Literature review on Pharmaceutical activities of Radix Achyrantes bidentatae

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Abstract

Pharmaceutical activities of radix Achyrantes bidentatae (RAB) were reviewed and summarized until September 2007. From the review, RAB has analgesic, anti-inflammatory, blood circulation invigoration, stagnant blood clearing, anti-stomach ulcer, secretion of bile enhancement, anti-procreate and anti-implantation, blood glucose level reducing, lipoprotein reducing, protein assimilation increasing, anti-tumor, memory and endurance improvement, anti-aging, bone growth promotion, bone resorption inhibition, anti-asthmatic and hepatoprotective activities. It also enhances immune system by macrophrages activation, increase in monocytes, activation of natural killer cells, suppress in spleenocyte, T-cells proliferation, induce secretion of IL-2, increasing C3bBb and ICR in immunosuppressed and normal mice peripheral blood, suppress in B-lymphocyte and immunoglobulin, with low toxicity. OA has hepatoprotective, anti-stomach ulcer, hypoglycemic, anti-hyperlipidemic, anti-hypertensive, cardiotonic, anti-dysrhythmic, anti-aggregation of blood platelet, anti-cancer, protection of renal toxicity, anti-inflammatory, anti-mycobial and anti-fertility activities with low toxicity from another published review [106].

Introduction

Achyrantes bidentatae is a perennial herb with 70-120 cm tall. It has green or tinged purple, angulate or quadrangular, appressed or spreading pubescent or nearly glabrous stems. Its branches are opposite. It has hairy petioles which are 0.5-3 cm long. Leaf blades are elliptic or elliptic-lanceolate with surface area of 4.5-12 x 2-7.5 cm^2 and rarely oblanceolate. Leaf blades also have annexed or spreading pubescent on both surfaces, cuneate or broadly cuneate base which are caudate. It also has terminal or axillary spikes of 3-5 cm long, white hairy rachis of 1-2 cm long and dense flowers of 5 mm long. The bracts are 2-3 mm long, reflexed after anthesis, broadly ovate and apex acuminate. It has spiny, two-parted base and apex curved bracteoles of 2.5-3 mm long. It also has shiny and lanceolate tepals of 3-5 mm long with apex acute midvein. The stamens are 2-2.5 mm long. Pseudostaminodes are slightly serrulate and apex rounded. Utricles are yellowish brown, shiny, oblong and smooth with 2-2.5 mm long. Seeds are light brown which are oblong with 1 mm thick. Florescence period: Jul-Sep, fruiting period: Sep-Oct [1].

Achyrantes bidentatae is grown at hillsides 200-1800 m above seashore. It can be found in Anhui, Fujian, Hebei, Hunan, Guangxi, Guizhou, Hubei, Jiangsu, Shaanxi, Shanxi, Sichuan, Taiwan, Xizang, Zhejiang, Bhutan, India, Indonesia, Japan, Korea, Laos, Malaysia, Myanmar, Nepal, New Guinea, Philippines, Russia, Sikkim, Thailand and Vietnam [1].

This review shows pharmaceutical activities of the root of *Achyrantes bidentatae*, radix *Achyrantes bidentatae* (RAB).

Pharmaceutical activities of RAB

Traditionally, RAB is used for nourishing liver, kidney, bone and tendons. It is also used as analgesic, diuretic and hypotensive agent. It is used to invigorate blood circulation and clear stagnant blood flow. It can strengthen bones by nourishing liver and kidneys. It is also used to descend the

flow of "Blood" and "Qi". As a blood and liver remedy, it is included in remedies for menstrual problems. It is more commonly used for pains in the back and lower limbs [1-5].

Evidence-based researches for pharmaceutical activities of RAB were reviewed and summarized as follow [6-14]:

Analgesic properties

Effective dosage (ED_{50}) of RAB extract on intramuscular injection (im) of formaldehyde-induced pain on mice by oral administration (po) was determined to be 1.8-5.7 g/kg [15]. With the same dosage, mice recovered in 10 minutes by RAB extract, compared with 20 minutes in other CHM extracts [15]. Analgesic effect of different processed products of RAB in mice were observed in hot plate and acetic acid-induced writhing test. Different processed product had analgesic activity and the wine-processed RAB extract had the most efficacies [16]. Achyranthes bidentata polysaccharides (ABPS) 30, 60, 120 mg/kg by smearing relieved ethanoic acid-induced and hot plate-induced pain in mice [17-18].

Anti-inflammatory activities

RAB extract could obviously inhibit croton fruit oil-induced ear inflammation of mice. Anti-inflammatory effect of wine-processed RAB was the most powerful [19]. RAB extract (sc) inhibited the croton fruit oil and formaldehyde-induced ear inflammation of mice [20]. Fifty g of RAB water extract applied to gonarthritis' patient 30 min./time for 2 times/day. The patient recovered for treatment after ten days [21].

Wine-processed RAB of 5-10 g/kg (po) treated albumen-induced inflammation of leg [22-23]. Five g/kg/day (po) for 5 days cured formaldehyde-induced inflammatory joint of leg [63]. Achyranthes bidentata saponins (ABS) 30, 100, 300 mg/kg (po) inhibited *p*-xylene-induced inflammation of ears of mice and albumen-induced inflammatory of legs of mouse [24].

Invigorate blood circulation

Water extract of RAB caused dilation of arteries on legs of mouse and increased arterial pressure on legs of mouse [25]. In addition, water extract and ethanol extract of RAB inhibited adrenalin-induced vasoconstriction [25]. Intravenous injection (iv) of RAB extract reduced and then raised blood pressure of rabbit. Blood pressure of rabbit after injection for 1 hour was lower than before injection [26]. ABPS 0.3 mg/kg (iv) reduced blood pressure of rabbit [27]. RAB extract caused dilation of ear blood capillaries of rabbit to enhance blood flow *in-vitro* [29]. RAB extract 5 g/kg (po) increased number of dilated blood capillaries in mice and inhibited 0.8 mg/kg/time for 2 times (sc) adrenalin-induced vasoconstriction [30].

Clearing stagnant blood

Extract of RAB reduced blood viscosity and density of red blood cells, and increased international normalized ratio (INR) and prothrombin time (PT) [31]. ABPS could reduce blood coagulation time (CT) and PT after oral administration for 1 hour. This means it can improve blood circulation for non-woulded mouse. It could also increase the number of blood platelet in serum [32]. This means it could increase rate of blood stasis and wound healing when the mouse had a cut.

Anti-stomach ulcer activities

Intravenous injection (iv) of RAB extracts 0.15-0.4 g/kg on dog and rabbit could stimulate peristalsis but *in-vitro* study showed inhibit on peristalsis in mice intestine [33]. 1.2×10^{-3} g/ml of RAB stimulated peristalsis in rabbit intestine *in-vitro* [27].

Increasing secretion of bile

EDS 5 mg/kg/day (po) for 7 days increased secretion of bile in mice and increase cholic acid and bilirubin contents and reduced cholesterol content. It also increased the rate of liver recovery on inflammation induced by heliotrine [34].

Anti-procreate and anti-implantation activity

Intragastric injection of ABPS 300 mg/kg had anti-procreate effect and anti-implantation effect on mouse [35]. Oral administration of 0.5, 2.0, 4.0 g/kg/day of RAB butanol extract for 15 days for before fertilization of mice had no effect on sperm density, mobility and abnormality of male mice. The weight, length, diameter and weight/body ratio of male mice were also not affected. The pregnant ratio, termination of pregnancy and number of embryo implantation and viable fetuses per pregnant female mice

were not been affected [36]. Oral administration of 0.5, 1.0, 1.5, 2.0 ml /day for 7 days of RAB extract had anti-implantation effect on mice. The number of mast cell in the transverse section of uterus of mice also increased with RAB extract (po) [37]. ABS 0.125 mg/ml to 1.0 mg/ml (po) excited the uterine smooth muscle of mice *in-vivo*. The contraction rhythm, frequency and amplitude were increased [38]. The effect was more significant in old mouse than young mice [39]. The exciting action of RAB on the uterine smooth muscle in virgin rats partly contributes to α adrenergic receptor and H_1 histamine receptor, which exist in the membrane of the uterine smooth muscle cell [40]. ABS 60, 120, 240 mg/kg (po) for 5 days has proportional relationship on anti-procreation and anti-implantation activity on mice [41]. One to five days after fetation, ABPS 500 mg/kg (po) had anti-implantation effect on mice. But ABPS 250 and 500 mg/kg (po) had no anti-procreation effect on mouse. Fetation of 2 g/kg/day (po) for 14-19 days had no anti-procreation effect on mouse [42]. ABPS 0.5 mg/ml (po) to rabbit. After 1-4 minutes, the contraction of uterus was vigorous but released after 25 minutes [38]. ABS 0.06, 0.12, 0.24 g/L (po) to rabbit caused excitation of uterine smooth muscle. The amplitude, frequency and rhythm of contraction were proportional to concentration of ABS intake [43].

Reducing blood glucose level

ABPS 1.0, 0.5, 0.25 mg/kg/day (po) for 14 days had no significant effect on the blood glucose of normal mice, but could markedly decreased blood glucose at alloxan-induced and adrenalin-induced diabetic mellitus mice and increased the amount of hepatic glycogen of alloxan mice [44]. Oleanolic acid (OA) reduced blood glucose of adrenalin-induced, glucagon-induced and alloxan-induced diabetic mellitus mice [45]. EDS and inkosterone had no significant effect on the blood glucose of normal mice, but could markedly decrease it at glucagon-induced diabetic mellitus mice for 0.1-10 mg/kg (ip) or 1-100 mg/kg (po) [46-47]. EDS reduced blood glucose of adrenalin-induced, glucagon-induced diabetic mellitus mice but it required the presence of insulin. It stimulated the formation of glycogen from glucose in the liver of mice [48].

Reducing lipoprotein

RAB reduced total cholesterol (TC), triglyceride (TG) and lipid peroxide (LPO) levels of quall [49]. EDS 20 mg/day (po) for 12 weeks can inhibit cholesterol induced high TC, TG and LPO [51]. EDS 10 mg/kg (po) in rabbit can inhibit WR-1339 induced high TC, TG and LPO contents [51].

Increasing protein assimilation

Injection or oral administration of EDS or inkosterone greatly increased the protein and RNA in the liver of mice [48]. Glycogen in liver and skin of mouse greatly increased by EDS [52].

Effect on immune system

Effect on macrophages

Activation of macrophages

Lactate dehydrogenase (LDH) and acid phosphatase (ACP) are the characteristic enzymes of macrophages. The activities of LDH and ACP increased with the activation of macrophages, and vice versa [53-54]. The activities of lactate dehydrogenase and acid phosphatase in thoracic cavity macrophages were increased induced by ABPS after the macrophages were cultured with 0.312 mg/ml to 5.000 mg/ml ABPS for 24 hours *in-vitro*. Expression levels of TNF-α and IL-6 in the macrophages induced by ABPS increased markedly too, and expression of TNF-α had dose-dependent relation [55]. ABPS could induce secretion of IL-1 and TNF-α of macrophages. It enhanced induction of synthesis and secretion of IL-1 by 5 μg/mL lipopolysacharrides but no effect on induction of secretion of TNF-α by 10 μg/mL lipopolysacharrides. ABPS 200 μg/mL enhanced secretion of IL-1 with the maximum after 24 hours *in-vitro*. ABPS 25, 50 mg/kg (ip) could increase lipopolysacharrides induced secretion of IL-1. One hundred mg/kg (ip) could induce secretion of TNF-α with comparable results with BCG [56]. Sixty % of ethanol extract of RAB 2.5-10 mg/ml caused apoptosis to BGC823 stomach cancer cell by blocking G_0/G_1 cell cycle *in-vitro*. It also stimulated the proliferation of macrophage and enhanced its phagocytosis but also improved the production of TNF-α and

IL-6 [57]. ABPS 50-800 μg/ml did not inhibit growth of S_{180} tumor cells *in-vitro* but increased the activity of macrophages to inhibit S_{180} tumor cells *in-vivo* [58-59]. ABPS increased activity of macrophages to inhibit S_{180} tumor cells *in-vitro* [60]. ABPS increased synthesis of IL-1 and secretion of TNF-α of macrophages *in-vitro* but could not increase synthesis of IL-1 by LPS-induced suppression *in-vitro* [61]. ABPS 25, 50 mg/kg (ip) increased IL-1 synthesis on LPS-induced suppression on mice. One hundred mg/kg (ip) increased TNF-α synthesis [62].

Effect on monocytes

ABPS induced increasing of lysosomes number and plasma level, phago-ability of monocytes (CD_{14}^+ number cell of PBMC) with no proliferation. It could up-regulate $HLA\text{-}DR_\alpha$ surface molecules expression of the monocytes in dose and time-dependent manner *in-vitro* [63-64]. The phagocytosis and number of lysosomes were increased induced by ABPS after the monocytes were cultured with 0.312 mg/ml to 5.000 mg/ml ABPS for 12 hours *in-vitro*. ABPS could significantly induce the expression of TNF-α and IL-6 in monocytes [65].

Effect on natural killer cells

Activation of natural killer (NK) cells

NK cells are a form of cytotoxic lymphosyte which constitue a mojor component of the innate immune system. NK cells play a major role in the host-rejection of both tumors and virally infected cells. ABPS 60 mg/kg/day (ip) for 10 days significantly increased the activity of NK in immunosuppressed and normal mice peripheral blood [66]. ABPS 50-800 mg/L could activate NK cells *in-vitro* [62]. ABPS 50 mg/kg/day (ip) for 7 days could increase activity of NK cells on S_{180} tumor mice from 22.5 ± 8.0 % to 49.7 ± 6.6 % [67]. ABPS 50-800 mg/L could increase activity of NK cells in mice *in-vitro*. ABPS 100 mg/kg/day (ip) for 5 days could increase activity of NK cells from 14.0 – 39.3 % [58]. ABPS 100 mg or 200 mg/kg/day (ip) for 8 days could increase cancericidal activity of NK and LAK cells on H_{22} liver cancer cells-mediated mice [68]. Given their strong cytolytic activity and the potential for auto-reactivity, natural killer cell activity is variety of forms, the most important of which are listed below.

Effect on Spleenocyte

ABPS 5-50 mg/kg/day (ip) for 5 days or 250-1000 mg/kg/day for 5 days enhanced Con A induced proliferation of spleenocyte [61]. ABPS 0.05-0.8 g/L proliferated murine spleenocytes *in-vitro* [69]. ABPS 50, 100 mg/kg/day (ip) for 6 days increased activity of TNF-β protein of spleenocytes. ABPS 5-50 mg/kg/day (ip) for 5 days or 250-1000 mg/kg/day (po) for 5 days increased activity of Con A suppressive spleenocyte [70].

Effect on T-cells

Proliferation

ABPS prolifered T-cell directly but it proliferated stimulated T-cells which were stimulated by IL-2 [70-71].

Induce secretion of TNF-α protein

Tumor necrosis factor (TNF) refers to a group of cytokines family which can cause apoptosis. TNF-*α* is a cytokine involved in systemic inflammation and is a member of a group of cytokines that all stimulate the acute phase reaction. TNF causes apoptotic cell death, cellular proliferation, differentiation, inflammation, tumorigenesis, and viral replication. However, dysregulation on overproduction of TNF have been implicated in a variety of human diseases as well as cancer. ABPS 60 mg/kg/day (ip) for 10 days significantly increased the activity of TNF in immunosuppressed and normal mice peripheral blood [66].

Induce secretion of TNF-β protein

ABPS 50, 100 mg/kg (ip) raised the synthesis of TNF-β protein and enhanced the secretion in Con A induced suppression of TNF-β protein [62].

Induce secretion of IFN-γ proteins and anti-induce secretion of IL-4 by human T-cell

In-vitro study showed ABPS can induce secretion of IFN-γ protein, and the effect is time and dose dependent from 100-400 μg/ml and the maximum level was obtained at 400 μg/ml, and can anti-induce the expression of IL-4 protein [73]. *In-vivo* study showed positive rate of IFN-γ mRNA expression in PBMC from patient with asthma or lung cancer increased from 6/25 to 14/25 and from 3/22 to 10//22.

Positive rate of IL-4 mRNA expression dropped from 17/25 to 9/25 and from 14/22 to 5/22. There was significant correlation between IFN-γ mRNA or IFN-γ protein and the concentration of ABPS, respectively. The optimal stimulation concentration of ABPS were 400 mg/L or 800 mg/L and IL-4 protein was restrained. ABPS could rectify the unequilibrium of Th1/Th2 cytokines in PBMC from patient with asthma or lung cancer. It induces IFN-γ secretion in a time and dose dependent manner. ABPS may up-regulate Th1-type cytokine and down – regulate Th2 – type cytokine at transcriptional and translational levels [74].

Induce secretion of IL-2

ABPS 60 mg/kg/day (ip) for 14 days induced secretion of IL-2 on S_{180} tumor cell-medicated mice [75]. ABPS 100, 200 mg/kg/day (ip) for 8 days can increase IL-2 synthesis of H_{22} tumor cells-mediated mice [76].

Enhance CD_3 and CD_4 expressions on T-lymphocyte cell and decrease CD_8 expression on T-lymphocyte cell.

Cluster of differentiation (CD) is a protocol used for the identification of investigation of cell surface molecules present on leukocytes. CD molecules can act in numerous ways, often acting as receptors or ligands (the molecule that activates a receptor) important to the cell. A signal cascade is usually initiated, altering the behavior of the cell. Some CD proteins do not play a role in cell signaling, but have other functions, such as cell adhesion.

CD_3 antigen is a protein complex composed of 4 distinct chains (CD_3-γ, CD_3-δ and 2 x CD_3-ε), in mammals, that associate with molecules known as T cell receptor (TCR) and ε-chain to generate activation signal in T-lymphocytes. CD_4 is a glycoprotein expressed on the surface of T helper cells, regulatory T cells, monocytes, macrophages, and dendritic cells. On T cells, CD_4 is the co-receptor for TCR. It amplifies signal generated by TCR by recruiting tyrosine kinase that is essential for activating many molecules involved in the signaling cascade of an activated T-cell.

CD_8 is a transmembrane glycoprotein which serves as a co-receptor for TCR. It binds to a major histocompatibility complex (MHC) molecule, but is specific for the class I MHC protein. It is predominantly expressed on surface of cytotoxic T cells, but can also be found on natural killer cells [77].

ABPS 60 mg/kg/day (ip) for 14 days increased the percentage of CD_3 and CD_4 in S_{180} tumor cell-medicated mice peripheral blood and decrease CD_8 in mice peripheral blood [78]. The activation of CD_4^+ T-lymphocyte can lead to activate T-helper cells and T-cells to secrete IL-2, IFN and TNF and modulate MHC II so as to inhibit tumor and virus growth.

Effect on Red Blood Cells

Increased C3bBb and ICR in immunosuppressed and normal mice peripheral blood.

In immunology, soluble C3-convertase catalyzes the proteolytic cleavage of C3 into C3a and C3b as part of the alternative complement system. C3b may bind to microbial cell surfaces within an organism's body. This could lead to the production of surgace-bound C3 convertase and thus more C3b components. Also known as C3bBb, this convertase is similar to soluble C3-convertase except that it is membrane bound. Alternatively, bound C3b may aid in opsonization of the microbe by macrophages. ABPS 60 mg/kg/day (ip) for 10 days significantly increased C3bBb and ICR in 80 mg/kg (ip) cycophosphamide-induced immunosuppressed and normal mice peripheral blood [78].

Effect on B-lymphocyte

ABPS 50 mg and 100 mg/kg (ip) activated LPS (10 μg/kg, ip) - induced suppressive B-lymphocyte *in-vivo* [69].

Effect on immunoglobulin (Ig)

ABPS 50 mg/kg/day (ip) for 5 days raised total IgG and IgM levels in serum. ABPS 50 and 100 mg/kg/day (ip) for 5 days had significant antagonistic effect on cyclosporine A-induced suppression of plague forming cell and IgG generation [69]. ABPS 50 mg/kg/day (ip) for 17 days increased serum IgG from 19 ± 5 to 40 ±10 mg/ml [67].

Anti-tumor effect

In-vitro study showed ABPS 10^{-2} g/L had 100 % inhibition on EAC tumor cells after 48 hours and 10^{-1} g/L had 100 % inhibition on EAC tumor cells after 24 hours. ABPS 5.0

mg/kg/day (po) for 7 days had 56 % inhibition on sarcoma 180 tumor cells and 2.5 mg/kg/day (po) for 7 days had 46.2 inhibitions on Heps tumor cells in mice *in-vivo* [79]. ABPS 25-100 mg/kg/day (po) for 7 days had 31-40 % inhibition on the growth of S_{180} tumor cells on mice [60]. ABPS 1-2 μg/ml (ip) could inhibit growth of S_{180} and K_{562} tumor cells on mice *in-vivo* [67]. ABPS 50-800 μg/ml did not inhibit growth of S_{180} tumor cells *in-vitro* but increased activity of macrophages to inhibit S_{180} tumor cells *in-vivo* [58-59]. ABPS increased activity of macrophages to inhibit S_{180} tumor cells *in-vitro* [60]. ABPS 50 mg and 100 mg/kg/day (ip) for 7 days increased activities of TNF-β and lymphokine activated killer (LAK) cells [67].

Memory and endurance

RAB water extract 20 g/kg (ip) improved the memory and endurance of mice in which the memory restricted mice were induced by 10 g/kg (ip) of amobarbital sodium [80].

Anti-aging effect

Harman discovered the free radical theory in 1956 [82]. Experiment showed aging related to free radical theory [82]. Free radical reaction included electron enters the oxygen molecules to form oxygen free radical ($O_2^{\cdot-}$), superoxide dismutase (SOD) reacts with O_2 to form H_2O_2, H_2O_2 reacts with $O_2^{\cdot-}$ to form hydroxide radical ($OH^{\cdot}$). $O_2^{\cdot-}$ and $OH^{\cdot}$ can form other radicals such as lipid radical and lipid peroxide by chain reaction. These free radicals can form malondialdehyde (MDA) and cause cellular membrane damage. MDA contents can reflect the level of lipid peroxidation in our body. Free radicals can be divided into activated oxygen free radicals ($O_2^{\cdot-}$, $OH^{\cdot}$ and H_2O_2) and lipid radicals. (LO, LOO and LOOH). Fortunately, our body contains SOD and catalase which scavenge ultra-oxygen anion free radical and hydrogen peroxide and may inhibit lipid peroxidation. Lipid radicals can be inhibited by GSH-Px, Se, vitamins C and E. With increasing ages, MDA content in body increases and GSH-Px, SOD and catalase activities decreases, and the free radical chain reactions are becoming more generous. These free radical chain reactions can cause damages to body's cells and cause the degradation of cells' functions, leading to aging [83].

Oral administration of 20 % water extract of RAB 0.3 ml/day for 45 days to mice increased SOD and GSH-Px activities with the decreased in lipid peroxide (LPO) activity [84].

ABPS 2-5 mg/g increased lifespan of drosophila by 2.61 % - 3.16 % [85].

Promote bone growth and inhibition on bone resorption

75% EtOH extract of RAB prolifered osteoblast-like UMR 106 cells by 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide (MTT) assay. Ethyl acetate and petroleum ether extract of ethanoic extract of RAB prolifered osteoblast-like UMR 106 cells [86]. EDS prolifered osteoblast-like UMR 106 cells by MTT assay [87]. Methanol extract of RAB had inhibitory effect on osteoclast formation at 44 μg/mL. ABPS had inhibitory effect on $1\alpha,25(OH)_2D_3$-induced tartrate-resistant acid phosphatase-positive osteoclast formation [88]. RAB extract 7.1 g/kg and 14.2 g/kg (po) prevented calcium and phosphorus missing within bone of osteoporosis rats induced by 70 mg/kg retinoic acid. It also increased voluntary activity and bone density of the treated rats [89].

Treatment of Asthma

Asthma is a chronic disease of the respiratory system in which the airway occasionally constricts becomes inflamed, and is lined with excessive amounts of mucus, often in response to one or more triggers. Eosinophils (EOS) play an important role on this inflammation process [90]. EOS could accumulate at the bronchitis of asthmatic person and secrete inflamed substance which damaged the epithelial cells and triggers secretion of mucus. EOS apoptosis is one of the solutions to airway inflammation in asthma [91]. In asthmatic rat, the expressions of the genes fas and bcl-2 mRNA in EOS were changed evidently and the ratio of EOS apoptotosis reduced greatly. ABPS 50 mg/kg/day (ip) in asthmatic rats could enhance the apoptosis of EOS by upregulating the expression of the genes fas and bcl-2 mRNA [92].

Signal transducer and activator of transcription 6 (STAT6) proteins and its mRNA were found strongly in bronchus of asthma rat model, the epithelial cells were the chief expression cells. ABPS 50 mg/kg (ip) had an inhibitory

effect on airway inflammation cells infiltration such as esinophils (EOS), it significantly depressed STAT6 and its mRNA expression, and thus reduced the synthesis of IL-4 might be the key in modulating mechanism of asthma [94]. The reduction of the synthesis of IL-4 and the raise of synthesis of IFN-γ by intraperitoneal injection of ABPS can modulate the imbalance of Th1/Th2 [94]. Clinical study showed that oral administration of ABPS has 83.3 % improvement on infantile recurrent respiratory infection [95]. Peripheral blood mononuclear cell (PBMC) from patient with asthma or lung cancer.

Hepatoprotective

Small dose (8.1 % water extract) of RAB could significantly elevate activity of SOD and/or lower the T/K ratio, markedly reduce MDA content and significantly decreased the activities of ALT and AST for the mild chronic hepatic damage induced by carbon tetrachloride, demonstrating that it is effective in combating oxygen free radicals in chronic liver damage. But high dose (32.4 % water extract) of RAB could raise the ALT, AST, TXB_2 and T/K levels. This means high dose of RAB could cause liver damage [96]. Degradation of CCl_4 and alcohol and ishemiareperfusion in liver are all factors that may induce production of OFR and lead to liver damage. LPO is produced in lipid peroxidation and its level is closely correlated to the extent of hepatic dysfunction. MDA is the main degradation product of LPO that may seriously damage the cellular membrane and reflect the degree of cellular membrane damage caused by activated oxygen. SOD is the scavenger of ultra-oxygen anion free radical and may inhibit lipid peroxidation. The small dose of RAB significantly decreased MDA level; ALT and AST activities were not accompanied with the marked increase of SOD activity indicate that there must be other route in decreasing the LPO level.

Thromboxane A_2 (TXA_2) and prostaglandin I_2 (PGI_2) are important bio-active regulators of vascular tension. Their half-lives in the body are very short, and will be quickly decomposed into the stable degradation products of TXB_2 and 6-keto-PGF1α. In liver damage induced by CCl_4, the increased TXB_2 and T/K ratio will cause vascular constriction, leading to decreased blood flow in the liver and the liver will be ischemic and anoxic. The neutrophils will be activated and produce a large amount of OFR, which conversely enhances lipid peroxidation. In the study the significant decrease of TXB_2 and T/K ratio along with sig. decrease in MDA, ALT and AST was found in small dose RAB but a reverse for large dose. So drugs for activating blood flow should be preferably prescribed at small doses in treating mild chronic hepatitis.

Toxicity

N-butanol extract 2.0 and 4.0 g/kg/day for 15 days (po) on mice had acute inflammatory response on spleen, liver and kidney but no inflammatory response at 0.5 g/kg [97]. LD_{50} (ip) of EtOH extract of RAB on mice were determined to be 1.277-3.531 g/kg. The maximum tolerance level is greater than 50 g/kg [98].

LD_{50} (ip) of ABPS on mice were determined to be 18.87-13.27 g/kg [99].

LD_{50} (ip) and LD_{50} (po) of ABPS on mouse were determined to be 1.5 g/kg and 20 g/kg respectively. ABPS 1, 2 and 5 g/kg/day (po) for 90 days on mouse did not have any abnormal effect [100].

LD_{50} of EDS and inkosterone on mice were 6.4 g/kg and 7.8 g/kg (ip) respectively [46].

LD_{50} of water extract of RAB on mice is 146.49 g/kg (po). Seventy-five g/kg/day (po) for 3 days did not have any abnormal effect. Sixty g/kg/day (po) for 7 days and 48 g/kg/day (po) for 30 days did not have any abnormal effect [27].

When saponins are consumed, the sugar molecule is usually cleaved off by enzymatic action either in the gut or in the blood stream [101]. ABPS or OA saponins in RAB may be cleaved off to OA and polysaccharides when consumed. OA has hepatoprotective, anti-stomach ulcer, hypoglycemic, anti-hyperlipidemic, anti-hypertensive, cardiotonic, anti-dysrhythmic, anti-aggregation of blood platelet, anti-cancer, protection of renal toxicity, anti-inflammatory,

anti-mycobial and anti-fertility activities with low toxicity from another published review [106].

In addition, RAB contains trace elements which were reviewed and tabulated (Table 1 and 2).

Recommended nutrient intake (RNI) or adequate intake (AI) and tolerable upper intake level (TUIL) for different trace elements were reviewed and tabulated (Table 3). It also showed the usage and symptoms of overusing different trace elements [102-103].

	Metal contents (μg/g)						
Source of samples	**Fe**	**Cu**	**Mn**	**Zn**	**Co**	**Cr**	**Ni**
Henan Wubu (河南武陟)	269	5.04	78.36	22.51	0.535	0.672	0.125
Sichuan Daxuan (四川達县)	347	9.06	35.69	23.43	0.385	0.838	1.50
Hebei Shenze (河北深澤)	218	3.53	48.13	10.81	0.321	0.531	0.693

Table 1 Table showing metal contents of RAB done by Lau F. L. in 1988 [104].

	Metal contents (μg/g)						
Source of samples	**Hg**	**As**	**Cu**	**Pb**	**Cd**	**Zn**	**Fe**
Henan Wubu – first class medicine (河南武陟一等藥材)	0.042	0.40	5.49	ND	0.075	28.28	161.56
Henan Wubu – first class unprocessed medicine (河南武陟一等生品)	0.052	0.46	18.18	ND	ND	23.79	91.42
Hebei Anguo – medicine (河北安國藥材)	0.063	0.34	15.80	ND	ND	13.97	354.63
Hebei Anguo – unprocessed medicine(河北安國生品)	0.114	0.93	34.10	ND	0.226	16.38	414.75
Shanxi bingcheng – medicine (山西芮城藥材)	0.020	0.24	5.41	ND	ND	36.16	97.89
Shanxi bingcheng – unprocessed medicine (山西芮城生品)	0.054	0.26	14.90	ND	0.164	15.60	307.50
Normal unprocessed medicine (普通包裝生品)	0.065	0.60	16.19	ND	ND	12.50	420.88
Henan Wubu – wine-processed medicine (河南武陟酒炙)	0.068	0.39	22.88	ND	ND	17.57	331.13
Hebei Anguo – wine-processed medicine (河北安國酒炙)	0.013	1.34	7.93	ND	ND	50.79	155.38
Shanxi bingcheng – wine-processed medicine (山西芮城酒炙)	0.075	0.25	23..23	0.52	ND	11.83	151.55
Normal wine-processed medicine 普通包裝酒炙	0.082	1.35	16.23	ND	ND	21.00	231.18

Table 2 Table showing trace element analysis of RAB done by Zhang ZL [105].

	AIs	TUILs	Usage	Overuse
Ca /mg	300-1000	2000	Essential for formation and maintenance of bones and teeth (prevention of osteophoresis); contraction of muscles (including the heart muscle); supports normal nerve function; aids blood clotting; may reduce risk of colon cancer; body electrolyte.	Hypercalcaemia (anorexia, nausea, vomiting, constipation, abdominal pain, muscle weakness, mental disturbances, polydippsia, polyuria, nephrocalcinosis, renal calculi, (in severe cases) cardiac arrhythmias and coma)
P / mg	150-1000	3000-3500	Needed for strong bones and teeth; involved in helping the body release energy.	Local gastrointestinal irritation with intense thirst, pain, nausea, vomiting, and diarrhea. The breath may smell of garlic and vomitus and excreta are luminescent. Shock, delirium, convulsions, coma, and death may occur. Hepatic and renal damage, haemorrhage due to hypoprothrombinaemia and low fibrinogen concentrations, cardiovascular collapse,and CNS involvement including confusion, convulsions, and coma.
K/ mg	500-2000	8000	Body electrolyte; helps ransmit nerve impulses; contraction of muscles (including the heart muscle; may help maintain normal blood pressure.	Hyperkalaemia (paraesthesia of the extremities, muscle weakness, paralysis, cardiac arrhythmias, heart block, cardiac arrest, and confusion.)
Na/ mg	200-2200	5000-7000	Maintains fluids in body; helps in nerve transmission and muscle contraction; helps control rhythm of heart muscle.	Electrolyte imbalances; Pulmonary and peripheral oedema; Hypernatraemia (dehydration of brain which causes somnolence and confusion progressing to convulsions, coma, respiratory failure, and death. Thirst, reduced salivation and lachrymation, fever, sweating, tachycardia, hypertension or hypotension, headache, dizziness, restlessness, irritability, weakness, and muscular twitching and rigidity.
Mg /mg	30-35	200-700	Works in hundreds of chemical reactions in the body that metabolize food and transmit messages between cells; body electrolyte	Hypermagnesaemia (respiratory depression and loss of deep tendon reflexes, both due to neuromuscular blockage. Nausea, vomiting, flushing of skin, thirst, hypotension due to peripheral vasodilation, drowsiness, confusion,

				slurred speech, double vision, muscle weakness, bradycardia, coma, and cardiac arrest.
Fe /mg	0.3-25	10-50	Helps carry oxygen in bloodstream; essential for formation of red blood cells; Formation of haemoglobin in blood, muscle protein myoglobbin, enzymes and cytochromes.	Gastrointestinal irritation, notably vomiting and diarrhea. Cardiovascular disorders such as hypotension, metabolic changes including acidosis and hyperglycaemia, and CNS depression ranging from lethargy to coma. Gastrointestinal toxicity recurs together with shock, metabolic acidosis, severe lethargy or coma, hepatic necrosis and jaundice, hypoglycaemia, coagulation disorders, oliguria or renal failure, and possible myocardial dysfunction. Gastrointestinal obstruction and possibly late hepatic damage. Haemochromatosis (pigment deposition in skin and other organs, mild liver dysfunction, endocrine dysfunction (failure of adolescent growth spurt, hypogonadism, sometimes diabetes and hypothyroidism), and heart disease (pericarditis, heart failure, and arrhythmias).
I /μg (RNI)	50-150	800-1000	Essential constituent of the hormones produced by the thyroid. Prevent iodine deficieny disorders (enlargement of thyroid, endemic cretinism (a syndrome characterized by deaf-mutism, intellectual deficit, spasticity, and sometimes hypothyroidism), impaired mental function in children and adults, and an increased incidence of still-births as well as perinatal and infant mortality.	Hypersensitivity reactions (urticaria, angioedema, cutaneous haemorrhage or purpuras, fever, arthralgia, lymphadenopathy, and eosinophilia.) Iodism (metallic taste, increased salivation, burning or painful mouth; acute rhinitis, coryza-like symptoms, and swelling and inflammation of the throat.) Eyes may be irritated and swollen and there may be increased lachrymation. Pulmonary oedema, dyspnoea, and bronchitis. Skin reactions (acneform or, severe eruptions (iododerma).) Depression, insomnia, impotence, headache, gastrointestinal disturbances, notably nausea,

				vomiting, and diarrhea.
Zn /mg (RNI)	1.5-19	13-45	Used in sperm production; needed for growth and production of energy; helps immune function and blood clotting; activity of many enzymes. Prevention of growth retardation and defects of rapidly-dividing tissues such as skin, immune system, and intestinal mucosa.	Copper deficieny with associated sideroblastic anaemia and neutropenia; Gastrointestinal irritation (abdominal pain, dyspepsia, nausea, vomiting, diarrhea, gastric irritation, and gastritis.)
Se /μg	15-50	55-400	Works as an antioxidant (protects cells from damage); essential for healthy heart muscle; Needed for enzymes in red blood cells; Formation of glutathione peroxidase, which pretects intracellular structures against oxidative damage. Prevention of endemic form of cardiomyopathy, Keshan disease.	Loss of hair, nail changes, diarrhea, dermatitis, metallic taste, garlic odour of breath, irritability, fatigue, and peripheral neuropathy.
Cu /mg	0.4-2.0	1.5-8.0	Essential in formation of skin and connective tissure; needed for many chemical reactions related to energy; essential for heart function; associated with certain enzymes. Prevention of anaemia, neutropenia and bone demineralization.	Hepatotoxicity; haemolysis; haematological reacions with kidney involvement. Wilson’s disease (hepatolenticualr degeneration)
F /mg	0.1-1.5	0.4-3.0	Structure of bones and teeth; resistance of teeth to decay. Treatment of esteoporosis.	Salty or soapy taste, increased salivation, gastrointestinal disturbances, abdominal pain, weakness, drowsiness, faintness, and shallow brething. Hypocalcaemia, hypomagnesaemia, hyperkalaemia, tremors, hyperreflexia, tetany, convulsions, cardiac arrhythmias, shock, respiratory arrest, and cardiac failure. Skeletal fluorosis, manifestations (increased density and coarsened trabeculation of bone and calcification in ligaments, tendons, and muscle insertions. Bone pain, stiffness, limited movement, and in severe cases, crippling

				deformities. Prolonged excessive intake by children during the period of tooth developemtn before eruption can result in dental fluorosis characterized by mottled enamel.
Cr /μg	10-50	200-500	Works with insulin to convert carbohydroates and fat into energy.	Renal failure
Mn / mg	3.5-50	10	Associated with certain enzymes; proteins, DNA and RNA synthesis.	Cholestatic liver disease, and possibly changes in the basal ganglia; damage to central nervous systems
Mo /μg	15-60	110-800	Associated with certain enzymes	Affect assimination of copper; growth retardation, anaemia.

Table 3 Table showing the recommended nutrient intake (RNI) or adequate intake (AI) and tolerable upper intake level (TUIL) for different trace elements. It also shows the usage and symtomps for overusing of different trace elements.

Results and Discussion

From the review, RAB has analgesic, anti-inflammatory, blood circulation invigoration, stagnant blood clearing, anti-stomach ulcer, secretion of bile enhancement, anti-procreate and anti-implantation, blood glucose level reducing, lipoprotein reducing, protein assimilation increasing, anti-tumor, memory and endurance improvement, anti-aging, bone growth promotion, bone resorption inhibition, anti-asthmatic and hepatoprotective activities. It also enhances immune system by macrophrages activation, increase in monocytes, activation of natural killer cells, suppress in spleenocyte, T-cells proliferation, induce secretion of IL-2, increasing C3bBb and ICR in immunosuppressed and normal mice peripheral blood, suppress in B-lymphocyte and immunoglobulin, with low toxicity. OA has hepatoprotective, anti-stomach ulcer, hypoglycemic, anti-hyperlipidemic, anti-hypertensive, cardiotonic, anti-dysrhythmic, anti-aggregation of blood platelet, anti-cancer, protection of renal toxicity, anti-inflammatory, anti-mycobial and anti-fertility activities with low toxicity from another published review [106]. Therefore, OA is one of the active ingredients of RAB. However, the establishment was partial because there are still a lot of ingredients of RAB not realized. In addition, a lot of activities and mechanism of actions still not be known. Furthermore, the synergistic and inhibition effects of ingredients were not realized. They just provide a rough guide of using this herb.

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