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MiniReview

Emerging Roles of Anacardic Acid and Its Derivatives: A Pharmacological Overview

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Abstract: Anacardic acid (AA) is a bioactive phytochemical found in nutshell of *Anacardium occidentale*. Chemically, it is a mixture of several closely related organic compounds, each consisting of salicylic acid substituted with an alkyl chain. The traditional Ayurveda depicts nutshell oil as a medicinal remedy for alexeritic, amebicidal, gingivitis, malaria and syphilitic ulcers. However, the enduring research and emerging evidence suggests that AA could be a potent target molecule with bactericide, fungicide, insecticide, anti-termite and molluscicide properties and as a therapeutic agent in the treatment of the most serious pathophysiological disorders like cancer, oxidative damage, inflammation and obesity. Furthermore, AA was found to be a common inhibitor of several clinically targeted enzymes such as NFκB kinase, histone acetyltransferase (HATs), lipoxygenase (LOX-1), xanthine oxidase, tyrosinase and ureases. In view of this, we have made an effort to summarize the ongoing research on the therapeutical role of AA and its derivatives. The current MiniReview sheds light on the pharmacological applications, toxicity and allergic responses associated with AA and its derivatives. Although the available records are promising, much more detailed investigations into the therapeutical properties, particularly the anti-cancer and anti-inflammatory activities, are urgently needed. We hope the present MiniReview will attract and encourage further research on elucidating and appreciating the possible curative properties of AA and its derivatives in the management of multifactorial diseases.

Plant medicines play a vital role in human health and diseases. According to the WHO, in recent times, more than 80% of the world's population in developing countries depends primarily on herbal medicines for basic healthcare needs. The ancient ayurvedic preparations and usage have proven the healing abilities of plants, undoubtedly. Hence, a large proportion of drugs used in modern medicine are either directly isolated from plants or synthetically modified from a lead compound of natural origin. Medicinal plant extracts and their isolated compounds are often used as an alternative for the drugs with associated complications in the treatment of many disorders [1,2].

In the course, anacardic acid (AA) (fig. 1A) and its related compounds from *Anacardium occidentale* (an angiosperm belonging to the *Anacardiaceae* family) seed (Cashew nutshell) have received great attention by the chemicobiology researchers and pharmaceutical companies. AA alone constitutes about 90% of the cashew nutshell liquid (CNSL), and the remaining part is constituted by AA-related compounds such as cardanol, cardol and 2-methyl cardol (fig. 1B–D) [3]. It is a yellow liquid partially miscible in alcohol and ether but nearly immiscible in water [4,5]. Chemically, AA is a mixture of several closely related organic compounds each consisting of a salicylic acid substituted with saturated or unsaturated alkyl chain that has 15–17 carbon.

Outstandingly, AA claims a lion's share in the medicinal value of the nutshell as it offers a better protection against several pathophysiological disorders ranging from oxidative damage to cancer (Table 1). On the other hand, cardanol and cardol are the phenolic components that are less effective in all sorts of biological activity exhibited by AA. However, these are largely employed in the chemical industry in the preparation of resins, coatings, frictional materials, surfactants and pigment dispersants for water-based inks. Friction particles are made by polymerizing the unsaturated side chain of cardanol, followed by cross-polymerization with phenol to yield a cardanol-formaldehyde resin by a process analogous to the formation of phenol-formaldehyde resin such as Bakelite.

Ethnopharmacology

Many parts of the *Anacardium occidentale* have been exploited for its medicinal value. The fruit juice and the nutshell oil are both said to be folk remedies for cancerous ulcers, elephantiasis and warts. The oily substance from pericarp is used for cracks on the feet. Old leaves are applied to skin afflictions and burns. In the Gold Coast, the bark and leaves are used for sore gums and toothache. Decoction of the astringent bark is suggested for severe diarrhoea and thrush. In India, bark is used in herbal tea for asthma, cold and congestion and as an antidote for snake envenomations.

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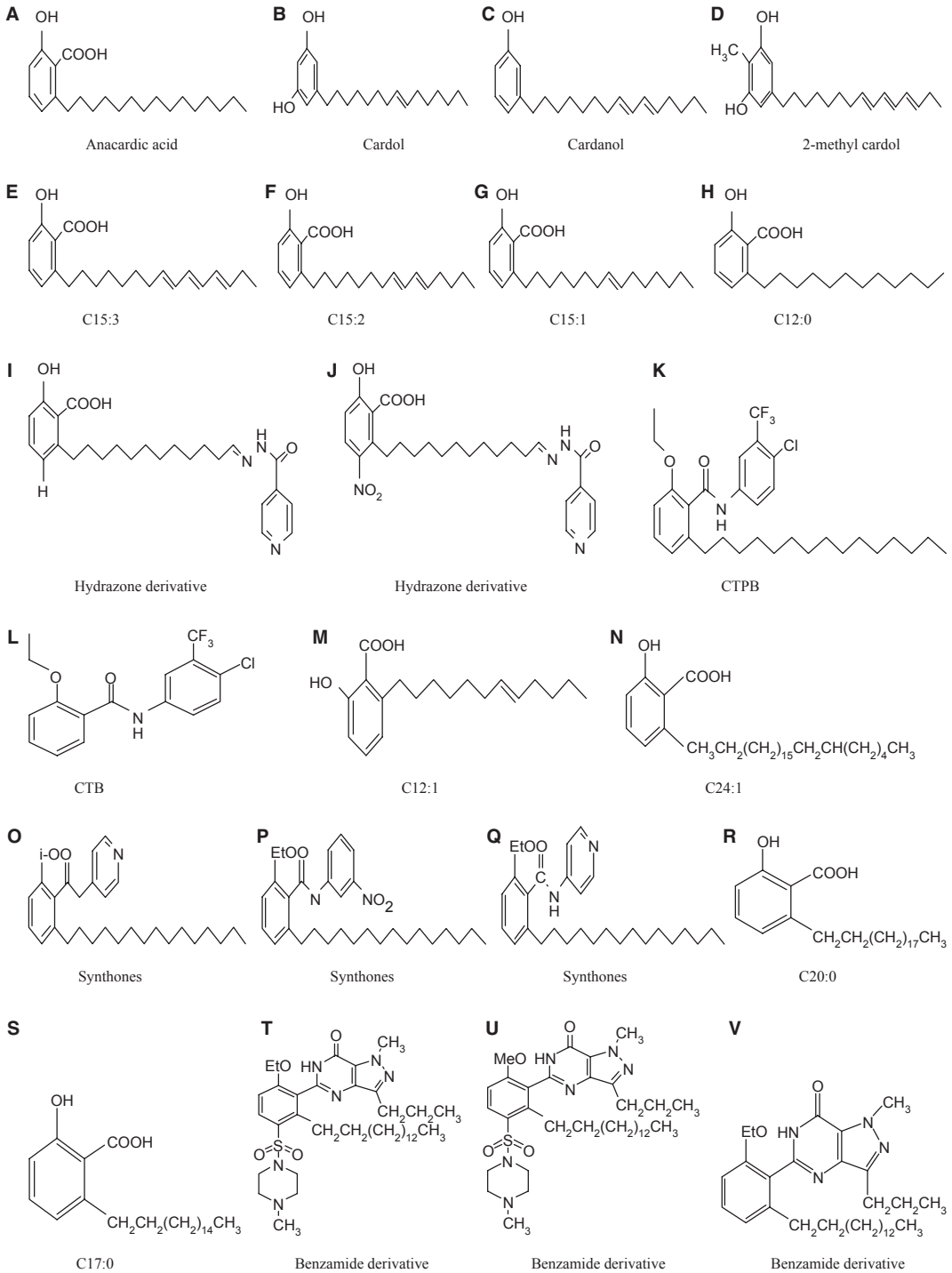

Fig. 1. Structures of anacardic acid and its derivatives.

The seed oil is believed to be alexeritic and amebicidal and is used to treat gingivitis, malaria and syphilitic ulcers. Traditional Ayurveda recommends the fruit for cancer, oxidative stress, anti-helminthic, aphrodisiac, ascites, haemoptysis, dysentery, fever, inappetence, leucoderma, piles and obstinate ulcers and hyperglycaemia. Sap from young leaves and budding fruits is considered fungicidal and insect repellent [3,5].

Pharmacology and Medicine

Antioxidant activity.

Cellular injury from ROS (reactive oxygen species) has been implicated in the development and progression of several diseases [6]. AA (fig. 1A) effectively prevents cell damage induced by H_2O_2 because this can be converted to ROS, hydroxyl radicals, in the presence of metal ions. AA acts as

Table 1.

Pharmacological roles of AA and its derivatives.

Compounds	Pharmacological role	Model used to test	Dose
Anacardic acid (C15:0)	ROS generation inhibition		0.053 ± 0.005 mM
	Xanthine oxidase inhibition		0.0043 ± 0.0005 mM
	Anti-bacterial	*Propionibacterium acnes*	0.0015 mg/mL
		Corynebacterium xerosis	0.0062 mg/mL
		S. aureus	0.025 mg/mL
		MSSA ATCC12598	0.0031–0.0062 mg/mL
		MRSA	
		B. subtilis	
	Zoosporicidal activity	*Aphanomyces cochlioides*	
	Anti-parasitic activity	Colorado potato beetle larvae	
	HATs inhibition		0.0085 mM
	p300		
	PCAF		0.005 mM
	Cytotoxicity		0.008 mM
	PfGCN5 HATs inhibition		
	NFκB inhibition		
	Anti-cancer activity	Pituitary adenoma cells, melanoma cells	
	Aurora kinase A activation		
	Anti-obase activity	Wistar rats	
	Tyrosinase inhibition	Mashroom tyrosinase	0.18 mg/mL
	Urease inhibition		0.12 mg/mL
	Selective metal ion chelation		
Anacardic acid (C15:1)	LOX-15 inhibition	Soybean lipoxygenase	0.05 mM
	Cytotoxicity	HeLa cells	0.01 mM
Anacardic acid (C15:2)	Anti-bacterial	*Helicobacter pylori*	0.2 mg/mL
Anacardic acid (C15:3)	Anti-bacterial, Molluscidal activity	*H. pylori,* Snails	0.2 mg/mL
Anacardic acid (C24:1)	Anti-proliferation of ERα	MCF-10A, MCF-7, MDA-MB-231 breast cancer cells	
Cardol (C12:0)	Anti-bacterial	*H. pylori*	0.2 mg/mL
	LOX-15 inhibition	Soybean lipoxygenase	0.06 mM
	Cytotoxicity	HeLa cells	0.01 mM
N-isonicotinoyl-*N*'-8-[(2-carbohydroxy-3-hydroxy) phenyl] octanal hydrazone.	Anti-bacterial	*Mycobacterium smegmatis*	4 mg/mL
N-isonicotinoyl- *N*'-8-[(2-carbohydroxy-3-hydroxy-6-nitro) phenyl] octanal hydrazone			5 mg/mL
N-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide	p300 activation		
N-(4-chloro-3-trifluoromethyl-phenyl) – 2-ethoxy-benzamide			
2-isopropoxy-6-pentadecyl-*N*-pyridin-4-ylbenzamide.	Cytotoxicity	HeLa cells	11.02 mM
2-ethoxy-*N*-(3-nitrophenyl)-6-pentadecylbenzamide.			13.55 mM
2-ethoxy-6-pentadecyl-*N*-pyridin-4-ylbenzamide			15.29 mM
6-n-pentadecyl salicylic acid	Amidolytic activity	sTF/VIIa	0.3–0.4 mmol/L
	GAPDH inhibition		0.028 mM
6-n-dodecylsalicylic acid			0.055 mM
6-n-heptadecylsalicylic acid	Amidolytic activity	sTF/VIIa	0.3–0.4 mmol/L
5-[2-Ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylphenyl]-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one	PDE5 inhibition		0.145 mM
5-[2-Methoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylphenyl]-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7- one			0.125 mM

AA, anacardic acid; HATs, histone acetyltransferase.

an antioxidant in multiple ways including inhibition of various pro-oxidant enzymes involved in the production of ROS and by chelating the divalent metal ions. The high selectivity of AA towards Fe^{2+} and Cu^{2+} ions could be the considerable advantage as an antioxidant [7]. However, it did not quench ROS of DPPH [8,9].

Because the radical scavenging activity of superoxide anion was low for AA, the effect on superoxide anion generation by xanthine oxidase was examined. Masuoka and Kubo [10] reported the sigmoidal inhibition of AA towards formation of uric acid by xanthine oxidase as well as the superoxide anion generation non-competitively with the EC_{50} of 0.0043 ± 0.0005 and 0.0536 ± 0.0051 mM. It was deduced that AA co-operatively binds near the molybdenum centre in the xanthine-binding domain of enzyme as does salicylic acid. Higher affinity of AA to the enzyme than that of salicylic acid suggests that there is a hydrophobic interaction of the alkenyl chain in AA with the enzyme. The difference of EC_{50} between superoxide anion generation and uric acid formation indicates that AA inhibits superoxide anion generation more strongly than uric acid formation. A concordant study by Trevisan *et al.* [11] showed the antioxidant and xanthine oxidase inhibitory potency of AA with IC_{50} of 0.6 and 0.35 mM, respectively, while cardols and cardanols also inhibited ROS generation and xanthine oxidase with IC_{50} of >4 mM. Hence, it could be pathologically important in various sorts of oxidative stress-induced physiological damage, neurodegenerative disorders, including cognitive deficits that occur during normal cerebral ageing and in the treatment of Alzheimer's and Parkinson's diseases.

Anti-microbial activity.

The immature CNSL exhibited methicillin-resistant *Staphylococcus* inhibitory activity to the extent of gallic acid. In addition, CNSL increased the survival time of antioxidant defence defective *S. cerevisiae* when treated with H_2O_2, suggesting the inhibition of further oxidative damage. The antioxidant and anti-microbial property of CNSL may be attributed to the presence of alkyl polyunsaturated phenols [12]. Kubo *et al.* [13] demonstrated the anti-bacterial activity of AA with varied length of side chain (C15:3), (C15:2), (C15:1), (C15:0) and (C12:0) (fig. 1E–H) against the Gram-negative bacterium *Helicobacter pylori,* which is known to cause acute gastritis. Among the compounds tested, (C15:3), (C15:2) and (C12:0) were the most potent, each having a minimal inhibitory concentration of 0.2 mg/mL, while (C15:0) (fig. 1A) did not exhibit any activity up to 0.8 mg/mL. The activity gradually increased with their side chain length and unsaturation that indicated the essentiality of double bond in the side chain to elicit the activity.

The CNSL and AA were tested against several strains responsible for cutaneous infection or olfactory disagreement. AA inhibited *Propionibacterium acnes, Corynebacterium xerosis* and *S. aureus* with minimal inhibitory concentrations of 0.0015, 0.0062 and 0.025 mg/mL, respectively. However, it did not inhibit *Pityrosporum ovale* fungus. AA also showed anti-bacterial activity against the strains of MSSA ATCC 12598 and MRSA with minimal inhibitory concentrations ranging from 0.0031 to 0.0062 mg/mL [14]. Further, the β-lactamase inhibitory potency was tested against two strains of *S. aureus*, one strain being resistant to penicillin and the other being insensitive to all β-lactamine antibiotics because of the modification of protein-linking penicillin (PLP-2a). Both AA and CNSL inhibited β-lactamase activity effectively, suggesting inhibition without affecting PLP-2a [15].

On the other hand, AA and its derivatives were also shown to inhibit Gram-positive *Bacillus subtilis* and a fungus, *Pythium vexans*. However, no effect was observed against the Gram-negative *E. coli*. In addition, both AA and cardanol exhibited significant zoospore lytic activity towards *Aphanomyces cochlioides* and inhibited the motility of zoospores. These results suggested the existence of certain links between the lysis-inducing activity to fungal zoospores and anti-bacterial activity against *B. subtilis.* Further, it was deduced that the free carboxyl group and unsaturated side chain were responsible for higher zoosporicidal and anti-bacterial effects. Structural modifications as 2-*O*-methyl derivative improved their inhibitory activities. Though, cardol, having no carboxyl group but the presence of two hydroxyl groups on the aromatic ring, could be responsible for noticeable anti-zoosporic and anti-bacterial activities against Gram-positive bacteria [16].

Furthermore, isonicotinoylhydrazones were synthesized from AA by converting its unsaturated side chain and 5-nitro derivatives into C8-aldehydes by oxidative cleavage. C8-aldehydes were then coupled with isoniazid (an anti-tuberculosis drug) to obtain *N*-isonicotinoyl-*N*'-8-[(2-carbohydroxy-3-hydroxy) phenyl] octanal hydrazone (fig. 1I) and *N*-isonicotinoyl- *N*'-8-[(2-carbohydroxy-3-hydroxy-6-nitro) phenyl] octanal hydrazone (fig. 1J). The synthesized isonicotinoylhydrazones showed potent inhibition against *Mycobacterium smegmatis* mc^2155 with minimal inhibitory concentrations of 4 and 5 mg/mL, respectively. The synergistic studies of both the compounds with isoniazid showed more inhibition compared with isoniazid alone. Both the derivatives also exhibited inhibitory activity against *Mycobacterium tuberculosis* H37Rv [17]. As a consequence, AA and its derivatives could be a potent tool in combating several multidrug resistance pathogenic microbial infections and the associated secondary complications.

HAT inhibitory activity.

Histone acetyltransferases (HATs) are a group of enzymes that play a significant role in the regulation of gene expression. These are covalently modifying the N-terminal lysine residues of histones by the addition of acetyl groups from acetyl-CoA. Dysfunction of these enzymes is often associated with the manifestation of numerous pathophysiological disorders, predominantly cancer [18].

Anacardic acid was found to be a compelling non-competitive inhibitor of p300 and p300/CBP-associated factor (PACF) HATs activities (fig. 2). Even though AA does not affect

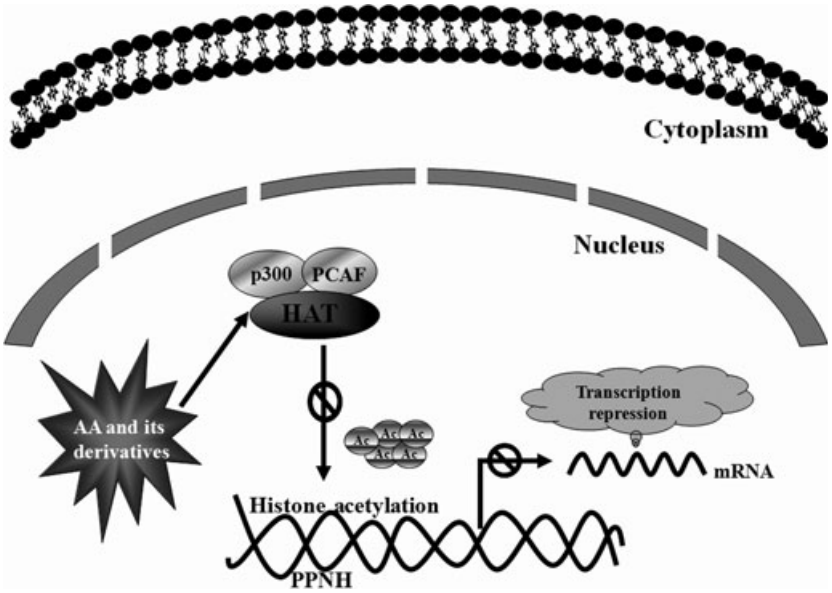

Fig. 2. Proposed mechanisms for the action of anacardic acid (AA) and its derivatives on histone acetyltransferase (HATs). AA and its derivatives interrupt non-competitively p300 and p300/CBP-associated factor (PACF) HATs activities. Even though AA does not affect DNA transcription directly, HAT-dependent transcription from a chromatin template was strongly inhibited. ⃠ represents the possible inhibitory targets of AA and its derivatives. *PPNH, promoter proximal nucleosomal histones.

DNA transcription directly, HAT-dependent transcription from a chromatin template was strongly inhibited. The IC_{50} values of AA for p300 and PCAF were found to be 0.0085 and 0.005 mM, respectively. Recently, it was demonstrated that AA also inhibits the MYST (named for members MOZ, Ybf2/Sas3, Sas2 and Tip60) family HAT member Tip60, thus blocking the acetylation and activation of the ataxia telangiectasia mutated protein kinase in HeLa cells. Therefore, AA and its derivatives would be useful as biological switching molecules for probing into the role of p300 and PCAF in transcriptional studies and may also be useful as new chemical entities for the development of anti-cancer drugs [19].

Moreover, two benzamide derivatives *N*-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide (CTPB) (fig. 1K) and *N*-(4-chloro-3-trifluoromethyl-phenyl) -2-ethoxy-benzamide (CTB) (fig. 1L) exhibited activation of the p300 HAT activity. Interestingly, between CTB and CTPB, the latter was found to be a better activator of p300. The enzyme kinetic analysis in the presence of compounds revealed that K_m decreases with increasing concentration of acetyl-CoA, whereas increased concentration of core histones increases the K_m, suggesting a strong binding of activator to the enzyme and alters the enzyme structure in such a way that it recruits more acetyl-CoA, which leads to the activation of the p300 HAT activity [20]. These results were further confirmed by SERS spectra and CD spectroscopy. However, CTPB did not affect the DNA transcription but enhanced the p300 HAT-dependent transcriptional activation in assembled chromatin template *in vitro*. CTPB also induced a decreased histone acetylation level in immortalized HEK cells and counteracted the action of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) in MCF7 breast cancer cells. These results suggest that the pentadecyl group may play a vital role in p300 activation and thus could be exploited for anti-cancer drug formulations.

Anti-cancer activity.

In view of HAT inhibition, CNSL, AA and their derivatives were evaluated for tumour suppressing activity. The petroleum ether extract of CNSL was found to be non-mutagenic up to a concentration of 0.003% with and without metabolic activation to *Salmonella typhimurium* (Ame's test) strains TA 1535, TA 100 and TA 98. On the other hand, the murine skin tumourigenesis model revealed that acetone extract of CNSL has no carcinogenic initiating effect at a concentration of 10%, while it may act as a weak promoter at a concentration more than 5% [21].

Anacardic acid with varied chain length induced cytotoxicity towards several human cancer cell lines *in vitro*. AA (C12:0) and (C15:1) exhibited nearly comparative cytotoxicity, suggesting the non-essentiality of unsaturation in the hydrophobic side chain. However, AA (C15:0) exhibited slightly more potent cytotoxicity with an IC_{50} of 0.008 mM than (C12:1) (fig. 1M). Indeed, AA (C15:0) had the higher molecular volume (V_m) of 341 cm^3/mol than AA (C12:1), which had the V_m of 298 cm^3/mol. Therefore, the molecular volume of the hydrophobic side chain is one of the main determinants of cytotoxicity. The anti-cancer activity exhibited by AAs could be due to their ability to act as surfactants, although the possibility of chelating the essential metals cannot be denied. Further, studies need to be undertaken for a comprehensive perspective on the involvement of surfactant and chelating properties of AA and its derivatives in the induction cytotoxicity [22].

An account of p300 HAT inhibition by AA, induction of hypertrophy in isolated neonatal rat cardiomyocytes, was also evaluated by Davidson *et al.* [23]. Hypertrophy was identified as an increase in cell size, the rearrangement of sarcomeres into a striated pattern, the induction of embryonic genes β-MHC (myosin heavy chain) and atrial natriuretic factor. AA effectively inhibited the induction of hypertrophy in response to phenylephrine or urocortin, a member of the corticotrophin-releasing-factor family, which stimulates specific G protein-coupled receptors. AA was equally effective as Spiruchostatin A, a known natural inhibitor of histone deacetylases. Therefore, it might eventually be possible to use phytochemical inhibitors such as AA and its derivatives in a therapeutic strategy to augment the survival of cardiomyocytes exposed to transient ischaemia by the inhibition of urocortin- and phenyephrine-induced hypertrophy.

In several cancers, AURA gene expression gets amplified to a varied extent. The link between over-expression of AURA and tumourigenesis is not well established. There are several mechanisms by which the activity of Aurora kinases is regulated. One of the most important factors is autophosphorylation. AA-induced autophosphorylation of Aurora kinase A was demonstrated using *in silico* approach. The possible mechanism of autophosphorylation could be the binding and induction of structural change of enzyme by AA, which acts as a signal for the autophosphorylation. It would be highly interesting to know the fact whether the similar principle is involved during autophosphorylation by substrate binding. However, AA did not activate the autophosphorylation of Aurora kinase B, suggesting the importance of autophosphorylation in the enhancement of Aurora kinase A activity. Similarly, small-molecule-mediated activation of Aurora kinase A *in vivo* would mimic the over-expression of AURA. Furthermore, the function of Aurora kinase A in cell division, maintenance of genomic integrity and chromosome segregation could be studied using small-molecule modulators like AA. As known today, AA can be considered one of the first natural and non-specific inhibitors of HAT, and it would be more interesting to find out the functional groups of AA responsible for the activation of Aurora kinase A and to establish its further link between over-expression of AURA and cancer. This phenomenon could be useful in understanding the role of Aurora kinase A in cell cycle and metastasis [24].

Recently, Schultz *et al.* [25] evaluated the effect of AA (C24:1) (fig. 1N) on proliferation of ERα-expressing breast cancer cells and compared the results with the ERα-negative cells such as HuMECs, MCF-10A breast epithelial cells and MDA-MB-231 breast cancer cells. AA (C24:1) displayed effective inhibition towards the proliferation of ERα-expressing breast cancer cells, regardless of endocrine/tamoxifen sensitivity, while no effect was observed in ERα-negative cells. In addition, AA inhibited cell cycle progression and induced apoptosis of ERα-expressing cells in an ERα-dependent manner by reducing ER-DNA interaction and inhibiting ER-mediated transcriptional responses. These results clearly suggested the direct interaction of AA (C24:1) with estrogen receptor DNA-binding domain (ERDBD). Further, AA also selectively inhibited ERα–ERE over ERβ–ERE (estrogen response element) binding to the estrogen receptor without affecting the ligand binding. These results were further supported by *in vivo* chromatin immuno-precipitation assay by using MCF-7 cells with ERE-containing promoter of the *TFF1* (pS2) gene. The fact that AA inhibited the proliferation of ERα-expressing breast cancer cells but not the primary HuMECs could be an interesting therapeutic perspective to delineate the diverse action(s) of AA-mediated inhibition.

Further, the effect of AA and cardols on the proliferation and melanin synthesis of murine B16-F10 melanoma cells was evaluated. Although AA and cardols were found to inhibit tyrosinase, a key enzyme in melanin synthesis, melanogenesis in melanocytes was not suppressed but rather enhanced. However, both AA and cardols exhibited moderate cytotoxicity [26].

Recently, Sukumari-Ramesh *et al.* [27] demonstrated a significant anti-proliferative and cytotoxic effect of AA against the pituitary adenoma cells. The AA-induced polymerase cleavage, sub-G1 arrest and annexin-V expression clearly suggested the classical apoptotic cell death. However, carbobenzoxy-valyl-alanyl-aspartyl-(*O*-methyl)-fluoromethylketone, a pancaspase inhibitor, failed to revert the AA-induced cell death, which suggested the possibility of non-canonical apoptotic mechanism of AA. It also reduced the expression of survivin and X-linked inhibitor of apoptosis protein and anti-apoptotic proteins associated with the cellular survivability. The pituitary adenoma is a common intracranial tumour associated with significant patient morbidity because of hormone secretion or as a complication of therapy.

Furthermore, several benzamide derivatives were synthesized from AA by alkylation followed by hydrolysis of the ester to obtain synthones like 2-ethoxy-6-pentadecylbenzoic acid and 2-isopropoxy-6-pentadecylbenzoic acid. These AA derivatives were then coupled with a variety of anilines to obtain novel benzamide compounds such as 2-isopropoxy-6-pentadecyl-*N*-pyridin-4-ylbenzamide (fig. 1O), 2-ethoxy-*N*-(3-nitrophenyl)-6-pentadecylbenzamide (fig. 1P) and 2-ethoxy-6-pentadecyl-*N*-pyridin-4-ylbenzamide (fig. 1Q). These synthesized compounds were tested for cytotoxic effect on HeLa cells. All these derivatives were found to be more potent with the respective IC_{50} values of 11.02, 13.55 and 15.29 mM. The anti-cancer potency of these derivatives was found to be similar to that of garcinol [28].

NF-κB inhibition.

On the other hand, the effect of AA on the NF-κB activation pathway and NF-κB-regulated gene products that regulate apoptosis was investigated. AA suppressed NF-κBα activated by carcinogens, growth factors and inflammatory stimuli through inhibition of IKK activation, IkB phosphorylation, IkB degradation, p65 phosphorylation and NF-κBα-dependent reporter gene expression. AA blocked NF-κB by inhibiting IKK and not by directly interfering with DNA binding, which led to the suppression of phosphorylation

and the degradation of IkB. Recent studies indicated that TAK1 plays a major role in the canonical pathway activated by cytokines through its interaction with TAB 1 and TAB 2. TAK1 has also been shown to be recruited by TNFR1 through TRADD, TRAF2 and receptor-interacting protein. AA inhibited TAK1-induced NF-κB activation, which suggested that TAK1 was the main upstream stimulatory kinase modulated by AA (fig. 3). Besides inducible NF-κB activation, AA also suppressed constitutive NF-κBα activation. Further, AA also potentiated the TNF-α and chemotherapeutic agents-induced apoptosis. Thus, AA and its derivatives may have a potential as anti-cancer and anti-inflammatory agents [29].

Anti-Inflammatory Activity

The anti-inflammatory efficacy of AA and its derivatives was also evaluated using soybean lipoxygenase-1 (LOX-15). Among those tested, AA C15:1 showed greater inhibition (IC_{50} = 0.05 mM), followed by cardol 8′Z, 11′ Z, 14′-triene (IC_{50} = 0.06 mM) compared with others. Saturated cardol and cardanol were ineffective, while the other unsaturated phenols showed moderate inhibition. However, inhibitory potency of saturated cardols and cardanols increased in the degree of unsaturation in their alkyl side chain [30]. The later on studies reported the time-dependent inhibition of AA (C15:1) against the LOX-15-induced linoleic acid peroxidation with an IC_{50} of 0.0068 mM. The kinetics revealed that AA was a slow, reversible competitive inhibitor with KI of 0.0028 mM. Although AA inhibited the linoleic acid peroxidation without being oxidized, it was dioxygenated at low concentrations as a substrate. In addition, the alk(en)yl side chain of AA was essential to elicit the inhibitory potency. However, the hydrophobic interaction alone was not enough because cardanol (C15:1), which possesses the same side chain as AA (C15:1), acted neither as a substrate nor as an inhibitor [31]. These findings suggest a significant physiological role for these compounds.

Anti-obese activity.

Toyomizo *et al.* [32] showed the uncoupling action of AA on oxidative phosphorylation. AA was found to be relatively more effective than that of dinitro phenol (DNP), but unlike DNP, AA exhibits dual effects, involving a stimulation of respiration rate in state 4 (i.e. uncoupling effect) and inhibition of the respiration rate in state 3 to a lesser extent. Similar effect was observed with arachidonic acid and other unsaturated long-chain free acids, but the addition of BSA blocked the increased respiration rate of state 4. However, the addition of BSA did not alter the uncoupling action of AA. These results were further substantiated by *in vivo* experiments using rat liver mitochondria. AA with an alkyl side chain exhibited uncoupling effect similar to the DNP, a classical uncoupler, while greater effect than the salicylic acid (no alkyl group) on ADP/O ratio, state 4 and respiratory control ratio was observed. Among the tested, AA (C15:1) was found to be the most effective uncoupler, whereas decorboxylated AA (cardanol) had no uncoupling effect regardless of the degree of unsaturation in its side chain. Overall, these results suggest that the alkyl side chain as well as the carboxyl group may play an important role in assisting the uncoupling activity of AA.

In view of this, AA could be used advantageously as a food supplement to reduce fat deposition through the

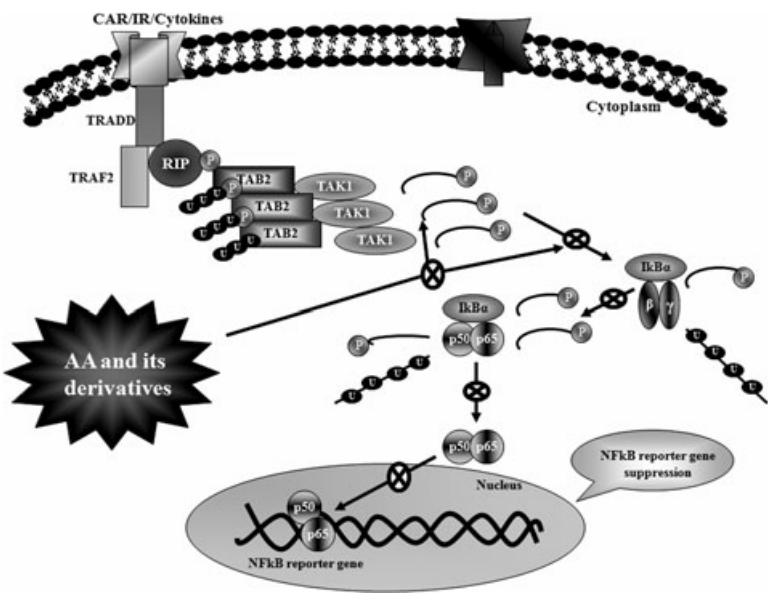

Fig. 3. Proposed mechanisms for the action of anacardic acid (AA) and its derivatives on NFκB reporter gene suppression. AA and its derivatives suppressed NF-κBα activated by carcinogens, growth factors, inflammatory stimuli and cytokines through inhibition of TAK1 phosphorylation, IKK activation, IkB phosphorylation, IkB degradation, p65 phosphorylation and NF-κBα-dependent reporter gene expression. ⊗ represents the inhibitory targets of AA and its derivatives. *CAR, carcinogen; IR, inflammatory response.

uncoupling action. Two sets of experiments were conducted specifically to evaluate the effects on growth, fattening and levels of blood serum parameters in rats fed normal and low protein–high carbohydrate (CHO) diets. According to the results, there were no significant differences in body-weight gain and feed consumption among the groups. Interestingly, AA (0.1% w/w) significantly decreased the total fat pad content in CHO diet-fed rats, but not in rats fed with normal diet. The dietary treatment of both AA and CHO did not affect the weight of heart, spleen and brown adipose tissue, but decreased weight of both liver and kidney was observed. In addition, AA supplementation had no effect on serum glutamic oxaloacetic transaminase, alkaline phosphatase and lactate dehydrogenase levels, suggesting its non-toxicity to liver or kidney [33]. Taken together, the results suggested a unique function of AA that potentially decreased the fat deposition on carbohydrate consumption and AA, and its derivatives could be used as anti-obese molecules.

Anti-parasite activity.

The anti-parasitic activities of AA and its derivatives were also observed against Colorado potato beetle larvae (CPB), *Trypanosoma cruzi*, CQ-sensitive and -resistant parasite strains and small pests such as aphids and spider mites. Schultz *et al.* [34] demonstrated the AA effect on the development of CPB based on the assessment of food preference (treated *versus* untreated). The results indicated the lower feeding rate on food-containing AA and suggested that AA could be applied as a spray or making transgenic plants; this may provide a new tool in the arsenal to minimize plant damage caused by pests and maximize the crop production. Approaches to genetically engineered pest resistance have proven to be effective for the Bt-endotoxin [35]. However, there is alarming concern that an increasing population of target insects will become resistant to Bt. Specifically in potato, work has been conducted to evaluate additional mechanism of resistance to CPB. Future studies will be needed to determine the usage of AA and its homologues as reliable pest control agents in modern agricultural practice.

The protozoan parasites such as *Trypanosoma cruzi, T. brucei* and *Leishmania* sp are considered to be causative agents for Chaga's disease, sleeping sickness and leishmaniasis, respectively. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme involved in the parasite's glycolytic pathway and plays a central role in controlling ATP production. Therefore, GAPDH has been considered an important target for drug development against these parasites. AA derivatives such as 6-n-pentadecyl- and 6-n-dodecylsalicylic acids (fig. 1R) showed potent inhibition against GAPDH with IC_{50} values of 0.028 and 0.055 mM, respectively. The inhibition found to be irreversible and not prevented by the addition of Triton X-100 suggests the absence of aggregate-based inhibition. In addition, detailed enzyme kinetics revealed that the AA derivatives exerted non-competitive inhibition with respect to both substrate and co-factor [36].

Moreover, AA showed significant anti-parasitic activity against both CQ-sensitive and -resistant parasite strains. In spite of less cell permeability, AA effectively blocked the development of intra erythrocytic parasites, suggesting its efficiency and accessibility to the parasites. The enhanced accessibility possibly owes to the increased permeability of the RBC membrane because of extensive remodelling by the parasite. Both *in vitro* and *in vivo* results strongly recommend the parasite-toxicity of AA that could be due to the inhibition of PfGCN5 HAT activity. Moreover, insignificant decrease of HAT activity was observed at which the concentration of AA inhibited the parasite growth. This was further supported by the observation of hypoacetylation of H3K9 and H3K14 at 0.02 mM of AA, at which concentration it did not inhibit the PfGCN5 HAT activity. It was demonstrated that H3K9 and H3K14 are the preferred sites for PfGCN5, whereas PfMYST preferentially acetylates H4K8 and H4K12 [37]. Recent research reveals the utilization of the technical CNSL component cardol as a new green larvicidal agent that can combat the spread of dengue [38].

Miscellaneous

Anacardic acid has been shown to form lipophilic metal derivatives with an unusually high degree of selectivity. AA metal derivatives in ratios of both 1:1 and 2:1 have been prepared and characterized. The order of the selectivity among the first row of transition metals is $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} = Co^{2+} = Mn^{2-}$ for the 2:1 derivatives [9]. Synthesis of linear, lipophilic metal derivatives with a high degree of selectivity (particularly to the important transition metal ions, Fe^{2+} and Cu^{2+}, and, to a lesser extent, Zn^{2+}) could not only find use in metallurgy, but may also explain the wide spectrum of biological activity for AA as a inhibitor of metal-dependent enzymes. The phenomena observed may also be used for the study of metal transport across membranes, paramagnetic properties, material sciences (e.g. molecular laminates) and chromatographic separation of metal ions on immobilized salicylic acid type stationary phases.

Kubo *et al.* [39] demonstrated the tyrosinase inhibitory activity by AA, 2-methylcardols and cardols. Among these, AA inhibited the mushroom tyrosinase on a competitive basis by specifically binding to the active site. In addition, AA also inhibited urease competitively with the IC_{50} of 0.125 mg/mL [13].

Anacardic acid derivates such as 6-(8′Z-pentadecenyl)- and 6-(10′Z-heptadecenyl)-salicylic acid (fig. 1S) inhibited the amidolytic activity of soluble tissue factor/activated factor VII complex (sTF/VIIa), with the IC_{50} value in the range between 30 and 80 M. The structure–activity relationship studies demonstrated that at least one cis double bond was essential for inhibitory activity and those fatty acids containing two or three cis double bonds were optimal. Evidence from pre-incubation studies implied that these AA derivates and other fatty acids might exert their effect by binding to VIIa and consequently preventing the binding of sTF to VIIa [40].

Phosphodiesterases (PDEs) are the key enzymes in the regulation of smooth muscle tone and play an important physiological role by regulating the intracellular level of

cyclic nucleotides. They are classified into five isoenzymes [41], PDE1, PDE2, PDE3, PDE4 and PDE5 according to their substrate specificity. Among the isoenzymes, PDE5 plays an important role in male erectile dysfunction by inhibiting the hydrolysis of cGMP [42]. Using saturated AA as a starting material, analogues of sildenafil [a potent PDE5 inhibitor and an orally active drug for the treatment of erectile dysfunction] were synthesized and tested for PDE5 inhibition. Analogues such as 5-[2-Ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)- 6-pentadecylphenyl]-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (fig. 1T) and 5-[2-Methoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylphenyl]-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (fig. 1U), which have a pentadecyl side chain on the phenyl ring, showed an IC_{50} of 0.145 and 0.125 mM, respectively. However, both were less effective than sildenafil that has shown 0.038 mM under similar experimental conditions. An analogue of 5-[2-Ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylphenyl]-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (fig. 1V) without the *N*-methylpiperazinesulfonamide moiety was found to be less effective than the other sildenafil derivatives with an IC_{50} of 0.16 mM. These results indicated the essentiality of the *N*-methylpiperazinesulfonamide functional group in the molecule [43].

Allergy and Toxicity

Anacardic acid, cardanol and cardols are believed to be the main allergens of cashew allergy [44]. Sullivan *et al.* [45] reported the toxicity of AA and related compounds to freshwater snails, *Biomphalaria glabrata*. The triene component was the most toxic form (LC_{50} 0.35 ppm), while the diene and monoene components were less toxic (LC_{50} 0.9 and 1.4 ppm), and the saturated component was found to be relatively non-toxic (LC_{50} > 5 ppm). Because cardanol and salicylic acid did not show molluscidal activity at concentrations up to 5 ppm, its toxicity could be attributed to the presence of both carboxyl and unsaturated side chain.

On the other hand, AA was found to be a good sensitizer, while cardanol failed to induce allergic contact dermatitis in experimental animals with no other cross-reactions. The cDNA screening and analysis of cashew nut revealed the presence of a 50-kDa major allergen Ana o 1, a vicilin-like protein. Epitope mapping revealed that among 11 linear IgE-binding epitopes, three appear to be immunodominant. Based on epitope homology, no similarities were observed between Ana o 1 and Ara h 1, a peanut vicilin allergen Wang *et al.* [46]. In addition, AA-induced dermatitis in human beings is like the known allergen uroshiol, which is commonly referred to as uroshiol-induced dermatitis Lepoittevin *et al.* [47].

Future perspective.

Based on available reported data, it is evident that AA and its derivatives have multi-therapeutic properties such as antioxidant, anti-cancer, anti-inflammatory, anti-microbial, anti-obese and insecticidal with greater efficiency. In view of this, AA has generated considerable interest among cancer and chemicobiology researchers, and the emerging research data suggest its ability to protect against chemically induced carcinogenesis, as well as its potential use as a chemopreventive agent. An interesting observation in this context is its ability to modulate NF-κB by acting on its upstream pathways. Because NF-κB is known to be a key player in the progression of human cancers and chronic inflammation, its suppression by AA indicates a putative potential molecular target of this compound. However, this requires a comprehensive inspection for establishing the scientific rationale for the use of AA as an anti-cancer and anti-inflammatory agent prior to its use as a novel therapeutic agent for the treatment of human malignancies. Moreover, AA has been shown to modulate various key signalling pathways, as discussed above, which is consistent with the pleiotropic activity of AA. The data on anti-microbial potentiality suggest the use of AA against microbial complications associated with human beings. In addition, future studies should lead to synthesis of these complex and fascinating chemical structures and their generics via modification/addition of different functional groups. It is also important to reveal the bio-efficacy of AA derivatives in combination with other herbs or drugs. Moreover, it is also necessary to study the effects and mechanisms of these molecules *in vivo* using suitable higher animals to ensure their potentiality and safety. Because there are no reviews on the therapeutical aspects of AA and its derivatives, the present MiniReview comprehensively enlists the remedial qualities and the molecular mechanism of action of AA and its derivatives. In summary, the available literature on AA points to its protective role against a number of human ailments and diseases, particularly cancer, inflammation, obesity and microbial infections; however, detailed mechanistic studies are needed to fully appreciate the potential beneficial effects in human health and diseases.

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