Arjunolic acid: A novel phytomedicine with multifunctional therapeutic applications

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Herbal plants with antioxidant activities are widely used in Ayurvedic medicine for cardiac and other problems. Arjunolic acid is one such novel phytomedicine with multifunctional therapeutic applications. It is a triterpenoid saponin, isolated earlier from Terminalia arjuna and later from Combretum nelsonii, Leandra chaetodon etc. Arjunolic acid is a potent antioxidant and free radical scavenger. The scientific basis for the use of arjunolic acid as cardiotonic in Ayurvedic medicine is proven by its vibrant functions such as prevention of myocardial necrosis, platelet aggregation and coagulation and lowering of blood pressure, heart rate and cholesterol levels. Its antioxidant property combined with metal chelating property protects organs from metal and drug induced toxicity. Its therapeutic multifunctionality is shown by its wound healing, antimutagenic and antimicrobial activity. The mechanism of cytoprotection conferred by arjunolic acid can be explained by its property to reduce the oxidative stress by enhancing the antioxidant levels. Apart from its pathophysiological functions, it possesses dynamic insecticidal property and it is used as a structural molecular framework in supramolecular chemistry and nanoscience. Esters of arjunolic acid function as gelators of a wide variety of organic liquids. Experimental studies demonstrate the versatile effects of arjunolic acid, but still, further investigations are necessary to identify the functional groups responsible for its multivariables effects and to study the molecular mechanisms as well as the probable side effects/toxicity owing to its long-term use. Though the beneficial role of this triterpenoid has been assessed from various angles, a comprehensive review of its effects on biochemistry and organ pathophysiology is lacking and this forms the rationale of this review.

Keywords: Antioxidant, Arjunolic acid, Cardioprotectant, Free radical scavenger, Terminalia arjuna

Ancient Indian medical knowledge known as Ayurveda goes back millennia. The Vedas and Puranas refer various materials of medical importance including herbs, plants and trees, etc. Medicinal plants are being investigated in the past to unravel the scientific principles behind their pharmacological properties. A scientific analysis of Ayurvedic herbal armamentarium will lead to the identification of potent drug molecules.

Terminalia arjuna (TA), a deciduous tree of the Combretaceae family, has been widely used in Indian system of medicine for cardiac ailments¹. Its cardioprotective property has been mentioned in ancient Indian medical literature including Charaka Samhita and Astang Hridayam². It is also believed to have the ability to cure hepatic, urogenital, venereal and viral diseases³. It also possesses antilipidemic, antioxidant⁴, antimflammatory, antinociceptive and immunomodulatory activities⁵. The various constituents present in the bark of TA include tannins, arjunic acid, arjunolic acid, arjungenin, arjunglycosides, flavonoids, ellagic acid, gallic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc and copper⁶.

Arjunolic acid (AA)

AA is used as a cardiac tonic in Ayurvedic medicine for centuries and it has been first isolated from TA⁷. Later, it has been isolated from Cochlospermum tinctorium⁸, Cornus capitata⁹, Leandra chaetodon¹⁰, Combretum leprosum¹¹, Campsis grandiflora¹², Syzygium guineense¹³, Combretum nelsonii¹⁴ etc. Ratsimamanga and Boiteau¹⁵ have filed a patent based on the hormonal, wound healing and bactericidal properties of AA.
AA, a triterpenoid saponin, is a major component of the extracts of the bark of TA (Fig. 1). Triterpenoids, are an important class of plant secondary metabolites derived from C$_{30}$ precursors$^{16}$ and they possess a wide range of biological activities$^{17}$. This chiral triterpenic acid has a rigid pentacyclic backbone with two equatorial hydroxyl groups and one equatorial hydroxymethyl group attached to the “A” ring. The carboxyl group is attached at the ring junction of the cis-fused “D” and “E” rings$^{18}$.

Research on AA aims to untie its multifunctional therapeutic applications. Being known for its cardioprotective effect over centuries, experimental studies have proved functions such as prevention of myocardial necrosis, platelet aggregation and coagulation, and lowering of blood pressure, heart rate and cholesterol levels, which lend support to the claim for its traditional usage. Apart from its cardioprotective effects, AA protects the cells from metal induced toxicity and it also possesses antiinflammatory, antidiabetic, antitumor, antimicrobial activity etc. AA is a potent antioxidant and a free radical scavenger. The free radical scavenging activity could be determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and its in vivo antioxidant potential could be assayed by ferric reducing/antioxidant power (FRAP) assay$^{19}$.

Though a vast repertoire of literature describes the therapeutic use of AA in experimental animal models and in vitro studies, a comprehensive review on the beneficial role of this triterpenoid saponin in organ pathophysiology is lacking and hence, this review deals with the multifunctional role of AA, a plant product.

**Extraction of arjunolic acid**

AA is extracted from the bark of TA$^7$. To state briefly, the bark (2 kg) of TA is sliced and ground into powder and extracted with methyl alcohol at room temperature. To the extract, lead acetate (100 g) is added and the precipitate material formed is filtered off. Water (10 l) is added to the filtrate and the greenish precipitate formed is separated by filtration. The mother liquor is concentrated under reduced pressure to have greenish yellow precipitate. The combined greenish yellow precipitate (Total 3.2 g) is subjected to separation on a silica gel column. Elution with chloroform:methanol yields 210 mg of arjunolic acid (mp: 296°–297°C) and the eluted fractions are examined by thin layer chromatography. The purity of the compound is checked by using standard tools such as nuclear magnetic resonance (H, C$^{13}$), infrared, mass spectroscopy and optical rotation studies.

**Cardioprotective effects**

Protection against myocardial necrosis—Sumitra et al.$^{20}$ have clearly portrayed the cardioprotective effects of AA by showing its effect on platelet aggregation, coagulation and myocardial necrosis. AA at an effective dose of 15 mg/kg body wt (pre- and post-treatment), when administered intraperitoneally to rats afflicted with isoproterenol (ISO) induced myocardial necrosis, effects a decrease in serum enzyme levels such as creatine kinase, creatine kinase–MB and lactate dehydrogenase and the electrocardiographic changes get restored towards normalcy. AA treatment also prevents the decrease in the levels of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin and α-tocopherol and also reduces glutathione, ascorbic acid, lipid peroxide and myeloperoxidase. Histopathological studies on the heart tissue reveal an engorgement of coronary vessels due to the administration of ISO (Fig. 2a) while in liver, hepatocytes around the portal triad showed intense cytoplasmic staining (Fig. 2b). Normal architecture of heart (Fig. 3a) and liver (Fig. 3b) has been observed after AA pre- and post-treatment.$^{20}$ This study also proves the antiplatelet and anticoagulant activity of AA. The cardioprotective effect of AA (pre- and post-treatment) has been possibly attributed to the protective effect against the damage caused by myocardial necrosis.

Prevention of arsenic induced myocardial injury—Arsenic toxicity is one of the risk factors associated with cardiovascular diseases. Arsenic exposure is associated with direct myocardial injury, cardiac arrhythmias and cardiomyopathy$^{21}$. Manna et al.$^{19}$ have revealed the protective effects of AA against...
arsenic-induced cardiac oxidative damage. Oral administration of sodium arsenite (NaAsO$_2$), at a dose of 10 mg/kg body wt for two days, causes a significant accumulation of arsenic in cardiac tissues of the experimental mice in addition to reduction in cardiac antioxidant enzyme activities, viz. superoxide dismutase, catalase, glutathione-S-transferase, glutathione reductase and glutathione peroxidase. Arsenic intoxication also decreases the level of cardiac glutathione and total thiol contents and increases the levels of oxidized glutathione, lipid peroxidation end products and protein carbonyl content.

Treatment with AA at a dose of 20 mg/kg body wt for four days prior to sodium arsenite intoxication

Fig. 2—(a) Marked congestion of vessels and extravasation of erythrocytes (EE), hyalinisation of a few muscle fibers (MF) and mononuclear cell infiltration (→) in heart tissue in ISO administered rats (H & E; ×320); and (b) Hepatocytes (HP) around the portal triad showing intense cytoplasmic staining; diffuse degenerative changes in the hepatocytes; hypertrophy of Kupffer cells and focal mononuclear cell infiltration (MC) in the liver tissue in ISO administered rats (H & E; ×320).

Fig. 3—(a) Heart tissue showing normal architecture (MF) in ISO administered group, pre- and post- treated with 15 mg of arjunolic acid (H & E; ×320); and (b) Liver tissue showing normal architecture (HP) in ISO administered group, pre- and post- treated with 15 mg of arjunolic acid (H & E; ×320).
Protects the cardiac tissue from arsenic-induced oxidative impairment by restoring the levels of antioxidants. In addition to oxidative stress, arsenic administration increases total cholesterol level and it reduces high density lipoprotein (HDL) cholesterol level in the sera of the experimental mice. AA pretreatment, however, can prevent this hyperlipidemia. While sodium arsenite administration causes the disorganization of normal radiating pattern of cell plates in the heart, treatment with AA before sodium arsenite intoxication reduces such changes and helps to maintain the normal architecture of the heart. Treatment with AA prior to arsenic intoxication reduces the extent of arsenic concentration as well as DNA fragmentation in the heart tissue and also it prevents the toxin induced reduction in the heart weight to body weight ratio. Results suggest that AA can prevent the reduction in the intracellular antioxidant potential and protect the myocardium from arsenic exposure by chelating the free arsenic in the system.

The cardioprotective effects of AA can be attributed to its powerful antioxidant, free radical scavenging and metal chelating properties.

**Protection against arsenic induced toxicity**

Arsenic, one of the ubiquitous environmental pollutants, induces tissue damage, which is a major concern to human population. Its exposure occurs from inhalation, absorption through the skin and primarily, by ingestion of contaminated food and drinking water. An impaired antioxidant defense mechanism followed by oxidative stress is the major cause of arsenic-induced toxicity. AA has been shown to protect arsenic induced cytotoxicity probably due to its free radical scavenging as well as metal–chelating properties and thereby, diminishing the arsenic burden in the cells.19

**Hepatic protection (in vitro)**—Manna et al.23 have further reported that the incubation of isolated murine hepatocytes with sodium arsenite (1mM) for 2 h caused reduction in the cell viability as well as activities of the intracellular enzymatic as well as nonenzymatic antioxidants. Treatment with sodium arsenite enhances lipid peroxidation and also increases the activities of alanine transferase and alkaline phosphatase. Administration of AA (100 µg/ml) before and also along with the toxin almost normalizes the altered activities of antioxidant indices. This study clearly reveals the ability of AA to maintain the integrity of cell membrane. AA is demonstrated to possess free radical scavenging activity and it can enhance the cellular antioxidant capability against sodium arsenite-induced cytotoxicity.

**Protection against oxidative insult in brain**—Sinha et al.24 have demonstrated the ability of AA to ameliorate arsenic-induced oxidative insult in murine brain. Dose of arsenic for disease induction as well as AA concentration for treatment are the same as reported earlier.22 Oral administration of arsenic in the form of sodium arsenite significantly decreases the levels of antioxidant enzymes, cellular metabolites, reduces glutathione and total thiols and increases the level of oxidized glutathione. In addition, it enhances the levels of lipid peroxidation end products and protein carbonyl content. Pretreatment with AA almost normalizes the above indices. Histological findings reveal that arsenic-treated brain tissue shows more frequent nuclear pyknosis. Treatment with AA prior to the arsenic intoxication reduces nuclear pyknosis and shows almost normal architecture similar to that of the control. The above effects can be attributed to the antioxidant activity of AA.

**Protection against nephrotoxicity**—Sinha et al.25 have reported the efficacy of AA against arsenic-induced nephrotoxicity in mouse model. Oral administration of sodium arsenite at a dose of 10 mg/kg body wt for 2 days causes a significant accumulation of arsenic in renal tissues and it alters the activities of serum markers, antioxidant enzymes...
and the levels of lipid peroxidation end products. Treatment with AA at a dose of 20 mg/kg body wt for 4 days almost normalizes above indices. Histological studies also indicate preventive role of AA against sodium arsenite induced nephrotoxicity.

Testicular protection—Manna et al. reported the preventive role of AA against arsenic-induced testicular damage in mice. Dose of arsenic for induction of disease and AA concentration for treatment are the same as reported earlier. Administration of arsenic (in the form of sodium arsenite) significantly decreases the intracellular antioxidant activity, the activities of the antioxidant enzymes and the levels of cellular metabolites. In addition, arsenic intoxication enhances testicular arsenic content, lipid peroxidation, protein carbonylation and the level of glutathione disulfide. Exposure to arsenic also causes significant degeneration of the seminiferous tubules with necrosis and defoliation of spermatocytes. Pretreatment with AA prevents the arsenic-induced testicular oxidative stress and injury to the histological structures of the testes which can be due to its intrinsic antioxidant property.

Mechanism of action

It can be speculated that the preventive role of AA against arsenic-induced cardiac oxidative stress may be due to formation of five-membered chelate complex between arsenic and two equatorial hydroxyl groups of AA. This chelate formation probably removes the free toxin from the system and thereby inhibits it from causing any further oxidative damage to the tissue. In addition, the presence of one carboxylic hydrogen atom may be responsible for its free radical scavenging activity. Therefore, AA acts as a good chelator against arsenic-induced toxicity.

Manna et al. have demonstrated that AA exhibits preventive role similar to that of vitamin C. So, it can be expected that AA can also be oxidized by reactive oxygen species to the corresponding keto-derivative like vitamin C, since it has one primary as well as two secondary hydroxyl groups. Besides, AA also contains one carboxylic hydrogen atom which can easily be abstracted by any free radical. This property of AA may explain its DPPH radical scavenging activity.

Protection against sodium fluoride induced toxicity

Fluoride is utilized in a number of industrial practices and is an ubiquitous ingredient of drinking water, foodstuffs, and dental products. Excess intake of fluoride, however, causes fluorosis, a slow progressive degenerative disorder. Along with other toxic effects, fluoride accumulation induces oxidative stress. Hence, Ghosh et al. have conducted a study to investigate the effect of AA against sodium fluoride (NaF)-induced cytotoxicity and necrotic cell death in murine hepatocytes. Dose-dependent studies suggest that incubation of hepatocytes with sodium fluoride (100 mM) for 1 h significantly decreases the cell viability as well as intracellular antioxidant potential. Incubation with AA (100 µg/ml) both prior to and in combination with sodium fluoride almost normalizes the altered activities of antioxidant indices. The increased cell viability and reduced cellular reactive oxygen species (ROS) in AA treated hepatocytes prove the anticytotoxic effect of AA. AA treatment enhances the cellular antioxidant capability and protects hepatocytes against sodium fluoride-induced cytotoxicity and necrotic death.

Protection against acetaminophen (APAP) induced toxicity

Renal protection—Acetaminophen (APAP) is a widely used analgesic and antipyretic drug and it is safe at therapeutic doses. But accidental or intentional overdose causes acute liver and kidney failure. Rats exposed to a nephrotoxic dose of APAP exhibit alterations in the levels of a number of biomarkers (blood urea nitrogen, serum creatinine levels, etc.) related to renal oxidative stress, a decrease in antioxidant activity and elevation in renal TNF-α and nitric oxide levels. AA treatment both pre- and post to APAP exposure protects the alteration of these biomarkers, compensated deficits in the antioxidant defense mechanism and suppressed lipid peroxidation in renal tissue. Experimental evidence suggests that APAP-induced nephro-toxicity is a caspase-dependent process that involves the activation of caspase-9 and caspase-3 in the absence of cytosolic cytochrome C release. These results provide evidence that inhibition of nitric oxide overproduction and maintenance of intracellular antioxidant status may play a pivotal role in the protective effects of AA against APAP-induced renal damage. AA represents a potential therapeutic option to protect renal tissue from the detrimental effects of acute acetaminophen overdose.

Hepatic protection—Another study has been undertaken to explore the protective role of AA against APAP induced acute hepatotoxicity. Exposure
of rats with a hepatotoxic dose of APAP (700 mg/kg body wt, ip) altered a number of biomarkers and induced necrotic cell death. Pre-treatment (80 mg/kg body wt, orally) with AA affords significant protection in liver injury. AA also prevents acetaminophen-induced hepatic glutathione depletion and APAP-metabolites formation. Results of this study suggests that this preventive action of AA can be due to the metabolic inhibition of the specific forms of cytochrome P450 that activates acetaminophen to NAPQI. In addition, administration of AA 4 h after APAP intoxication, reduces acetaminophen-induced JNK (Jun N-terminal Kinase pathway) and downstream Bcl-2 and Bcl-xL phosphorylation and thus, prevents mitochondrial permeabilization, loss in mitochondrial membrane potential and cytochrome C release. AA affords protection against acetaminophen-induced hepatotoxicity through inhibition of P450-mediated APAP bioactivation and inhibition of JNK-mediated activation of mitochondrial permeabilization.

Wound healing activity

Use of Arjuna bark in wound healing has been mentioned in Susruta Samhita. Effect of topical application of phytoconstituents (fraction I, II and III) fractionated from a hydroalcohol extract of the bark of TA, has been assessed for the healing of rat dermal wounds. Fraction III mainly consists of saponin from the bark (AA is a major triterpenoid saponin in the bark of TA). Wounds created on the back of rats under anaesthesia have been treated with various fractions applied topically as simple ointment. Results prove that Fraction III prepared as 1% simple ointment shows complete epithelialization on day 20, whereas Fraction I shows complete epithelialization on day 9, which essentially consists of tannins.

Antiinflammatory activity

Facundo et al. have extracted AA from Combretum leprosum and demonstrated that when AA administered at 100 mg/kg body wt is able to inhibit significantly the carrageenan-induced rat paw edema by 80.8%, but at this dose, AA has been found to be gastrototoxic. At a lower dose of 10 mg/kg, toxicity has not been observed and AA significantly inhibits the edema by 37.6%. AA (10 mg/kg body wt) inhibited the acetic acid-induced constrictions by 30.3% in mice. Arachidonic acid and 12-O-tetradecanophorbol-13-acetate (TPA) induced ear edemas are widely used to evaluate the antiinflammatory activity of cyclooxygenase (COX) and lipoxygenase (LOX) inhibitors. AA shows a similar profile as that of indomethacin and it inhibits the arachidonic acid-induced ear edema by 55.5% without interfering with the TPA-induced one. The overall results suggest that AA affects the arachidonic acid metabolism by cyclooxygenase, thus exerting its antiinflammatory and antinociceptive activities.

Anticholinesterase activity

Inhibition of acetyl and butyryl cholinesterase enzymes by AA has been proved by modified Ellman’s method using thin layer chromatography. Inhibition of Acyl-CoA:Acyltransferase (ACAT), which catalyses acylation of cholesterol to cholesteryl esters with long chain fatty acids is a very attractive target for the treatment of hypercholesterolemia and atherosclerosis. ACAT-1 and ACAT-2 are the two isoforms of ACAT in mammals. AA isolated from Campsis grandiflora, exhibits relatively high hACAT-1 inhibitory activity of 60.8% at a concentration of 100 µg/ml, while it is not shown to inhibit hACAT-2. Microsomal fractions of Hi5 cells containing baculovirally expressed hACAT-1 or hACAT-2 and rat liver microsomes are used as the source of enzyme. Presence of three hydroxyl groups at C-2, C-3 and C-23 in the A ring of AA is attributed to its high hACAT-1 inhibitory action.

Antidiabetic activity

Manna et al. have investigated the prophylactic role of AA against streptozotocin (STZ) induced diabetes in the pancreatic tissue of Swiss albino rats. STZ administration (at a dose of 65 mg/kg body wt, injected into the tail vein) causes an increase in the production of both ROS and reactive nitrogen species (RNS) in the pancreas of experimental animals. Formation of these reactive intermediates decreases the intracellular antioxidant defense, increases the levels of lipid peroxidation, protein carbonylation, serum glucose and TNF-α. Treatment of animals with AA (at a dose of 20 mg/kg body wt, orally) both pre- and post- to STZ administration effectively reduces these adverse effects by inhibiting the excessive ROS and RNS formation as well as by down-regulating the activation of phospho-ERK1/2, phospho-p38, NF-κB and mitochondrial dependent signal transduction pathways leading to apoptotic cell death, thus showing the beneficial role of AA against STZ-induced diabetes.
Manna et al.\textsuperscript{36} have reported the efficacy of AA against STZ induced diabetic nephropathy in rats. Diabetic renal injury is associated with increased kidney weight to body weight ratio, glomerular area and volume, blood glucose (hyperglycemia), urea nitrogen and serum creatinine. This nephro pathophysiology increases the production of ROS and RNS, enhances lipid peroxidation and protein carbonylation and decreases intracellular antioxidant defense in the kidney tissue. Treatment of AA (20 mg/kg body wt) effectively ameliorates diabetic renal dysfunctions by reducing oxidative as well as nitrosative stress and deactivated the polyol pathways. Histological studies reveal multiple foci of hemorrhagic necrosis and cloudy swelling of tubules in the kidney of toxin control and this is reduced after treatment with AA, suggesting that AA may act as a ameliorating agent against the renal dysfunctions developed in STZ-induced diabetes.

α-Glucosidase and α-amylase inhibitors are widely used in the treatment of patients with type II diabetes\textsuperscript{37}. AA isolated from the leaves of Lagerstroemia spectiosa inhibits α-glucosidase to some extent, while it shows weak inhibitory activity against α-amylase\textsuperscript{38}.

**Antiasthmatic activity**

Mast cells release mediators such as histamine, acetylcholine etc. Mast cell disruption is induced in rat by compound 48/80 (a condensation product of p-methoxy-N-methyl phenyl amine). Rats treated with AA (50 and 100 mg/ kg body wt) and alcoholic extract of TA (250 and 500 mg/kg body wt) shows significant protection against mast cell disruption. While the control group demonstrates 62% degranulation, the AA treated groups (50 and 100 mg/kg body wt) show 42 and 33% degranulation, respectively. Animals treated with 250 and 500 mg/kg body wt of alcoholic extract of TA exhibit 51 and 39% degranulation, respectively, while the standard drug disodium chromoglycate (50 mg/kg body wt) produces 22% degranulation. The results reveal that AA and alcoholic extract of TA have significant mast cell stabilization activity and specifically, AA exhibits comparatively better stabilization activity than alcoholic extract of TA\textsuperscript{39}.

TA and AA also protect the guinea pig against histamine as well as acetylcholine induced bronchospasm. Histamine is the most implicated mediator in bronchoconstriction that accompanies asthma\textsuperscript{40}. Acetylcholine can cause bronchoconstriction by activating efferent cholinergic fibers, secondary to the stimulation of histamine\textsuperscript{41}. AA at a dose of 50 and 100 mg/kg body wt, shows 49 and 64% protection, respectively against histamine challenge and 40 and 51% protection, respectively against acetylcholine challenge. The alcoholic extract of TA at 250 and 500 mg/kg body wt, exhibited 28 and 40% protection, respectively against histamine challenge and 25 and 32% protection, respectively against acetylcholine challenge. The standard drug chlorpheniramine maleate (2 mg/kg body wt) produced 81% protection against histamine challenge and atropine sulphate (2 mg/kg body wt) produced 72% protection against acetylcholine challenge. The above results clearly reveal that both AA and alcoholic extract of TA show greater percentage of protection against histamine challenge than against acetylcholine challenge. This antiasthmatic and antianaphylactic activity of TA and AA may be due to the mast cell stabilizing potential and inhibition of antigen induced histamine and acetylcholine release\textsuperscript{39}.

**Antitumour activity**

AA, isolated from the rhizome of Cochlospermum tinctorium and its triacetate derivative and their methyl esters have been tested using the short term in vitro assay on Epstein Barr virus-EA activation in Raji cells induced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Their inhibitory effects on skin tumor promoters are greater than those of previously studied natural products\textsuperscript{48}.

Hemisynthetic derivatives of AA isolated from Mitragyna ciliata, have been tested in vivo on a two-stage carcinogenesis assay in mouse skin. The activities have been evaluated by rate (%) of papilloma-bearing mice and average number of papillomas per mouse. In the group of mice treated with hemisynthetic derivatives of AA, the occurrence of papillomas is delayed, compared to the control. Hence, Diallo et al.\textsuperscript{42} have suggested that AA derivatives can be valuable compounds as antitumour-promoters.

**Antimicrobial activity**

AA isolated from the leaf extracts of Syzygium guineense\textsuperscript{13} shows the most significant antibacterial activity against Escherichia coli (3 µg/spot), Bacillus subtilis (0.5 µg/spot) and Shigella sonnei (30 µg/spot). The hydroxyl group present at C-23 position may be responsible for its expression of antibacterial activity. AA shows inhibitory activity against Cryptococcus neoformans with an IC\textsubscript{50} of 20 µg/ml\textsuperscript{10}.
Moderate antifungal activity against *Candida krusei*, *C. albicans* and *C. parapsilosis* has been exhibited by a mixture of AA [2α, 3β, 23-trihydroxy-olean-12-en-28-oic acid/2α, 3β, 23-trihydroxy-urs-12-en-28-oic acid] with minimum inhibitory concentration (MIC) values in the range of 50–200 µg/ml. The MIC values of a mixture of asiatic acid and AA against five fungal animal pathogens ranged from 0.2 to 1.6 µg/ml, viz., *Candida albicans* (0.9 µg/ml), *Cryptococcus neoformans* (0.4 µg/ml), *Aspergillus fumigatus* (1.6 µg/ml), *Microsporum canis* (0.2 µg/ml) and *Sporothrix schenckii* (0.2 µg/ml).

**Insecticidal property**

Bhakuni *et al.* have identified another important property of AA viz. insecticidal property. AA isolated from the stem of *Cornus capitata* exhibits significant inhibitory activity towards fourth instar larvae of *Spilarctia obliqua*. A dose-dependent relationship of activities has been observed. Effective concentration to reduce feeding and growth of the larvae has been found to be 617.8 and 666.9 ppm, respectively.

**Analogs of AA**

AA has potential to be used as a structural framework for the design of molecular receptors and supramolecular architectures. The design and synthesis of a chiral arjuna-18-crown-6, with two additional functional groups at the 23 and 28 positions and its high association constants make it attractive for the design of molecular receptors, biomimetic systems and supramolecular systems capable of performing specific tasks. The nanosized chiral triterpenoid on derivatization can immobilize varieties of organic solvents at low concentrations. Arjunolic acid-derived crown ether shows efficient binding to monovalent cations, including a primary ammonium ion paving the way for chiral recognition of amino acids. Its use in the field of supramolecular chemistry and nanoscience are also prospective.

Though triterpenoids have been recognized renewables in nanoscience, a major difficulty in their use is their availability in pure form. The mixture of the triterpenic acids extractable from TA contains AA as the major component along with asiatic acid, as a minor component having a close structural resemblance. Hence, Bag *et al.* have reported a simple method of separation for the two nano-sized triterpenic acids. Arjuna-bromolactone and the nine esters of AA synthesised with alkyl chains were found to function as excellent gelators of a wide variety of organic liquids.

**Conclusions**

The scientific basis for the use of AA in Ayurvedic medicine has been clearly proved by its versatile effects viz. prevention of myocardial necrosis, platelet aggregation, anticoagulant property, free radical scavenging property, antioxidant property, metal chelating property, antimicrobial activity etc (Table 1). Though a large database could be cited to prove the efficacy of AA in vivo (in experimental animals) and in vitro, preliminary clinical trials (alone or combined with conventional drugs) have to be undertaken to establish its therapeutic effectiveness.

Studies on bioavailability and route of administration have to be furnished. Also, further investigations are necessary to identify the functional group(s) of AA responsible for its cytoprotective role. More studies have to be undertaken to unravel the molecular mechanisms by which AA exerts its preventive role against various cellular stress conditions. Though it has been observed that the use of AA has little side effects, further work has to be initiated to evaluate its toxicity/side effects due to its long term use, as well as to delineate the side effects due to the use of AA analogs.

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