

Ashitaba Chalcone Powder

A Comprehensive Scientific Review

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I. Summary

This paper describes the biological activity and safety of chalcones from the sap of Ashitaba, *Angelica keiskei*. The paper focuses on a proprietary product produced by Japan Bio Science Laboratory Co, Ltd of Japan (JBSL), known as Ashitaba Chalcone Powder. Ashitaba Chalcone Powder is a concentrated preparation containing a minimum of 8.0% total chalcones (measured as 4-hydroxyderricin and xanthoangelol). The beneficial properties of JBSL Ashitaba Chalcone Powder on the symptoms of metabolic syndrome have been demonstrated in a human study as well as animal and in vitro studies. The safety of Ashitaba Chalcone Powder has been demonstrated in several preclinical toxicity screening tests.

Activity

Preliminary studies indicate that the chalcones may ameliorate many of the signs and symptoms associated with metabolic syndrome. Evidence from a human clinical study and from animal studies indicates that the chalcones may help reduce visceral fat. Animal studies also indicate that the chalcones may have a beneficial effect on lipid metabolism. A chalcone extract increased serum high-density lipoprotein (HDL) cholesterol and decreased triglyceride levels in the livers of spontaneously hypertensive rats. There is also evidence that 4-hydroxyderricin (one of the chalcones) lowers blood pressure in hypertensive animals. And there is evidence that 4-hydroxyderricin and, to a lesser extent, xanthoangelol reduce the progression of diabetes in animals that develop hyperglycemia upon aging. In addition, there is preliminary evidence from animal and in vitro studies that the chalcones may increase the production of the adipokines: leptin and adiponectin. Other studies with chalcones demonstrate anti-inflammatory and antioxidant effects.

There is evidence from animals and in vitro studies that the chalcones has anti-cancer activity. Mechanistic studies indicate that xanthoangelol may inhibit tumor angiogenesis and 4-hydroxyderricin may have ameliorating effects on the immune system. In addition, xanthoangelol and 4-hydroxyderricin may inhibit tumor promotion and induce apoptosis (cell death) to cancer cells.

The chalcones may also help prevent ulcers by inhibiting gastric acid secretion and having an antibacterial effect on *Helicobacter pylori*.

Safety

Ashitaba has a long history of use, as the fresh leaves of Ashitaba are used as food and preparations of the plant have been used as traditional medicines. JBSL Ashitaba Chalcone powder is a concentrated preparation of the yellow sap of the plant. This preparation was determined to be non-mutagenic in the Ames assay with 4 strains of bacteria and in a chromosomal aberration study conducted in CHO cells. The Chalcone powder also did not cause toxicity to Wistar rats given a single oral dose of 2,000 mg /kg body weight. Further, animal studies conducted with various preparations of Ashitaba have not reported any signs of toxicity.

Description

Ashitaba is the common name for *Angelica keiskei* (Miq.) Koidz. (syn. *Archangelica keikei* Miq.), a plant native to the Izu islands on the Pacific coast of Japan. Ashitaba has been consumed as a vegetable and medicine for many hundreds of years by the local islanders. The fresh leaves and dried plant material are used in foods. The plant is nutritious as it is high in vitamin and mineral content. In traditional medicine, Ashitaba is used as a tonic, to improve digestion, as well as to prevent and cure infectious diseases. When the stems or roots of the plant are broken, a sticky yellow sap gushes out. This sap has been used to treat various skin conditions, including boils, cysts, pustules and athletes foot (Baba K et al. 1998).

The sap contains compounds called chalcones, which belong to a larger class of chemical compounds known as flavonoids. About 20 different chalcones have been identified in the plant, with the most abundant being 4-hydroxyderricin and xanthoangelol. The edible portion of the leaf (raw leaves) contain 0.2 to 0.3% chalcones (Nagata et al. 2007). JBSL Ashitaba Chalcone Powder is a concentrated preparation containing a minimum of 8.0% total chalcones.

II. JBSL Ashitaba Chalcone powder

JBSL grows Ashitaba in Indonesia using organic farming techniques. Ashitaba preparations sold by JBSL include the juice, dried leaves and chalcone powder. The yellow sap from the plants is prepared using a series of steps including sterilization and filtration. Ashitaba chalcone powder has a yellow color, no smell, and contains not less than 8.0% total chalcones (measured as xanthoangelol and 4-hydroxyderricin).

III. Chalcones and Metabolic Syndrome

Metabolic syndrome is a group of metabolic risk factors that signal conditions for cardiovascular disease and diabetes mellitus type 2 (Blaha et al. 2008). Metabolic syndrome has been defined by various means including serum lipid profiles, elevated blood pressure and elevated fasting glucose. However, central to metabolic syndrome is abdominal fat.

Fat is no longer considered just an excess of weight. Adipose (fat) tissue is now known to act as an endocrine organ, which produces hormones known as adipokines (adipocytokines). The adipokines include resistin, leptin and adiponectin (Kamada et al. 2008). These adipokines play roles in satiety, lipid metabolism and sensitivity to insulin. Also associated with abdominal fat are inflammatory mediators including interleukin 6, tumor necrosis factor alpha and C-reactive protein (Inadera 2008). In addition, secretion of plasminogen activator inhibitor-1 (PAI-1) by adipose tissue increases the risk of thrombosis, or heart attack and stroke.

Preliminary evidence indicates that Ashitaba chalcones may help to alleviate some of the risk factors associated with metabolic syndrome. Although it is unlikely that the chalcones affect body weight per say, there is evidence from a human clinical study and from animal studies that the chalcones may help reduce visceral fat. Animal studies also indicate that the chalcones may have a beneficial effect on lipid metabolism. A chalcone extract increased serum high-density lipoprotein (HDL) cholesterol and decreased triglyceride levels in the livers of spontaneously hypertensive rats. Low HDL cholesterol is a risk factor for heart disease and high triglyceride levels are associated with heart disease and diabetes.

There is also evidence that one of the chalcones, 4-hydroxyderricin, lowers blood pressure in hypertensive animals. And there is evidence that 4-hydroxyderricin, and to a lesser extent xanthoangelol, reduce the progression of diabetes in animals that develop hyperglycemia upon aging.

In addition, there is preliminary evidence from animal and in vitro studies that the chalcones may increase the production of the adipokines: leptin and adiponectin. Leptin is a hormone that has a central role in fat metabolism and appears to play a role in satiety. Although leptin is a circulating signal that reduces appetite, leptin levels are increased in obese people. Those who are obese are thought to be resistant to the effects of leptin, in a similar manner as people with type 2 diabetes are resistant to the effects of insulin. It is thought that the high sustained concentrations of leptin from the enlarged adipose tissue may result in leptin desensitization (Knudson et al. 2008). Chalcones have been shown to increase leptin levels in the adipose tissue of mice. This effect may be beneficial to individuals who have not yet become resistant to leptin. Adiponectin increases insulin sensitivity and has anti-inflammatory properties. The secretion of adiponectin decreases with abdominal obesity. Circulating levels of adipokines can be used to assess obesity-related health problems, including low grade inflammation (Inadera 2008). In vitro studies have demonstrated that chalcones increase the levels of adiponectin secreted by adipocytes. Other studies with chalcones directly demonstrate anti-inflammatory and antioxidant effects.

Preliminary evidence indicates that the chalcones may ameliorate many of the signs and symptoms associated with metabolic syndrome. Additional studies in humans are indicated to explore this potential.

IV. Studies Conducted on Chalcones

Body weight / visceral fat

The effects of chalone preparations on body weight and visceral fat have been tested in one human study and several animal studies. The human study, conducted with JBSL Ashitaba Chalone powder, indicated a trend towards a decrease in visceral fat especially for those with greater quantities of fat. An animal study conducted with Ashitaba Chalone powder (0.75% of diet) indicated a decrease in body weight and quantity of visceral fat in male mice. However this result was not repeated in other studies.

Body weight / visceral fat - Human study

A placebo controlled study was conducted on healthy men to determine the effect of JBSL Ashitaba Chalone powder on body fat. Fifteen (15) men, average age 38 years old and average BMI of 24.0 +- 3.2, were given 200 mg/day Chalone powder or placebo (dextrin) for 8 weeks. Visceral fat was measured using a CT scan. Administration of the Chalone powder was associated with a decrease in the cross-sectional area of visceral fat. There was a greater decrease in visceral fat in men who had greater girth at baseline (13.3 and 10.1% decreases in 2 cases). There was no change in the placebo group (JBSL 2009).

Body weight / visceral fat - Animal Studies

A placebo-controlled animal study was conducted to determine the effect of JBSL Ashitaba Chalone powder on body weight and adipose tissue content. Mice (C57 BL/6n; male and female) were administered food with or without Ashitaba Chalone powder (0.75% of diet) for 5 weeks. Body weight was measured twice weekly during the study. At the end of the 5 weeks, the animals were euthanized and the weight of the adipose tissue in the back, abdominal region and mesentery were determined individually. Body weight increased for all animals the beginning of the experiment at 7 weeks of age, until the end at 12 weeks of age. At the last determination of body weight, after 5 weeks of treatment, the male animals given chalone were significantly lighter in weight compared to the control male animals. There was no difference in the female animals. The male animals given chalone also had significantly less adipose tissue (measured using weight) in the back, abdomen and mesentery compared to controls. Again there was no significant difference in the female animals (JBSL 2009).

The experiment described above was repeated with the addition of a high fat diet. Also, this study included two doses of Ashitaba Chalone powder (0.25% and 0.75% of the diet) in addition to the control group. Again there was a trend towards a reduction in body weight in the male animals given the highest concentration of Ashitaba at the 5th week. There was no difference in weight in the female animals. There was no significant difference in visceral adipose tissue with either dose with either male or female animals (JBSL 2009).

The effects of a chalone powder characterized as containing 0.3% chalcones was determined on rats given 17, 170 or 1700 mg/100g body weight for 28 days along with a high fat diet. At the end of that time, there were no significant decreases in body weight

or adipose tissue weights in the groups (Nagata et al 2007). There was a significant increase in perirenal adipose tissue weight in rats fed 170 mg powder per 100 g body weight compared to control animals ($p < 0.05$), but no trend was evident as this effect was not observed in animals given the higher dose.

Spontaneously hypertensive rats (SHRSP) given diets containing 0.02% or 0.1% xanthoangelol for 7 weeks had no changes in body weight (Ogawa et al. 2007). In addition, SHRSP animals fed diets containing 0.07% 4-hydroxyderricin for 7 weeks showed no change in body weight (Ogawa et al. 2005c). SHRSP animals fed a diet including 0.1% laserpitin, a coumarin, for 7 weeks had a reduction in body weight gain without any changes in daily food intake ($p < 0.05$) (Ogawa et al. 2005a).

Lipid Levels

In one group of experiments, Ashitaba chalcone powder given to rats in amounts up to 1700 mg/100g body weight for 28 days along with a high fat diet did not affect serum total cholesterol or high-density lipoprotein (HDL) cholesterol. Another series of experiments were conducted with stroke-prone spontaneously hypertensive rats (SHRSP) suggested that Ashitaba might have beneficial effects on lipid metabolism. An ethyl acetate extract increased serum HDL and decreased liver triglycerides. Components of the extract were also studied in this model. Chalcones 4-hydroxyderricin and xanthoangelol as well as the coumarin laserpitin, all individually decreased liver triglyceride content. Their individual effects on serum lipids were as follows: 4-hydroxyderricin decreased levels of VLDL, Xanthoangelol reduced levels of LDL and laserpitin increased levels of HDL.

Lipid Levels – Animal Studies

In an experiment with rats, Ashitaba chalcone powder characterized as containing 0.3% chalcones did not appear to directly affect lipid metabolism. The effects the powder was determined on rats given 17, 170 or 1700 mg/100g body weight for 28 days along with a high fat diet (Nagata et al 2007). The chalcone powder did not affect serum total cholesterol or high-density lipoprotein (HDL) cholesterol. It also did not affect liver cholesterol or triacylglycerol concentrations. Serum triacylglycerol levels in the rats fed 1,700 mg/kg was significantly higher ($p < 0.05$) than that of the group fed 17 mg/kg, but not that of control animals. Fecal weights of the rats fed 1700 mg/100 kg were significantly higher than those in the control group ($p < 0.05$). Bile acid excretions of the rats fed 170 mg and 1700 mg/100 kg were significantly higher than those in the control group ($p < 0.05$). The authors of the study suggested that the increase in bile acids might be due to the fiber content of product and that this increase in bile acid production might result in a decrease in serum cholesterol levels over time. However this effect was not observed in this assay.

An experiment with stroke-prone spontaneously hypertensive rats (SHRSP) suggested that Ashitaba may enhance transport of cholesterol from the peripheral tissues to the liver and suppress accumulation of lipids in the liver. Stroke-prone spontaneously hypertensive rats (SHRSP) were fed a control diet or a diet containing 0.2% extract (ethyl acetate: water (4:1) of the sap from the stems) for 6 weeks (Ogawa et al. 2003). The extract

produced an elevation of serum HDL levels and a reduction in liver triglycerides. Serum levels of cholesterol and phospholipids were elevated in the treatment group compared to the control group. There was also an increase in serum apolipoproteins (Apo) A-I and E compared to control, with no effect on Apo B. The authors suggested that the changes in lipid levels were due to increases in high-density lipoprotein (HDL) containing Apo A-I and Apo E.

Experiments with 4-hydroxyderricin in SHRSP animals suggested that it may suppress hepatic lipid accumulation through decreases in proteins involved in lipid metabolism (Ogawa et al 2005c). 4-hydroxyderricin (0.07% of diet for 7 weeks) reduced serum very low-density lipoprotein (VLDL) levels (without any effects on HDL levels) and a reduction in serum concentrations of free fatty acids. There were also significant decreases in liver weight and liver triglyceride content. Experiments measuring expression of proteins involved in lipid metabolism in the liver revealed that mRNA expression of microsomal triglyceride transferprotein (MTP), fatty acid synthase (FAS) and adipocyte determination and differentiation factor 1 (ADD1) decreased significantly whereas HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzymeA reductase) mRNA increased significantly. There were no significant effect to serum levels of total cholesterol, phospholipids and triglyceride. There were also no significant differences in serum levels of apolipoproteins (Apo A-1, ApoB, ApoE) compared to control.

Experiments with xanthoangelol in SHRSP rats demonstrated a reduction in serum low-density lipoprotein (LDL) levels along with decreases in total cholesterol and triglyceride contents in the liver (Ogawa et al 2007). A diet containing 0.02% or 0.1% xanthoangelol for 7 weeks caused a dose-dependent decrease in serum LDL levels (cholesterol and phospholipid fractions). There were no significant effects on serum total cholesterol, phospholipid, triglyceride, free fatty acids or apolipoproteins (ApoA-1, B and E). In the liver, there were significant dose-dependent decreases in weight and total triglyceride content. There was a significant decrease in total cholesterol content in the livers of the higher dose group. Experiments measuring expression of proteins involved in lipid metabolism in the liver revealed a significant increase in peroxisome proliferator-activated receptor (PPAR) alpha mRNA expression, which was associated with a tendency for increases in acyl-coenzyme (CoA) synthase and acyl-CoA oxidase mRNA expression in the high dose group.

Laserpitin, a coumarin isolated from the yellow sap of the plant, increased serum HDL levels, especially apoE-HDL, and decreased hepatic triglyceride content in SHRSP rats (Ogawa et al. 2005b). The animals were fed a control diet or a diet including 0.1% laserpitin for 7 weeks. Serum total cholesterol, phospholipid and apo E levels were significantly increased. In the liver there were significant decreases in weight and triglyceride content. Hepatic mRNA expression of proteins involved in lipid metabolism indicated a significant decrease in hepatic triglyceride lipase which may be the cause of the increase in serum HDL levels. A marked decrease in adipocyte determination and differentiation factor 1 may be associated with the decrease in hepatic triglyceride content. The authors suggested that laserpitin, not 4-hydroxyderricin, may be responsible for the increase in serum HDL observed with the extract. The reductions in liver weight

and triglyceride content were almost equivalent to that of 4-hydroxyderricin, suggesting that both compounds contribute those effects observed with the extract.

Blood Pressure

Animal experiments suggest that there are chemical constituents in Ashitaba that reduce blood pressure in hypertensive rats. When the two major chalcones were tested in animals individually, 4-hydroxyderricin effectively lowered blood pressure while xanthoangelol had no effect. In vitro experiments indicate that many of the chalcones may cause vasorelaxation through increases in production of endothelium-derived relaxing factor (EDRF), also known as nitric oxide (NO). Alternatively, xanthogenolol D was shown to modulate the activity of endothelin-1 (ET-1), a potent vasoconstrictor peptide.

Blood pressure – Animal studies

Blood pressure was lowered slightly, but insignificantly compared to controls, in stroke-prone spontaneously hypertensive rats (SHRSP) that were fed a diet containing 0.2% extract of the stem sap (ethyl acetate: water (4:1) for 6 weeks (Ogawa et al 2003).

An 80% ethanol extract of the leaves, but not the stems, caused a reduction in blood pressure in spontaneously hypertensive rats (SHR) (Shimizu et al. 1999). Activity guided fraction of the extract produced a fraction that caused a decrease in blood pressure when given to the rats in drinking water at a concentration of 21.8 mg/kg/day for 10 weeks. Result was a significant decrease in blood pressure from the 6th to 10th week of treatment ($p < 0.05$). This fraction had an angiotension converting activity (ACE) inhibiting activity in vitro with an IC_{50} of 4.1 mcg/ml. Further isolation of the activity suggested that it was similar to nicotianamine.

In another experiment, 4-hydroxyderricin lowered systolic blood pressure in SHRSP rats fed a diet containing 0.07% 4-hydroxyderricin for 7 weeks ($p < 0.05$) (Ogawa et al 2005c). However the other major chalcone, xanthoangelol, had no effect. Xanthoangelol, fed to rats at 0.02% or 0.1% of the diet for 7 weeks, did not cause any change in blood pressure (Ogawa et al 2007).

The coumarin laserpitin had no effect on blood pressure in SHRSP animals when given in their diet 0.1% laserpitin for 7 weeks (Ogawa et al 2007).

Blood Pressure - In vitro

Chalcones may reduce blood pressure through relaxation of vascular blood vessels. A 50% ethanol extract of the roots inhibited phenylephrine-induced vasoconstriction of rat aortic rings in a concentration dependent manner with an ED_{50} of approximately 500 mcg/ml. (Matsuura et al. 2001) This activity appeared to be due to the presence of five chalcones: xanthoangelol, 4-hydroxyderricin, xanthoangelol B, xanthoangelol E and xanthoangelol F. The degree of activity at a concentration of 100 mcg/ml was in the following order: xanthoangelol B > 4-hydroxyderricin > xanthoangelol > xanthoangelol E > xanthoangelol F. Mechanism of action studies indicated that the chalcones caused the vasorelaxation through increases in production of endothelium-derived relaxing factor (EDRF) also known as nitric oxide (NO). The action of xanthoangelol B was an

exception as it was not mediated through increases of NO and instead appeared to directly inhibit smooth muscle contraction.

Another paper investigated whether chalcones might modulate the activity of endothelin-1 (ET-1), a potent vasoconstrictor peptide that is thought to play a role in vascular disorders. ET-1 is modulated through nuclear factor-kappa B (NF-kB), a transcription factor involved in regulating gene expression. It was found that xanthogenol D suppressed TNF-alpha induced NF-kB activation in cultured porcine aortic endothelial cells (PAEC's). Xanthogenol D suppression of NFkB was accompanied by a decrease in ET-1 release from PAECs (under basal and TNF-alpha-stimulated conditions). The authors of the study suggested that the results indicate that xanthoangelol has a beneficial effect on ET-1 mediated vascular disease. Further that inhibition of NF-kB is a mechanism for treatment of vascular and inflammatory diseases. The effect observed with xanthogenol D was not observed with other chalcones; namely xanthoangelol, xanthoangelol E or xanthoangelol F (Sugii et al. 2005).

Blood Glucose

Animal experiments indicate that 4-hydroxyderricin, and to a lesser extent xanthoangelol, reduce the progression of diabetes in mice that are genetically disposed to develop hyperglycemia upon aging. Xanthoangelol had no effect on blood sugar levels in hypertensive animals. In vitro studies showed that 4-hydroxyderricin and xanthoangelol enhanced glucose uptake by differentiated 3T3-L1 adipocytes.

Blood Glucose – Animal studies

A placebo-controlled animal study was conducted to determine the effect of JBSL Ashitaba Chalcone powder on blood sugar levels. Mice (C57 BL/6n) were given a control diet or a diet including Ashitaba Chalcone powder (0.75% of diet) for 5 weeks. At the end of treatment, there was a trend towards reduction in blood sugar in the male animals and a significant reduction in the female animals (JBSL 2009).

The effects of chalcones 4-hydroxyderricin and xanthoangelol were studied on mice (KK-A^y) that develop hyperglycemia (insulin resistant diabetes) upon aging. Mice were fed a diet with or without 0.15% of each chalcone. After 2 weeks both compounds ameliorated the elevation of blood glucose levels; with a 50% reduction by 4-hydroxyderricin (p<0.01) and a 33% reduction with xanthoangelol (p<0.05). The polydipsia (thirst) associated with diabetes was only reduced in the 4-hydroxyderricin group. There was no difference in body weight in any of the groups (Enoki et al. 2007).

In another experiment, 4-hydroxyderricin (0.15% of the diet) was given to KK-A^y mice for 7 weeks. The effects of 4-hydroxyderricin on the progression of diabetes were compared to control animals and to mice given the antidiabetic drug, pioglitazone (0.05%). Administration of 4-hydroxyderricin led to a significant suppression of blood sugar elevation and amelioration of thirst (polydipsia) compared to control animals. In comparison, Pioglitazone was more effective. It almost completely suppressed the hyperglycemia and polydipsia. A normal increase in body weight was observed with pioglitazone but not in the 4-hydroxyderricin or control groups. The authors of the study

concluded that in this model, 4-hydroxyderricin had a preventative effect on the progression of diabetes (Enoki et al 2007).

In a rat study with SHRSP animals, a diet containing 0.02% or 0.1% xanthoangelol for 7 weeks had no significant effect on serum glucose (Ogawa et al 2007). However a coumarin present in sap, laserpitin, significantly reduced blood sugar in SHRSP when given in the diet at a dose of 0.1% for 7 weeks ($p < 0.01$) (Ogawa et al 2005b).

Blood Glucose – In vitro

A 100% ethanol extract of the roots of the plant produced two insulin-like activities in cultured 3T3-L1 cells. The extract caused a dose dependent (0.0042 to 0.03% extract) conversion of the cells from preadipocytes into adipocytes as indicated by triglyceride content. The extract also significantly enhanced glucose uptake by differentiated 3T3-L1 adipocytes in a dose related manner (0.0167 and 0.03%). When these experiments were repeated with 4-hydroxyderricin and xanthoangelol, both compounds were similarly effective in the conversion assay. Both compounds were also active in the glucose uptake assays, but 4-hydroxyderricin was almost twice as effective. Peroxisome proliferator-activated receptor (PPAR)-gamma is a key molecule in promoting the differentiation of preadipocytes to adipocytes. PPAR-gamma agonists promote this conversion and increase uptake of glucose. However mechanistic experiments showed the chalcones were not active via this pathway (Enoki et al 2007).

Adiponectin

Adiponectin is a hormone that is produced and secreted by fat cells (adipocytes) and which regulates the metabolism of lipids and glucose. Adiponectin plays a role in the sensitivity of cells to insulin. People who are obese have low plasma levels of adiponectin. An in vitro experiment with xanthoangelol and 4-hydroxyderricin indicates that the chalcones may stimulate the production of adiponectin by adipocytes.

Adiponectin - In vitro

The effect of xanthoangelol and 4-hydroxyderricin on adiponectin production by adipocytes (mouse 3T3-L1 cells differentiated into adipocytes) was measured in vitro. Xanthoangelol increased the production of adiponectin in the cell supernatant in a dose-related manner with doses of 0.059, 0.159 and 1.59 mcM by approximately 22 fold. 4-hydroxyderricin also increased adiponectin product but to a much lesser degree. It increased production by approximately 3.7 fold at a concentration of 0.185 mcM with no further increase with a dose of 1.85 mcM (JBSL 2009).

Leptin

Leptin is a hormone that has a central role in fat metabolism and may be key in controlling body weight. An animal experiment with Ashitaba Chalcone powder indicates that it may assist in weight management through an increase in leptin levels.

Leptin – Animal studies

Mice (C57 BL/6n) were given control diets or diets containing Ashitaba Chalcone powder (0.75% of diet) for 5 weeks. At the end of the experiment adipose tissue in the

back, abdomen and mesentery was removed and assays for leptin content. In male animals there was a significant increase in leptin levels in adipose tissue in the back and abdomen in treatment animals compared to control animals. In the female animals there were significant increases in the treatment group in all three tissues (JBSL 2009).

Anti-inflammatory effects

In vitro results suggest that xanthoangelol has the ability to modulate arachidonic acid metabolism in platelets but had no effect on production of prostaglandins in gastric tissue. This is a characteristic of selective anti-inflammatory agents which act on COX-2 but not on COX-1 (Singh and Mittal 2008).

Anti-inflammatory - In vitro

Several chalcones (xanthoangelol B, C and E) and a coumarin (selinidin) displayed anti-inflammatory effects on mast cells in vitro. However other chalcones (xanthoangelol and 4-hydroxyderrcin) displayed pro-inflammatory effects, while xanthoangelol D had no effect.

Xanthoangelol E (0.05 to 1.0 mM) effectively inhibited the production of thromboxane b₂ and 12-hydroxy-5,8,10-heptadecatrienoic acid from exogenous arachidonic acid in platelets, with an IC₅₀ of 5 mcM. Xanthoangelol E had no effect on the production of prostaglandin E₂, F₂ alpha or their metabolites in gastric antral mucosal slices (Fujita et al. 1992).

Six chalcones isolated from the EtOAc extract of the dried roots were tested for inhibition of chemically induced histamine from rat peritoneal mast cells in vitro. Xanthoangelol B, xanthoangelol C and xanthoangelol E were active at a concentration of 100 mcM. Xanthoangelol and 4-hydroxyderrcin (100 mcM) enhanced histamine release and xanthoangelol D had no effect (Nakata K and Baba K 2001).

Selinidin, a coumarin isolated from the plant was reported to attenuate mast cell degranulation following activation with IgE and antigen. Further selinidin was reported to inhibit the release of beta-hexosaminidase, synthesis of leukotriene C₄ and production of tumor necrosis factor alpha. Selindin suppresses IgE-mediated mast cell activation by inhibiting multiple pathways involved in allergic inflammation (Kishiro et al. 2008).

Antioxidant Activity

Anti-oxidant activity has been demonstrated by preparations of Ashitaba in vivo and in vitro. In addition, a small clinical study indicates that an Ashitaba green drink might protected the DNA of smokers from damage. Further, protection of DNA from antioxidant damage was demonstrated in vitro.

Anti-oxidant – Human ex-vivo study

A green drink based on *Angelica keiskei* was protective to the DNA of smokers in a small clinical study. It is known that smoking increases free radical-mediated damage of DNA. Twenty smokers were given 240 ml of commercially available green drink every

day for 8 weeks. There was a significant reduction in lymphocyte DNA damage as assessed using the single cell gel electrophoresis (COMET) assay (Kang et al. 2004).

Anti-oxidant – Animal study

A methanol extract of the aerial parts of *Ashitaba* displayed anti-oxidant activity in vivo. The elevation of lipid peroxide produced by bromobenzene was significantly reduced by the extract and cynaroside (a component of the extract). The extract and cynaroside also restored the epoxide hydrolase activity that was decreased by the treatment with bromobenzene (Park et al. 2002).

Anti-oxidant – In vitro

A methanol extract of the *Ashitaba* vegetable displayed antioxidant activity in the both the ferric reducing/antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays at concentrations of 1 to 20 mcg/ml. The extract also displayed anti-mutagenic activity. It caused a dose-dependent inhibition (25 mcg/ml to 200 mcg/ml) of three heterocyclic amine mutagens in the presence of S9, a metabolic activation system. The extract was not active in the absence of S9. The methanol extract also inhibited DNA nicking (induced by iron and hydrogen peroxide) (~50% at 20 mcg/ml) and hydrogen peroxide-induced genotoxic damage to human HCT116 colon cancer cells in the “comet” assay (5 to 20 mcg/ml; $p < 0.01$) (Kwon et al. 2006).

Three recently identified chalcones (xanthokeismins A, B and C), isolated from the stems of the plant, have been identified as having superoxide-scavenging activity (Aoki et al. 2008). Superoxide radicals were generated in vitro by the hypoxanthine/xanthine oxidase system. Activity guided fraction of a methanol extract resulted in the isolation of xanthoangelol B and xanthokeismins A, B and C. The 4 compounds exhibited superoxide-scavenging activity with IC_{50} values in the range of 0.51 to 1.1 mcM with the most active compound being xanthokeismins A. This activity is comparable to that of resveratrol (IC_{50} 5.3 mcM). Xanthoangelol and ascorbic acid had little activity in this assay, with IC_{50} 's greater than 40 mcM.

Anti-Cancer

An ethyl-acetate soluble fraction of an ethanol extract was shown to have anti-cancer activity in the Lewis lung carcinoma mouse model. Xanthoangelol and 4-hydroxyderricin were also active in this model. Mechanistic studies indicated that xanthoangelol may inhibit tumor angiogenesis and 4-hydroxyderricin may have ameliorating effects on the immune system. Xanthoangelol and 4-hydroxyderricin inhibited tumor promotion in a two-stage mouse skin carcinogenesis assay. Xanthoangelol and 4-hydroxyderricin have also been shown to induce apoptosis (cell death) to cancer cells.

Anti-cancer – Animal studies

An ethyl-acetate soluble fraction of the 50% ethanol extract was tested for anti-cancer activity in Lewis lung carcinoma (LLC)-bearing mice. The extract inhibited tumor growth in LLC-bearing mice at a daily dose of 100 mg/kg, prolonging survival time and inhibited metastasis to the lung after surgical removal of the primary tumors. Two active substances were identified: xanthoangelol and 4-hydroxyderricin (Kimura and Baba

2003). Xanthoangelol (50 mg/kg) and 4-hydroxyderricin (50 mg/kg twice daily) orally inhibited tumor growth in subcutaneously LLC-implanted mice; prolonging survival time and inhibited metastasis to the lung after surgical removal of the primary tumors (Kimura et al. 2004). Mechanistic studies revealed that xanthoangelol and 4-hydroxyderricin inhibited DNA synthesis in LLC cells at concentrations of 10 and 100 μ M, respectively. These compounds had no effect on DNA synthesis in human umbilical vein endothelial cells, indicating a selective effect on cancer cells. Xanthoangelol inhibited tumor-induced neovascularization in vivo at doses of 10 and 20 mg/kg and inhibited Matrigel-induced formation of capillary-like tubes by endothelial cells at concentrations of 1 to 100 μ M. Xanthoangelol also inhibited binding of vascular endothelial growth factor (VEGF) to endothelial cells. 4-hydroxyderricin inhibited Matrigel-induced formation of capillary-like tubes by endothelial cells at concentrations of 10 to 100 μ M. In addition, 4-hydroxyderricin appeared to affect the immune system by ameliorating the reduction in the number of lymphocytes and natural killer cell in the spleen of the mice who had had their tumors removed (Kimura et al 2004; Kimura & Baba 2003).

Xanthoangelol and 4-hydroxyderricin were shown to inhibit tumor promotion in a two-stage mouse skin carcinogenesis test using DMBA (7,12-dimethylbenzylanthrane) as an initiator and TPA (12-O-tetradecanoylphorbol-13-acetate as a promoter (Okuyama et al. 1991). Isobavachalcone exhibited inhibitory effects on skin tumor promotion in the same model (Akihisa et al. 2006).

Anti-cancer – In vitro

Chalcones, coumarins and flavanones isolated from the ethyl acetate-soluble extract were tested for inhibition of tumor-promoter and tumor initiation activity in vitro. Two chalcones (xanthoangelol F and isobavachalcone) and six coumarins (lasepitin and others) showed potent inhibitory activity in both assays (Akihisa et al. 2003).

Six chalcones from *Ashitaba* were found to be cytotoxic in vitro to human neuroblastoma cells. Two of them, isobavachalcone and xanthoangelol H, had no toxicity to normal primary rat cerebellar granule cells (Nishimura et al. 2007).

Xanthoangelol and 4-hydroxyderricin induced apoptosis (cell death) in human stomach cancer KATO III cells in vitro (Takaoka S et al. 2008).

Xanthoangelol also induced apoptosis (cell death) in vitro in neuroblastoma and leukemia cells (Tabata et al. 2005). Further studies indicated that xanthoangelol induced caspase-3-dependent apoptosis in neuroblastoma cells. Xanthoangelol acted by increasing reactive oxygen species through targeting the DJ-1 protein. Xanthoangelol had a cytotoxic effect on both drug-sensitive (IMR-32 and SK-N-SH) as well as drug resistant cell lines (LA-N-1 and NV-39) (Motani et al. 2008).

Anti-Ulcer

Ashitaba may help to prevent gastric ulcers. Xanthoangelol and 4-hydroxyderricin inhibited gastric acid secretions in proton transport experiments in vitro. Xanthoangelol and 4-hydroxyderricin inhibited pig gastric hydrogen, potassium (H^+/K^+) ATPase with

IC₅₀ values of 1.8 and 3.3 mcM respectively. Xanthoangelol significantly inhibited the formation of gastric lesions in restrain-stressed Wistar male rats with an oral dose of 100 mg/kg. 4-hydroxyderricin was ineffective in this model with a dose of 200 mg/kg (Murakami et al. 1990).

A preparation of Ashitaba sap is reported to have anti-microbial activity against the bacteria associated with ulcers: *Helicobacter pylori*. The minimum inhibitory concentration was reported at 0.5 mg/ml (US patent application 0050186292; Fukuo, Y and Satoh K)

Antibacterial

Xanthoangelol and 4-hydroxyderricin had antibacterial activity against gram-positive pathogenic bacteria (Inamori et al. 1991)

Bioavailability

No data is available on the absorption of Ashitaba chalcones from the digestive tract (Nagata et al 2007)

V. Safety

Summary

The fresh leaves of Ashitaba are used as food and preparations of the plant have been used traditionally for medicinal purposes (Inamori et al 1991). JBSL Ashitaba Chalcone Powder (8% chalcone) is a concentrated preparation of the yellow sap of the plant. This preparation was determined to be non-mutagenic in the Ames assay with 4 strains of bacteria and in a chromosomal aberration study conducted in CHO cells. The Ashitaba Chalcone powder was tested for toxicity in Wistar rats given a single oral dose of 2,000 mg /kg body weight. As a result, there were no deaths or signs of gross pathology. Further, animal studies conducted with various preparations of Ashitaba did not report any signs of toxicity.

Mutagenicity profile

The mutagenic potential of JBSL Ashtiba Chalcone Powder (8% chalcone) was tested in five strains of bacteria: *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2uvrA. Ashtiba Chalcone Powder was tested with all strains in the absence and presence of a metabolic activation system (liver homogenate enzymes; S9-mix) in five concentrations ranging from 12.3 to 1000 mcg chalcones/plate. (Note: the concentration was calculated as the weight of the constituent chalcones not for the whole powder). Negative and positive controls were included. The tests were conducted in compliance with the OECD guideline adopted July 1997 and the Japanese Ministry of Health and Welfare guidelines, 1994. As a result, Ashtiba Chalcone Powder did not cause mutagenic effects, defined as a two-fold or more increase in the mean number of reverting colonies. The test could not be conducted with bacterial strain TA100 due to toxicity caused to the bacteria by the powder. The authors concluded that Ashtiba Chalcone Powder was not mutagenic under the conditions of this study (Krul C.A.M 2002).

Chromosomal aberration tests were conducted using Chinese hamster ovary (CHO) cells. JBSL Ashtiba Chalcone Powder was tested at concentrations from 10 to 2500 mcg/ml; with and without the S9-mix. The first test included a pulse treatment of 4 hours (with and without S9-mix) with harvesting of the cells after 18 hours and continuous treatment for 18 hours (without S9-mix). The second test was performed in the absence of S9-mix for 18 and 32 hours continuously. Both positive and negative controls were included. In both tests, higher concentrations of Ashtiba Chalcone Powder caused inhibition of cell growth, an indication of toxicity. Thus, chromosomal aberration tests were performed at concentrations lower than those that caused this effect. Tests with Ashtiba Chalcone Powder at concentrations that were not toxic to the cells found no significant increases in the number of aberrant cells and thus no metagenicity. The authors concluded that Ashtiba Chalcone Powder did not induce any genotoxic effects but did exhibit cytotoxicity (de Vogel N 2003).

Animal Studies

Acute Single-Dose Study

JBSL Ashitaba Chalcone powder (8% chalcone) was tested for toxicity in Wistar rats given a single oral dose. A group of 6 rats (3 male and 3 female) was given 2,000 mg /kg bodyweight by oral gavage. The animals were observed for 14 days and at the end of that time they were examined for gross pathology. The study methodology was based upon guidelines established by the EC Directives on acute oral toxicity (1996; 2001). As a result of treatment, there were no deaths. Normal bodyweight gains proceeded during the observation period and no abnormalities were observed at necropsy. The oral LD50 is therefore greater than 2000 mg/kg (Prinsen MK 2002).

Short-term Observations in Efficacy Studies

A rat study in which the animals were fed a high fat diet in addition to a large amount (1700 mg/100g body weight) of a Ashtiba powder containing chalcones (318.9 mg/100g product) and dietary fiber (31.2g/100g product) for 28 days reported no pathological effect on liver or kidneys as measured by serum biochemical indices and histopathological examinations (Nagata et al 2007).

A study in SHRSP rats that were fed a diet containing 0.2% extract (ethyl acetate: water (4:1) of the stem sap (chalcone content not given) for 6 weeks produced no differences in growth rate or food intake. There were also no differences in liver enzymes measured in the plasma of the animals (Ogawa et al 2003).

Male genetically diabetic KK-Ay mice fed a diet with 0.15% of 4-hydroxyderricin for 7 weeks did not exhibit any side effects (Enoki et al 2007).

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The logo for Japan Bio Science Laboratory features a stylized, light blue figure of a person with arms and legs outstretched, forming a large 'J' shape. The figure is composed of several overlapping light blue shapes. Below the figure, the text "Japan Bio Science Laboratory" is written in a light gray, sans-serif font.

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