed for NK cell preparation. A cell suspension was prepared and NK cells were purified using an Easy Sep Mouse NK Cell Enrichment Kit. Purified NK cells were analyzed by FACS. The result showed that treatment with A4+ didn't enhance NK activity *in vivo*. There may have been a tendency for decreased NK activity with A4+ treatment, but the number of experiments was too low to show statistically significant differences. In this short term experiment the A4 extract were not toxic to the mice. Since the antiviral activity was only shown in lymphocyte free cell culture, it was concluded that the antiviral effect of A4+ on HCV is a direct antiviral effect and is not mediated through a NK immune enhancement.

G) Cabanillas J., Fong F., "A4+ Interviews with Peruvian Medicine Experts", Peru, January 2009. Translated to English by Mr. Jorge Alvarado, Certified Translator from the Society of Translators and Interpreters of British Columbia, March 2009.

The following is a summary of five individual interviews with five different Peruvian Elder Expert Healers in Peru. Each confirms the traditional use of either *Cordia lutea*, *Curcuma longa* (Turmeric), and *Annona muricata* (Graviola) for maintaining liver health. The interviews were performed in Peru in January 2009 by Dr. Jose Cabanillas together with Fenton Fong of PharmEng Technology.

The first three interviews focus on the traditional use of Graviola (aka. *Annona muricata*/Guanabana) with additional references to the use of Turmeric or *Curcuma longa* (aka. Curcuma, Curcumin, "Guisador"). All three experts interviewed indicated that Graviola and Turmeric have been used for centuries to treat conditions of the liver, commonly referred to in the interviews as Hepatitis. All agreed that the use of Graviola and Turmeric is safe and that there are no known side effects. They also confirmed that the preparation and use of alcohol extracts of Graviola and Turmeric is a common practice.

The fourth and fifth interviews focus on the traditional use of *Cordia lutea* (Overal) in traditional Peruvian culture for treating liver illnesses. All agreed that the use of *Cordia lutea* is safe and has no side effects.

Summary, Interview 1: Marcos Pandoro Mesa, age 43. Mr. Mesa indicated that both Guanabana and Curcuma have been used for longer than 50 years; they have been used since 'the time of his grandparents'. He indicated that both ingredients are used to treat the liver as well as blood problems and malaria. Mr. Mesa knew of no side effects, recommended to avoid fatty foods, meat, and acidic foods. The method of preparation he was most familiar with was chopping the roots of the Curcuma plant and boiling in water. He also said that you can chop the root, put it in alcohol, mix it for 3 days then you can drink it. He indicated a use of half a cup every morning before breakfast for 9 days.

Summary, Interview 2: Mr. Ruiz, 56 years old. Mr. Ruiz is employed as a forestry engineer, and said he often works with botanists, shamans and healers in using plants from the Amazon as natural medicines. He said people have been using Guanabana and Curcumin since at least the 1800's, and he knew of no side effects. He indicated that Curcumin is traditionally used for treating the liver, and Guanabana is also used for treating the liver but mostly for cancers, breast and liver cancers and problems of the female reproductive system. He prepares Curcumin by grating the root and soaking in alcohol for 3 days then uses the alcohol in hot water for drinking, and crushes the leaves of the Guanabana and mixes with hot water to prepare a tea-like beverage. He also included that alcohol is commonly used in the Amazon to preserve plant medicines.

Summary, Interview 3: Ramon Aredano Huaman, age 74. Mr. Huaman said that besides treating others, he personally has been using these ingredients for over 60 years, and indicated no side effects. He said Curcuma is always used to treat liver related conditions like yellow fever. He also indicated Curcumin is usually boiled in water with the water later being drunk like a tea, or soaking in alcohol for 3 days and then adding the alcohol to water to drink. For Guanabana he suggested crunching the leaves and mixing them with hot water to be drunk.

The following two interviews focused on *Cordia lutea* (Overal) in traditional Peruvian culture. The two experts interviewed all indicated that the use of Cordia was common in teas for treatment of the liver.

Summary, Interview 4: Hernan Alemen, 62 years old. Mr. Alemen indicated said he uses Cordia lutea himself but also has harvested the flower and sends it to others to be used in teas to treat jaundice and liver problems. He said this plant has been used for well over 100 years, and was used by his grandparents. He knew of no side effects and said pork, and shellfish should be avoided. He recommended taking the tea 3 times per day to treat liver conditions. He recommended preparing from fresh water and perhaps adding honey.

Summary, Interview 5: Rigoberto Leon Carrasco, 75 years old. In this final interview from Mr. Carrasco, the healer said he relies on the teachings of his grandparents and said his grandmother used Cordia when he was a child to treat family members with hepatitis and jaundice. He personally uses the Cordia tea when he feels ill, fatigue, or has inflammation of the liver and claims he has used Cordia to successfully treat his granddaughter who was ill with hepatitis, having not had the hepatitis vaccine. He said there can be different methods of taking this medicine for different conditions but for liver problems it is taken as a tea. He also said this flower can be soaked in alcohol for 8 to 15 days with the alcohol later being used in hot water.

It should be noted that the interview accounts above, show that each of the three constituent plants in A4+ have been used individually in traditional Peruvian medicine for similar, overlapping treatment purposes or healing paradigms (namely: promoting healing of the liver and maintaining liver health) for well over 50 years. Therefore, according to NHPD, the above interview accounts can be considered as acceptable expert opinion report evidence for the efficacy of A4+. The above interview evidence is being used to support each ingredient component of A4+, and A4+ final product because they all have a common therapeutic purpose.

H) Sabell Corporation's Medical Research & Advisory Board

Sabell Corporation is committed to developing a safe and effective treatment for liver diseases using natural/herbal products. Sabell Corporation has recognized the potential of A4+ and chosen to invest in the development and execution of a number of clinical, in vitro, in vivo, toxicology, and product chemistry development studies to prove the safety and efficacy of A4+.

Sabell Corporation has a medical research and advisory board comprised of the following highly distinguished doctors - two of which are recipients of the Order of Canada;

Francis Green, M.D, Ph.D. Department of Pathology & Laboratory Medicine, Faculty of Medicine, University of Calgary Edmonton, Canada	Medical Advisory Committee Chair for Sabell Corporation
Steven K.H. Aung, M.D, College of Integrated Medicine, Faculty of family Medicine, University of Alberta Edmonton, Canada	Recipient of the **Order of Canada** & WHO Consultant. Medical practitioner of Eastern, Western and Integrated Medicine, Advisor on traditional herbal medicine.
John Wallace, *PhD, MBA, FRSC,* Farncombe Family Digestive Health Research Institute, McMaster University, Canada	Evaluation of the Anti-Inflammatory, Anti-Oxidant and Mucosal Protective/Reparative Actions of A4+ and Its Constituents
Lorne Tyrrell,M.D, PhD, FRCP, Professor and CIHR/GSK Chair in Virology, Director, Li Ka Shing Institute of Virology University of Alberta Edmonton, Canada	Recipient of the **Order of Canada**; former Dean , Faculty of Medicine , University of Alberta. Investigations of the Herbal Compound A4+ and its constituents as a Potential Antiviral Therapy for Hepatitis B and Hepatitis C Viruses
Mark G. Swain, MD, MSc, FRCPC, Professor of Medicine, University of Calgary	Hepatologist. Engaging in studies with mice to determine effect of A4+ and Cordia flowers in fibrosis, and the anti-inflammatory effects in liver disease.
Sam Lee, M.D. & Alex Aspinal, M.D, Faculty of Medicine, University of Calgary	Hepatologists. Investigation and preparation for a potential double blind placebo controlled human clinical trial involving

Edmonton, Canada	A4+ in treating the symptoms and quality of life of chronic Hepatitis C Genotype 1 patients
Hugh A. Semple, DVM, PhD, Toxtest, Alberta Innovates – Technology Solutions in Vegreville, Edmonton, Canada	Heading four new GMP A4+ toxicology testing studies at AITF, consulting on product development.
Brian Duff Sloley, PhD, Senior Scientist, Phytovox Inc.	Analytical chemistry research for characterizing marker compounds in the A4+ formulation and individual constituents.
Raimar Loebenberg, PhD, Director, Drug Development and Innovation Centre and Associate Professor, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta,	A4+ Stability testing and formulation consultation
Dr Sheldon Roth, Professor, Department of Physiology & Pharmacology Faculty of Medicine, Hotchkiss Brain Institute	Advisor on toxicology and A4+ excipient formulation.

The herbal components of A4+ originate from the Amazon rainforest and the Coastal Plains of Peru. The unique formulation for A4+ has been developed by Dr. Jose Cabanillas, a western trained physician who is licensed in Peru and who is also a widely respected and highly regarded expert in natural medicinal plant medicines derived from the Amazon rainforest, the Andes and the Coastal Plains of Peru (http://sabell.ca/jose/bio.htm). Dr Cabanillas provides invaluable cultural expertise and knowledge into the medicinal plant ingredients for a variety of medicinal herbal formulas from Peru. Sabell Corporation has a long standing collaborative relationship with Dr. Cabanillas.

1.3 Dosage and Other Conditions of Use

A4+ is available as (250 mg) capsule in a bottle containing 84 capsules. Each capsule contains 77.8 mg *Cordia lutea*, 9.7 mg *Annona muricata* and 9.7 mg *Curcuma longa*.

Two capsules to be taken three times a day before meals. It is recommended that A4+ be taken daily for a minimum of 30 days. Users are encouraged to get a "daily dose" of sunlight on their bodies. Persons taking this herbal product are recommended to avoid fatty foods, shellfish, pork and red meat.

2.0 Safety Summary Report

2.1 Safety Overview:

Cordia lutea: *Cordia* is a genus of flowering plants in the borage family, Boraginaceae. It contains about 300 species of shrubs and trees. Many studies have shown the hepatoprotective potential of different species of *Cordia* and have been used for many years. The flower component of *Cordia lutea* has been used as a traditional medicine for centuries. It is used traditionally in Peru as a tea infusion or as an alcohol extract to treat jaundice, hepatitis and liver problems. Overall, *Cordia lutea* is a safe substance for human ingestion Peruvian medicine. There are no documented cases of adverse reactions.

Cautionary statement can be placed on the label:

- *Keep out of reach of children."*
- *"Consult a health care practitioner prior to use if you are pregnant, breastfeeding, or plan to become pregnant."*

***Curcuma longa* (Turmeric):** Based on Health Canada Monograph, for Turmeric (*Curcuma longa)* USDA 2008; Date: 2010-02-25

- **Caution(s) and Warning(s)**
  - Consult a health care practitioner prior to use if you are pregnant (ESCOP 2003, Brinker 2001, McGuffin et al. 1997).
  - Consult a health care practitioner prior to use if you have gallstones or a bile duct obstruction (ESCOP 2003, Brinker 2001, McGuffin et al. 1997).
  - Consult a health care practitioner prior to use if you have stomach ulcers or excess stomach acid (Brinker 2001, McGuffin et al. 1997).
  - Digestive aid; Relief of flatulent dyspepsia; Hepatoprotectant; Bile excretion; Anti-inflammatory; TCM; Ayurveda:

    Consult a health care practitioner if symptoms persist or worsen

- **Contraindication(s)**

  No statement is required

- **Known Adverse Reaction(s)**

  No statement is required

***Annona muricata* (Graviola):**

Annona is a genus of flowering plants in the pawpaw/sugar apple family, *Annonaceae*. It is the second largest genus in the family containing approximately 110 species of mostly neotropical and afrotropical trees and shrubs. Many studies have shown the hepatoprotective potential of different species of *Annona* and have been used for many years. *Annona muricata* has a long, rich history of use in herbal medicine as well as a lengthy recorded indigenous use. It is used traditionally by many Peruvian and Brazilian residents and elders from both countries to treat jaundice, hepatitis and liver conditions. The Rain Tree information source also has a compiled list of numerous scientific studies that have been conducted to characterize the many potential benefits of *Annona muricata* (Graviola). Overall, *Annona muricata* is a safe substance for human ingestion and there are no documented cases of adverse reactions and has no known food or drug interactions.

- Based on Raintree Nutrition, Tropical Plant Database, Monograph (Database File) for Graviola (*Annona muricata*).

  - **Caution(s) and Warning(s)**

    - It has cardiodepressant, vasodilator, and hypotensive (lowers blood pressure) actions.
    - Large dosages can cause nausea and vomiting. Avoid combining with ATP-enhancers like CoQ10.

  - **Contraindication(s)**

    - Graviola has demonstrated uterine stimulant activity in an animal study (rats) and should therefore not be used during pregnancy.
    - Graviola has demonstrated hypotensive, vasodilator, and cardiodepressant activities in animal studies and is contraindicated for people with low blood pressure. People taking antihypertensive drugs should check with their doctors before taking graviola and monitor their blood pressure accordingly (as medications may need adjusting).
    - Graviola has demonstrated significant *in vitro* antimicrobial properties. Chronic, long-term use of this plant may lead to die-off of friendly bacteria in the digestive tract due to its antimicrobial properties. Supplementing the diet with probiotics and digestive enzymes is advisable if this plant is used for longer than 30 days.
    - Graviola has demonstrated emetic properties in one animal study with pigs. Large single dosages may cause nausea or vomiting. Reduce the usage accordingly if this occurs.
    - Alcohol extracts of graviola leaf showed no toxicity or side effects in mice at 100 mg/kg; however, at a dosage of 300 mg/kg, a reduction in explorative behavior and

mild abdominal constrictions was observed. If sedation or sleepiness occurs, reduce the amount used.

Cautionary statement can be placed on the label:

- *Keep out of reach of children."*
- *"Consult a health care practitioner prior to use if you are pregnant, breastfeeding, or plan to become pregnant."*
- *"Consult a health care practitioner prior to use if you are taking antihypertensive drugs".*

In order to confirm the safety of A4+, the following studies were conducted by Sabell Corporation;

1.0 Semple H., "A 7-Day Repeat Dose (oral) Toxicity Screen on A4+ in Rats", ToxTest Alberta Innovates–Technology Futures, Vegreville, Alberta, 2010.

The purpose of this study was to determine adverse effect resulting from daily, oral administration of A4+, over the course of 7 days in male and female Sprague Dawley rats. A4+ was administered orally for 7 days to rats in aqueous suspensions at dosage of 2000 (high dose), 500 (mid dose), 125 (low dose) and 0 (Controls) mg/kg BW.

Species	:	Sprague Dawley rats
Route / Method	:	Oral / gavage
Number of animals	:	10 (5 male and 5 female) animals/ group
Sex	:	Male and female
Body weight	:	Male (323.3 – 355.7 g) & Female (212.2 – 238.4 g)

Treatment Group	Treatment	No. of Animals/group		Dose Level (mg/kg BW)	Dose Conc. (mg/mL)	Dose Volume (mL/kg BW)
		Male	Female			
1	Control	5	5	0	0	6
2	Low dose A4+	5	5	125	20.81	6
3	Mid dose A4+	5	5	500	83.25	6
4	High dose A4+	5	5	2000	333.0	6

There was no overt evidence of toxicity related to the test article at any dosage administered. There was no apparent effect on general health, including clinical observations, body weight and food consumption. Analysis of clinical pathology and hematology parameters revealed minor statistically significant group differences mostly within the normal range, that were not considered to be biologically relevant test article related effects. The pathologic examination revealed no treatment related macroscopic findings, and one high-dose animal exhibited

myocarditis on histopathological examination. Whether this was treatment related is an open question since only one animal was affected.

In summary, this study did not demonstrate any common or consistent adverse effects at the doses employed and under the conditions of the experimental protocol of this study.

2.0 Semple H., "A 28-day repeat dose oral toxicity study of A4+ in rats", ToxTest Alberta Innovates–Technology Futures, Vegreville, Alberta, 2010.

The purpose of this study was to determine the toxicity of A4+ in rats, when given daily for 28 days. Four groups, Vehicle Control (1), Low dose (2), Mid dose (3) and High dose (4) of 5 female and 5 male Sprague Dawley rats were administered test of reference item by oral gavage daily for 28 days.

Species	:	Sprague Dawley rats
Route / Method	:	Oral / Gavage
Number of animals	:	10 (5 male and 5 female) animals/group
Sex	:	Male and female
Body weight	:	Male (275.3 – 315.6 g) and Female (188.7 – 211.9 g)

Treatment Group	Treatment	No. of Animals/group		Dose Level (mg/kg BW)	Dose Conc. (mg/mL)	Dose Volume (mL/kg BW)
		Male	Female			
1	Control	5	5	0	0	6
2	Low dose A4+	5	5	125	20.81	6
3	Mid dose A4+	5	5	500	83.25	6
4	High dose A4+	5	5	2000	333.0	6

Animals were observed twice daily, weighed weekly and food consumption was measured weekly. During the last week of the study, functional observational batteries were conducted on all Groups 1 to 4 animals. On study day 29, Group 1 to 4 animals were exsanguinated under anesthesis and the collected blood was analyzed for clinical chemistry and hematology including coagulation. The euthanized animals were necropsied and tissues were collected. Selected organs were weighed. Tissues from the high dose and control groups were processed and histopathological examination was conducted.

No significant test item-related effects were observed among clinical observation, body weights, food consumption, functional observational battery, urinalysis, clinical pathology parameters, blood coagulation parameters, mortality or macroscopic and histopathological findings. Small but statistically significant differences in some hematology results and organ weights were deemed to have low biological significance. No animal deaths occurred during the study.

It was concluded that A4+ did not exhibit toxicity, under the conditions of this study and a no adverse effect level (NOAEL) of 2000 mg/kg can be assigned.

3.0 Semple H., "A Mammalian Erythrocyte Micronucleus Study of A4+ in Mice", ToxTest Alberta Innovates–Technology Futures, Vegreville, Alberta, 2010.

The purpose of this study was to evaluate the genotoxic potential of A4+ based upon its ability to induce micronuclei in rodent polychromatophilic erythrocytes (PCE).

Species	:	Balb/c mice
Inbred	:	Balb/C/CNPB
Number of animals	:	60 animals for this study
Sex	:	Male and female
Body weight	:	Male (18.2 – 23.1 g) and female (16.9 – 19.9 g)

Treatment Group	Treatment	No. of Animals/group		Dose Level (µg/g BW)	Dose Conc. (mg/mL)	Dose Volume (µL/g BW)
		Male	Female			
1	Vehicle Control (Reference Item 1)	5	5	0	0	6
2	Low dose A4+	5	5	125	20.81	6
3	Mid dose A4+	5	5	500	83.25	6
4	High dose A4+	5	5	2000	333.0	6
5	Water (Reference Item 3)	5	5	0	0	6
6	Cyclophosphamide (Positive control Reference Item 2)	5	5	40	4.0	10

Under the conditions of the experiment, A4+ was negative for the production of elevated micronucleus counts and did not exhibit bone marrow toxicity. If an acute micronucleus test is negative after 24 hours exposure, another cohort of animals should be examined 48 hours post dosing, or consideration should be given to testing for micronuclei after multiple dosing. In the case of A4+, because the extract is poorly water soluble, oral absorption is likely to be slow. Therefore, the results of an acute micronucleus protocol may not adequately predict the genotoxicity potential of A4+ in human. Therefore, integration of another micronucleus test into a repeated dose oral toxicity study is recommended.

4.0 Semple H., "A Repeated Dose Mammalian Erythrocyte Micronucleus Study of A4+ in Mice", ToxTest Alberta Innovates–Technology Futures, Vegreville, Alberta, 2010.

The purpose of this study was to evaluate the genotoxic potential of A4+ based upon its ability to induce micronuclei in rodent polychromatophilic erythrocytes (PCE).

Species	:	Balb/c mice
Inbred	:	Balb/C/CNPB
Number of animals	:	56 animals for this study
Sex	:	Male and female
Body weight	:	Male (16.2 – 22.2 g) and female 916.1 – 19.1 g)
Dose / Method	:	Treatment group: 1 to 4 (Oral / Gavage) Treatment group: 5 (Intraperitoneal / Injection)

Treatment Group	Treatment	No. of Animals/group		Dose Level (µg/g BW)	Dose Conc. (mg/mL)	Dose Volume (µL/g BW)
		Male	Female			
1	Control	5	5	0	0	6
2	Low dose A4+	5	5	125	20.81	6
3	Mid dose A4+	5	5	500	83.25	6
4	High dose A4+	5	5	2000	333.0	6
5	Cyclophosphamide (Positive control)	8	8	40	4.0	10

No abnormalities were observed during clinical observations. Animals were euthanized 24 hours after the final or in the case of the positive control group, only dosing, both femurs were collected, and the marrows were flushed with fetal bovine serum. From the suspended cells, bone marrow smears were made. The high dose group and both negative and positive control group smears were scored for PCE/200 erythrocytes and for micronucleated PCE 9MNPCE)/2000 PCE.

Statistical analysis of the bone marrow scores revealed that the positive control compound cyclophosphamide reduced PCE scores similarly in both sexes, an indicator of bone marrow toxicity. No changes in PCE scores were associated with high dose A4+ test item. MNPCE scores, the primary indicator of genotoxicity in this test, were significantly higher in the positive control animals of both sexes than in either the high dose A4+ treated animals or the negative controls. The A4+ treated animals of both sexes has similar low MNPCE scores to the control animals with no significant differences between groups.

It was concluded that under the repeated dose conditions of the experiment, A4+ was negative for the production of elevated micronucleus counts and did not exhibit bone marrow toxicity based on the results of this study.

5.0 Cabanillas Jose., "Toxicological Evaluation of the A4+ Formula", University of Iquitos, Lima, Peru, 2009.

Two A4+ toxicity studies conducted; a) The acute toxicity of A4+ was tested using limited dose test in albino rats and b) The acute toxicity of A4+ was tested using limited dose test in albino mice.

a) The acute toxicity of A4+ was tested using limited dose test in albino rats (Holtzmann). The A4+ and a control substance (a saline solution) were administered orally using an intragastric catheter.

Species	:	Albino rat (Rattus novergicus)
Inbred	:	Holtzmann
Number of animals	:	3 animals per experimental group
Sex	:	Male and female
Body weight	:	120 – 160 g
Group 1 (Treatment)	:	These animals were administered a dose of 2,000 mg/kg of A4+
Group II (control)	:	These animals were administered a saline solution (same as A4+ volume)

This experiment was performed using male and female rats, which underwent a week-long quarantine, were divided in two groups composed on three animals of each sex, and were weighed and marked for identification purposes. Before the evaluation, the animals underwent a fasting period of 12 hours; then, the A4+ and the saline were administered to both groups according to the dose. Immediately after the substances were administered, the animals were observed to look for toxic signs at system/organ level: Autonomous, behaviour, sensory, neuromuscular, respiratory, ocular, gastrointestinal, urinary, and others, such as body weight. The body weights of the animals were recorded on the 7^{th} and 14^{th} day after the administration.

After 14 days, the animals were sacrificed following the ethical principals for the animal experimentation; this was followed by a macroscopic study to analyse the size, colour and consistency of the following organs: heart, kidneys, liver, spleen, stomach, lung, brain, ovaries and testicles. The macroscopic analysis of the organs did not find any visible changes where the A4+ was administered at a dose of 2,000 mg/kg.

The results obtained show the innocuousness of the extract at a dose of 2,000 mg/kg A4+, since no mortality and no clinical signs or macroscopic changes were observed, thus finding no evidence of toxicity in the organs.

b) The acute toxicity of A4+ was tested using limited dose test in albino mice (Balb/C/CNPB). The A4+ and a control substance (a saline solution) were administered orally using an intragastric catheter.

Species	:	Albino Mice (Mus musculus)
Inbred	:	Balb/C/CNPB
Number of animals	:	10 animals per experimental group
Sex	:	Male and female
Body weight	:	20 – 25 g
Group 1 (Treatment)	:	These animals were administered a dose of 2,000 mg/kg of A4+

Group II (control) : These animals were administered a saline solution (same as A4+ volume)

The mice underwent a fasting period of 4 hours prior to the experiment; then, the A4+ and saline solution were administered accordingly and the animals were under continuous observation for 4 hours. Upon no occurrence of mortality, the observation period was extended to 14 days after the administered, and then up to 21 days, in order to perform an observation of the recovery of the animals and the reversibility of the effects. The body weight of the animals was recorded at the beginning of the experiment, as well as on the 7^{th} , 14^{th} and 21^{st} day, when possible after the substances were administered, in order to established whether there was a weight loss or gain.

At the end of the experiment, the animals were sacrificed through a cervical dislocation procedure. A necropsy was performed on all the animals that survived until the end of the experiment. The microscopic analysis of the organs did not find any visible changes in the Group I mice where the A4+ was administered at a dose of 2,000 mg/kg. The results obtained show the innocuousness of the A4+ at a dose of 2,000 mg/kg. p.c., since no mortality and no clinical signs or macroscopic changes were observed, and thus there was no evidence of toxicity in the organs.

Drug and Food Interactions:

There are no known interactions for *Curcuma longa* (Turmeric), *Annona muricata* (Graviola) or *Cordia lutea.*

2.2 Risk Information and Risk Mitigation:

According to the information in Section 2.1, the following cautions and warnings have been determined to be included on the product label:

- *"Keep out of reach of children."*
- *"Consult a health care practitioner prior to use if you are pregnant, breastfeeding, or plan to become pregnant."*
- *"Consult a health care practitioner prior to use if you are taking antihypertensive, blood thinning medication, have gallstones, ulcers, liver or bile obstruction."*

3.0 Combination Rationale (if applicable)

In accordance with NHPD guidance (NHPD Safety and Efficacy guidance document, Ver.2, Dec 2006) a combination rationale is not required for the A4+ formulation because "adequate evidence is provided above that support the safety and efficacy of the finished product".

4.0 Non-Medicinal Ingredient Information, if applicable

All non-medicinal ingredients are included in Health Canada's list of acceptable non-medicinal ingredients.

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