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Review

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Review

Azadirachta indica A. Juss (*neem*) against diabetes mellitus: a critical review on its phytochemistry, pharmacology, and toxicology

Shashank M. Patil[1], Prithvi S. Shirahatti[2], and Ramith Ramu[1,*]

[1]Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India
[2]Department of Biotechnology, Teresian College, Mysuru, Karnataka, India

*Correspondence: Ramith Ramu, Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru-570015, Karnataka, India. Tel: +91 9986 380 920; Fax: +91821-2548394; Email: ramithramu@jssuni.edu.in

Abstract

Objective We aim to provide a critical review focused on the various pharmacological activities of *Azadirachta indica* A. Juss related to diabetes management. We also emphasise on phytochemistry and toxicology of *A. indica*, which could provide a comprehensive approach for plant-based drug development in future.

Key findings From 2784 identified studies, only 83 were considered after double screening based on the inclusion criteria. Further, 63 pharmacological investigations were considered for review. Resultant studies deliberated on using different extracts and phytochemicals of *A. indica* on blood glucose level, lipid profile, oxidative stress, carbohydrate digestion enzymes, diabetic complications, glucose tolerance, and uptake of glucose.

Summary In the end, one can know the efficacy of *A. indica* as a potent antidiabetic herbal medicine. However, based on gaps in research, recommendations have been provided to evaluate *A. indica*. in a systematic manner to develop plant-based drugs, nutraceuticals, and to evaluate their clinical efficiency and safety against diabetes mellitus.

Keywords: *Azadirachta indica*; diabetic complications; oxidative stress; carbohydrate digestive enzymes; glucose uptake

Introduction

Diabetes mellitus (DM) is a group of chronic metabolic disorders with uncontrolled hyperglycaemia. Due to its extensive presence globally, it proves to be a socio-economic and health burden. The global incidence of DM was 108 million in 1980, which rose to 422 million in 2014 and expected to reach 592 million by 2035. The global prevalence of the disorder in adults is 8.5% in 2014, rose from 4.7% in 1980. An escalation of 5% of premature mortality has also been reported between 2000 and 2016.[1, 2] The autoimmune condition arises either due to the deficient production of insulin (type 1 DM, T1DM) or the disability of the body cells to utilise it (type 2 DM, T2DM). Gestational diabetes is a rare type, which occurs during pregnancy and vanishes after delivery. Although the exact reason for this is unknown, it is expected to be due to the interference of hormones (produced by the placenta during pregnancy) to the action of insulin.[3, 4] However, T2DM is reported to be more prevalent than T1DM with >90% of all DM cases. It is mostly seen in adults with a few incidences seen even in the case of children and adolescents.[5] The disorder continues to rise as a global threat due to the growing incidences of rapid urbanisation, unhealthy diets, tobacco usage, obesity, environmental factors, ageing populations and sedentary lifestyle. Chronic hyperglycaemia and insulin resistance in DM may lead to a series of associated illnesses, which are collectively called as diabetic complications. These include chronic impairment and dysfunction of body tissues, causing diabetic cardiomyopathy, nephropathy, neuropathy, retinopathy, diabetic liver, pancreas, adipose tissue and skeletal muscle.[6] In the absence of specific and complete cure, several oral hypoglycaemic agents including biguanides, thiazolidinediones, sulfonylureas, meglitinides, alpha-glucosidase inhibitors, and DPP-4 inhibitors with varied mechanisms have been put forth to minimise the effects of DM.[7, 8] With these drugs causing various adverse effects like lactic acidosis, keto-acidosis, anaemia, bone fractures, gastrointestinal aberrations, weight loss and cardiovascular complications, it becomes noteworthy to mention the antidiabetic potential of several Indian medicinal herbs, that have shown to be less toxic in comparison with that of the synthetic drugs.[9]

Out of several medicinal plants originated and available in India, *Azadirachta indica* A. Juss. or Neem is regarded the most potent, with its mentions in the Ayurveda, a traditional system of Indian medicine principally based on phytotherapy

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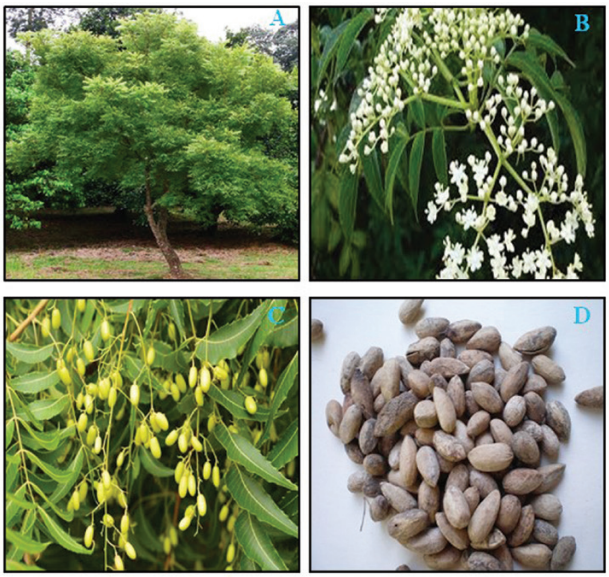

Figure 1 (a) Healthy *A. indica* tree. (b) A flowering twig. (c) A fruiting twig. (d) Seeds of the *A. indica* tree.

(Figure 1).[10] The plant was even traditionally used as an antidote to snake venom. The antimalarial activity of neem is reported in *Ayurveda* books by Charaka (2000 BC) and Sushruta (1500 BC), the two pioneers of Indian medicine.[11] It was used against dermatological diseases, where the leaf and bark extracts were used against leprosy, ringworms, scabies and other skin infections. Gastrointestinal aberrations like Chagas' disease, constipation, intestinal worms, biliary infections, stomach ache and stomach ulceration were treated with leaf, bark, twig extracts and oil. Respiratory diseases like asthma, phlegm, cough and epistaxis were treated with leaf and twig extracts. Several other maladies like piles, rheumatism, syphilis, anorexia, phantom tumour, anorexia, pyrexia, obstinate urinary disorder, spermatorrhoea and blood morbidity were dealt with different parts of the plant. The plant is also believed to possess significant contraceptive properties.[12, 13] It is noteworthy that the available literature depicts the antidiabetic properties of the plant in both clinical trials and preclinical studies with respect to anti-inflammation, antioxidant, anti-apoptosis and antifibrosis activities.[14–17] Therefore, to understand the complete role of *A. indica* in DM management, herein we review its phytochemistry, pharmacology and toxicology of the plant constituents. The present review also states the various pharmacological activities of extracts for which the phytochemical screening was performed at a much later stage. It is followed by the reporting of toxicity studies which may further benefit in conducting the clinical trials. Since this review is drafted to critically analyse and arbitrate the antidiabetic studies conducted so far using *A. indica*, suggestions have been provided to extenuate the gaps in research to develop *A. indica*-based antidiabetic drugs and to evaluate their clinical efficiency. The outcomes of this review may benefit in the development of *A. indica*-based drug as it establishes the plant as a potent antidiabetic herbal medicine.

Method

The drafting of the review article followed the recommendations and guidelines of Cochrane Collaboration and Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA). Complete set of procedures were followed by two authors independently before reaching the consensus on the framework and content of the article (Figure 2). It was followed by the resolving of discrepancies by a third author, who also reviewed the drafted article.

Literature search strategy

A thorough literature survey was conducted to gather all the essential information surrounding the antidiabetic potential of *A. indica* using electronic databases including Google Scholar, PubMed, Springer Link, Wiley-Blackwell and Web of Science. An extensive amount of studies published over 53 years (1967–2020) in peer-reviewed journals like Lancet, International Journal of Molecular Sciences, Phytochemistry Reviews, Scientific Report and Journal of Ethnopharmacology were collected. Further, authors searched for the data using keywords including '*Azadirachta indica*,' 'Antidiabetic activity of *Azadirachta indica*,' 'Ethnobotany of *Azadirachta indica*,' 'Chemical profiling of *Azadirachta indica*,' 'Pharmacological properties of *Azadirachta indica*,' 'Medicinal properties of *Azadirachta indica*,' 'Blood glucose level,' 'Diabetic complications,' 'Carbohydrate digestion enzymes,' 'Glucose tolerance', 'Glucose uptake', 'Toxicology of *A. indica*' etc., which resulted in the gathering of numerous studies done. A special search was conducted to get the ethnomedicinal details of the plant using words 'root,' 'stem,' and 'leaves'. Plant.org was used for the correct names of the plants used in the article. Also, chemical structures and International Union of Pure and Applied Chemistry (IUPAC) names were added using PubChem. The systematic arrangement of studies and their management till the end of drafting was completed using the software Mendeley.

Inclusion criteria

We searched different databases using keywords that led to a large number of research and review articles. The inclusion criteria were designed to select the studies available only in English language, studies with relevant title, abstract, keywords, studies related to blood glucose reduction, amelioration of oxidative stress, amelioration of diabetic complications, effect on carbohydrate digestion enzymes, effect on glucose tolerance and uptake of glucose by *A. indica*. Two authors completely screened the articles based on the above-cited inclusion criteria. The massive literature identified was divided into different sections based on the title and nature of the abstract. During this, articles with irrelevant title and abstract were removed. Later, a second screening was carried out, where studies with unique methodology covering maximum research aspects were retained. Further, a few of the review articles with a limited amount of information were removed. During the drafting of the review, a few research articles were further eliminated to retain the articles with a unique methodology and significant results. However, it was inevitable to retain a few of the articles despite their insignificant results and older methodology, due to lack of relevant information.

Data extraction and analysis

From the final amount of literature divided into different sections, data extraction and analysis was carried out using the standard protocols prepared by authors. All the manuscripts were analysed with Mendeley, and classified

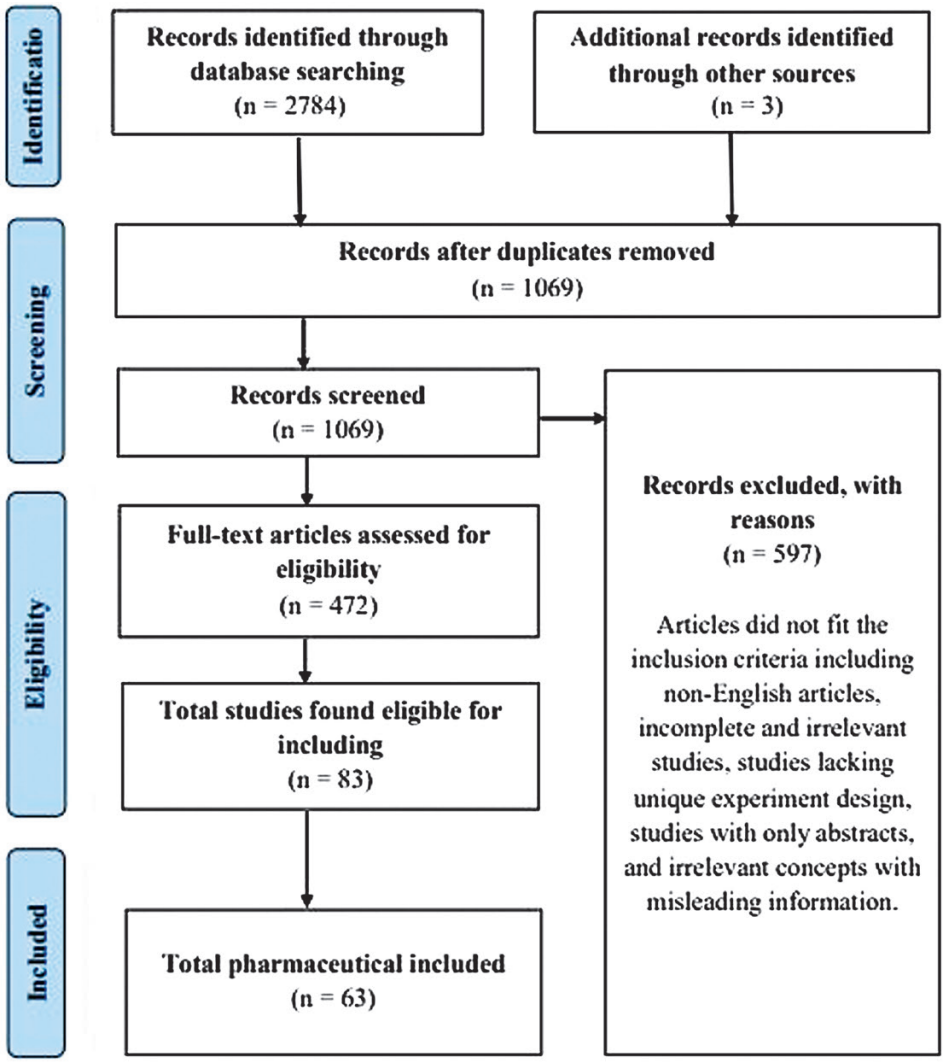

Figure 2 PRISMA flowchart showing the number of articles selected.

using the parameters such as year of publication, type of pharmacological effect, type of study (*in vivo* or *in vitro* or *in silico*), animal models used, plant part of *A. indica* used, type of extract obtained, specific phytochemicals present in the extract, dosage given, controls used, results obtained and underlying mechanism behind the respective pharmacological activity. Studies were also divided based on their toxicological effect, as it proves to be a key ingredient in the preparation of herbal drugs. As a result of data extraction, authors prepared a detailed summary of all the studies as stated in Table 1. Details of the data extraction and analysis are given along this review as representative figures.

Quality assessment

Simultaneous quality assessment of pharmacological investigations was completed alongside reviewing. Articles selected were read and marked under the particulars that determine the quality of a complete research article. For this assessment, authors used criteria that included aim and scope of the investigations, clarification on aim/objective of the study, identification of plant sample from recognised institute/department, faults in activity of extracts, usage of appropriate controls, selection and management of animal models, ethics committee approval, clarification on results obtained, dose-dependent evaluations, statistical significance of the studies and availability of data from toxicological studies. The articles analysed under the criteria were grouped as favourable (yes) and unfavourable (no).

Results and discussion

From 2784 identified studies, a total of 1069 studies were retained after the removal of duplicates. Out of 1069 studies, 597 studies were excluded for lacking the parameters set in the inclusion criteria. Subsequently, a second screening was carried out, where 83 studies were considered. Further, 63 pharmacological investigations were considered for reviewing. As a few studies performed multidisciplinary research using different parameters, they have been considered as individual studies due to their unique and unmatching experimental design. Most of the studies were completed between 2007 and 2012 (20), followed by 2013–2018 (18), 2019–present (9), 2001–2006 (8), 1995–2000 (5), 1989–1994 (1), 1983–1988 (1), and 1965–1970 (1). There were no studies recorded

Table 1 Summary of pharmacological evaluations in DM management

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
Effects on blood glucose and cholesterol								
in vivo	Alloxan induced diabetic rats	Seed	Petroleum ether	200 mg/ animal single dose	Non-alloxan rats and olive oil (placebo)	Possible increased peripheral glucose utilization, release of insulin and or inhibition of the proximal tubular reabsorption of glucose in the kidney	Decreased blood glucose levels 20.1% to 33.8% ($p<0.05$).	[18]
in vivo	Alloxan induced diabetic male albino rats	Leaf	Aqueous	200 and 300 mg/kg single doses	Glibenclamide (0.2 mg/kg) single dose	Not defined	Remarkable decrease in blood glucose and increase in cholesterol levels were seen. Toxic effects like loss of appetite, weight loss and mortality reported at high dose with high clotting time in diabetic patients.	[19]
in vivo	STZ induced IDDM and NIDDM male Wistar albino rats	Leaf	Aqueous	1g/kg single dose	Distilled water, diabetic and non-diabetic rats	IDDM: Increase in the uptake of glucose peripherally and inhibition of insulin action inhibiting glycogenesis in IDDM. NIDDM: increase in the uptake of glucose due to increased sensitivity of insulin receptor.	In IDDM rats, 80, 45.4, 38.02, 77.65% of reduction was found in insulin, neem extract, neem extract and insulin, and insulin with aminoguanidine for IDDM (100mg/kg). In NIDDM rats, extract showed more reduction activity (60%) than glibenclamide control (0.025 mg/kg, 53.95%) ($p<0.05$)	[20]
in vitro	Not defined	Leaf	Ethanol	25 mg/mL single dose	Freshly isolated rat pancreatic cells	Components are expected to inhibit serotonin, which is an inhibitor of insulin along with an elevation in insulin secretion.	Significant hypoglycemic activity by serotonin inhibition. Glucose + A. indica induced high amount of insulin secretion, compared to other formulations (>12.0 µU/mg/15 min).	[21]
in vivo	Alloxan induced diabetic and normoglycemic albino (male and female) rabbits	Seed, leaf	Aqueous and oil	500 mg/kg (Leaf extract) and 5 ml/kg(oil), single doses	Untreated alloxan induced rats	Pre-treatment may induce insulin production. Possible effect of plant products by increasing peripheral glucose utilization or direct metabolite effect on hepatic tissues.	Pre-treatment resulted in partial reduction of blood glucose (35.5% and 41.5%, respectively) ($p<0.05$).	[22]
in vivo	Alloxan induced diabetic Charles Foster male albino rats	Leaf	Ethanol	250 mg/kg double doses	Normoglycemic and diabetic untreated rats	Not defined	50% reduction in blood glucose level with reduction in urine sugar content from 1 mg/dl (initial level) to 0 mg/dl (100% reduction).	[23]
in vivo	Sprague-Dawley male rats	Leaf	Ethanol	50 and 300 mg/kg single doses	Normal and cholesterol fed-untreated rats	Not defined	Reduction in TC (38.96 and 51.41%), LDL (68.33 and 71.3%), and TG (36.64 and 53.39 %) levels except HDL in comparison with cholesterol control group. No significant changes in CRP concentrations were reported.	[24]
in vivo	STZ induced diabetic Wistar male albino rats	Leaf	Ethanol	500 mg/kg single dose	Normoglycemic and diabetic rats	Possible link of hypolipidemic activity with anti-atherosclerosis and antidiabetic activity	Reduction in TC (26%), TL (16.0%), TG (22.69%), LDL (38.98%), VLDL (27.5%) levels were observed in diabetic rats treated. Increasing level of HDL (12.5%) was found.	[16]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
in vivo	Alloxan induced diabetic Wistar albino rats (male and female)	Leaf	Ethanol	400 mg/kg in single dose, 200 mg/kg in double dose	Normal and diabetic rats treated with chlorpropamide (14.286 mg/kg) single dose	Not defined	Blood glucose levels in chronic treatment decreased in case of V. amygdalina + A. indica (71.05%), A. indica (44.95%), and chlorpropamide (75.83%) ($p<0.01$) compared to the initial levels. In the same order, GPT and GOT levels were decreased by 2.4, 2.2, and 3.5 times. Increasing protein breakdown reported with combined extracts.	[25]
in vivo	STZ induced diabetic Wistar male rats	Leaf	Ethanol	500 mg/kg double dose	Normoglycemic rats with metformin (350 mg/kg), Normoglycemic rats with saline treatment	Improved morphology of pancreatic islet, insulin secretion, modulatory effect on leptin production or activity. Possible action of flavonoids including quercetin, myricetin, kaempferol, rutin, and other glycosides	Lowering of blood glucose during initial 11-hour period for leaf extract (16.6%), and metformin (8.7%) compared to initial readings with no weekly reduction except the conversion of hyperglycemic animals to normoglycemic ($p>0.05$)	[26]
in vivo	STZ induced diabetic Wistar male rats	Leaf	Ethanol	200 mg/kg, double dose	Normal, diabetic, and insulin (5 unit/kg) treated rats	Possible insulin mimetic action	Significant reduction upon consuming the extracts was found in case of V. amygdalina+ A. indica (61%), A. indica (63.82%), and insulin (66.0%) compared to the initial levels ($p<0.05$)	[27]
in vivo	Alloxan induced Wistar albino rats (male and female)	Root	Ethanol	200, 400, and 800 mg/kg double dose	Glibenclamide 0.5 mg/kg single dose, distil water 2 ml/day	Possible anti-hyperglycemic activity shown by nimbin and nimbidin	Significant reduction observed at 800 mg/kg (76%) ($p<0.05$) compared to the control group. Reduction at 200 and 400 mg/kg was statistically not significant.	[28]
in vivo	Not defined	Leaf	Aqueous	200, 400, and 600 mg/kg single dose	Distilled water treated normoglycemic rats	Not defined	Blood glucose reduction by 29.52% in non-pregnant rats, and 25.07% in pregnant rats showed at 600 mg/kg ($p<0.05$). PCV in normal and pregnant rats decreased by 11.53 and 15.50%. Hemoglobin 14.25 and 17.29%, RBC 22.6 and 26.86%, WBC 37.42 and 31.11%, platelets 39.86 and 44.41%.	[29]
in vivo	Alloxan induced diabetic Wistar albino rats	Leaf	Aqueous	50, 100, and 150 mg/kg single doses	Diabetic rats treated with glibenclamide (0.214 mg/kg)	Not defined.	Blood glucose reduction of 52.9, 55.7, and 46.0% was observed with 50, 100, and 150 mg/kg doses. Lethal dose (LD_{50}) of 4.8 g/kg was also observed. Pre-treatment (100 mg/kg) 3 and 14 days before diabetic induction showed was 39% reduction on 3rd day.	[30]
in vitro		Fruit	Methanol	20 µL (2 mg/mL)	20 µL of DMSO instead of extract	Possible scavenging of superoxide ion,, which in turn induces insulin secretion due to the enhancement of pancreatic activity.	Inhibition of protein glycation at IC_{50} =18.02% observed at 20 µL (2 mg/mL) concentration of fruit extract compared to the control. Results were comparatively lower than the other plants used.	[31]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
in vivo	Alloxan induced diabetic male albino rats	Seed and Leaf	Ethanol	500 mg/kg of seeds and leaves extract	Diabetic untreated rats	Not defined	Highly significant blood glucose reduction was found in rats treated with leaf extract (51.07%) in comparison with seed extract (47%) ($p<0.001$)	[32]
Effect on oxidative stress								
in vivo	Alloxan induced diabetic male albino rats	Seed	Kernel powder	250 mg/kg + 0.25 mg/kg glibenclamide and 500 mg/kg in single doses	Saline (1.5 ml/kg), insulin (8 U/kg), 250 mg/kg + 0.25 mg/kg glibenclamide	Not defined	Significant reduction in seed kernel powder + glibenclamide (about 50.44%), seed kernel powder alone (38.93%), glibenclamide (43.36%), and insulin (44.24%), in comparison with diabetic control. In case of serum ACP, seed kernel powder + glibenclamide (34.4%), seed kernel powder (15.51%), glibenclamide (34.48%), and insulin (24.13%) was observed.	[33]
in vivo	STZ male albino rats	Leaf	Aqueous	500 mg/kg in single dose	Saline (10 mg/kg), glibenclamide (500 mg/kg)	Possible synthesis of antioxidant molecules and reduction of oxidative stress.	Comparatively lower but Significant increase in the levels of SOD observed with A. indica leaf extract (20.16 %), insulin (114.78%), and glibenclamide (70.58%), in comparison with diabetic control.	[34]
in vivo	Alloxan induced Wistar male albino rats	Leaf	Aqueous	200 mg/mL single dose	Healthy untreated and diabetic untreated rats	Synergistic effect of both extracts reduces free radical level and lipid peroxidation that may further reduce diabetic retinopathy.	A. indica extract reduced the LPO level by 68.0%. It also enhanced the levels of SOD (by 8.48%), CAT (6.04%), GPx (34.05%), and GST (121.33%) compared to diabetic control.	[35]
in vivo	STZ induced male Sprague-Dawley rats	Leaf	Aqueous	500 mg/kg single dose	Glibenclamide (5 mg/kg)	Regulation of interstitial drug receptor pump p-glycoprotein and in turn thehydraulic permeability in cells, activation of GSH synthase,	A. indica extract restored the reduced levels of plasma metals like copper about (128%), iron (36.0%), increased antioxidant enzyme levels like SOD (20.78%), CAT (15.45%), and GSH (11.53%)	[36]
in vivo	STZ induced male Wistar rats	Leaf	Not defined	100 mg/kg in single dose	Diabetic untreated rats with saline	Presence of flavonoids and tannins in multi-herbal mixture like Dihar is believed to produce antioxidant effects.	Significant increasing in the levels of SOD, GSH, CAT, and TBARS was observed in case of diabetic rats treated with Dihar, in comparison with diabetic untreated rats ($p<0.05$).	[37]
in vivo	STZ induced male Wistar albino rats	Leaf	Chloroform	300 mg/kg single dose	2% Tween-80 solution, glibenclamide (0.5 mg/kg)	Possible activity of A. indica polyphenols in reducing the lipid peroxidation and elevation of antioxidant enzyme activity.	Increased activity of antioxidant enzymes (75.19% in SOD, 17.82% in CAT, 28.64% in GSH, 36.76% in GSSG) and decreased protein levels (39.19% mg/protein) in TBARS was detected.	[38]
in vivo	STZ induced male Wistar rats	Leaf	Ethanol	CLE of 500 mg/kg	Metformin (150 mg/kg), diabetic untreated	Not defined	Combined therapy of A. indica and V. amygdalina decreased the antioxidant enzymatic activity:22.70% ALT, 65.91% AST, and 49.18% GPx.	[26]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
in vivo and in vitro	High fat induced Charles Foster male rats	Leaf	Aqueous	100, 200, and 400 mg/kg	Normal and high fat diet untreated groups	Not defined	The extract inhibited the O_2^- anions in xanthine-xanthine oxidase enzymatic system by 26.8% at 200 µg OH ions by 19.3% and 36.0% in enzymatic and non-enzymatic systems in vitro. Serum lipid peroxides were lowered by 28.0%, hepatic GSH by 31% at 400 mg/kg in vivo.	[39]
in vivo	STZ induced male Wistar albino rats	Leaf	Aqueous	500 mg/kg	Normal and diabetic untreated rats	Not defined	Decreasing lipid peroxidation and increasing SOD were observed ($p<0.05$). Although vanadate resulted in more reduction of lipid peroxidation, it resulted in more weight loss. Combined therapy of A. indica leaf extract with vanadate resulted in higher antioxidant activity with minimal toxic effects.	[40]
in vitro and ex vivo	Male Sprague-Dawley rats	Stem bark	Hexane, dichloromethane,ethyl acetate, butanol,aqueous.	15-240 µg/mL	Gallic acid	Not defined	All the fractions displayed significant in vitro antioxidant activity through DPPH radical scavenging, with aqueous extract showing IC_{50} of 16.98 µg/mL ($p<0.05$). Aqueous extract did not show significant results in different in vitro parameters like FRAP, and ex vivo parameters like SOD, CAT, and LPO,	[41]
in vivo	STZ induced male Sprague-Dawley rats	Leaf	Aqueous	250 mg/kg and 500 mg/kg single doses	Glipizide (5 mg/kg)	Possible reduction of oxidative enzyme activity by flavonol glycosides. Possible induction of CYP3A by quercetin.	A. Indica extract of 100 µg concentration induced the activity of CYP3A drug metabolizing enzyme ($p<0.001$). It also reduced ALP and AST levels at both concentrations (250 and 500 mg/kg) ($p<0.05$).	[42]
Effect on diabetic complications								
in vivo	Rabbits and guinea pigs	Leaf	Aqueous	5, 10, 40, 80, 100, and 200 mg/kg	Not defined	Possible effect of extract on vascular smooth muscle, giving rise to vasodilation due to the reduction in arteriolar tone. Possible inotropic and chronotropic effect of extract have been reported.	200 mg/kg reduced the heartbeat rate in both pre-drug and post-drug administration conditions in rabbits. It also decreased arterial blood pressure in anesthetized rabbits and was more effective on diastolic pressure than systolic. Yet 5 and 10 mg/kg extract increased the blood pressure in guinea pigs, whereas 40 mg/kg induced mortality. A double dose of 20 mg/kg normalized ouabain induced cardiac dysrhythmia within 8 minutes.	[43]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
in vivo	Male cats and frogs	Leaf	Ethanol	25, 50, 100, 200, 400 mg/kg single dose	Saline (2 mg/kg)	Possible role of flavonol-O-glycosides in the cardiac-depressant and hypotensive effect.	A. indica extract reduced the blood pressure at dose-dependent levels. Yet the extract at 200 mg/kg failed to alter the sensitivity of α- and β- adrenergic, cholinergic, or histaminergic receptors. No significant change in amplitude in cat respiration was observed. Temporary cardiac arrest in diastole observed at 1 mg-10 mg concentration in case of frogs.	[44]
in vivo	STZ induced albino Wistar rats	Leaf	Aqueous	250 mg/kg single dose	Diabetic untreated rats	Not defined	Reversal of diabetic retinopathy observed with normalization of dilated vessels of rat eyes. Retinopathic opaque eyes were turned into normal eyes.	[45]
in vivo	Charles Foster albino rats	Leaf	Aqueous	500 mg/kg single dose	Omeprazole (2 mg/kg)	The extract decreased acid-pepsin secretion and output in H^+/K^+ -ATPase attributed to the flavonoids present in the extract.	A. Indica extract reduced the H^+/K^+ -ATPase activity (proton pump inhibition) by 43.2% in accordance with the control used. This resulted in the prevention of acid-pepsin secretion and escalated mucin secretion.	[46]
in vivo	STZ induced male Wistar rats	Leaf	Ethanol	500 mg/kg single dose	Glibenclamide (600 µg/kg)	Role of ROS- and AGE-mediated mechanisms in the intestinal aberrations, causing arteriosclerosis of vessels of the small bowel, with thickening of vessel wall, narrowing of their lumen, and the resultant mucosal injury.	The intestinal epithelium was found to be intact and well-functioning in case of both A. indica and glibenclamide control during histological examination. Whereas, the diabetic untreated control showed intestinal cell necrosis, reduced mucin secretion, poor secretion of goblet cells, erosion of surface epithelium, absence of mucus lining on lumen.	[47]
in vivo	Albino Wistar rats	Leaf	Ethanol	400 mg/kg single dose	Chlorpropamide (7.14 mg/kg)	Not defined	Significant decreasing in WBC, neutrophils, CD4, lymphocyte levels upon consumption of A. indica leaf extract was observed ($p<0.05$).	[48]
in vivo	STZ induced Wistar rats	Leaf	Ethanol	500 mg/kg single dose	Glibenclamide (600 µg/kg)	Possible enhancement of islet-b cells by the phytochemicals through the upregulation of glucose-6-phosphate dehydrogenase (G6PD) produced in islet-b cells, causing increased nucleotide synthesis because of increasedribose-5-phosphate (R-5-P).	A. Indica extract significantly reduced the fibrosis and necrosis of the islet-b cells at the end of the experiment (50d). Rats treated with the extract showed highest viability of islet-be cells (50%), in accordance with control ($p<0.05$).	[49]
in vivo	STZ induced male Wistar rats	Leaf	Ethanol	500 mg/kg single dose	Metformin (350 mg/kg)	Flavonoids like quercetin and its glycosides have been attributed to ameliorate the oxidative responsible for diabetic nephropathy.	Histological examinations revealed that A. indica and metformin treated rats showed absence of glomerular lesions including nodular glomerulosclerosis (Kimmelstiel-Wilson disease).	[17]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
in vivo	STZ induced Charles-Foster albino rats	Leaf	Ethanol	500 mg/kg single dose	Glibenclamide (0.6 mg/kg) and Pentoxyphylline (10 mg/kg)	Wound healing activity of A. indica is attributed to the presence of rutin, a flavanol glycoside, phytosterols (sitosterols, sigmasterol, and cam- pasterol), flavonoids, and triterpenoids. These compounds are believed to possess antioxidant, anti-inflammatory, and cytoprotective activities.	A. indica treated rats showed an increase in the VEGF level along with the decreasing TNF-α and IL-1β ($p < 0.1$ to $p < 0.01$) levels. The extract also levelled up the dry granulation tissue weight through collagen synthesis ($p<0.05$ to $p<0.001$).	[50]
in vivo	STZ induced male Sprague-Dawley rats	Leaf	Aqueous	200-1000 mg/kg single doses	Diabetic untreated rats	Possible antioxidant and anti-inflammatory activities of terpenoids which include azadirachtin, azadiradione, nimbin, nimbolin, nimbolide, nimbinene, desacetylnimbin, azadirone, and salanin.	A.indica extract significantly elevated the cardiac content of reduced GSH by about 38% ($P<0.001$) while the lipid per oxidation (LPO) was reduced by about 27% ($p<0.001$).	[16]
in vivo	Male Wistar rats	Leaf	Methanol	100 and 200 mg/kg single doses	Vitamin C (100 mg/kg and 200 mg/kg)	Phytochemicals activate ERK 1/2 signaling pathway that further activates the RISK pathway, ultimately healing IIRI. A. indica phytochemicals prevented IRI-induced cardio-renal dysfunction via reduction in oxidative stress, improvement in antioxidant defense system and increase in the ERK1/2 expressions.	About 16.07% and 12.05% reduction in Xanthine oxidase levels at 100 and 200 mg/kg observed, respectively in comparison with untreated rats. About 51.82% and 67.41% of reduction on the levels of myeloperoxidase was observed in case of 100 and 200 mg/kg of extract, respectively ($p<0.05$).	[51]
in vivo	Male Wistar rats	Leaf	Aqueous	100, 200, and 400 mg/kg single doses	Pregabalin (10 mg/kg)	Possible antioxidant, anti-inflammatory, anti-apoptotic potential ofA. indica. No specific mechanism deduced.	The extract increased the paw withdrawal threshold, tail withdrawal latency and motor nerve conduction velocity. Neural calcium levels were decreased with a corresponding increase in Na-K-ATPase level. Elevated levels of neural TNF-α, IL-1β and NF-κB were reduced, along with the downregulation of neural Bax, Caspase-3, and iNOs mRNA expression. ROS was significantly decreased, induced axonal degeneration and histopathological alterations were reverted.	[52]
in vivo	STZ induced male Wistar rats	Flowers	Dried flower powder	250, 500, and 750 mg/kg	Diabetic untreated rats, Vitamin C (100 mg/kg)	Possible antioxidant potential of quercetin.	The extract (750 mg/kg) significantly increased SFI on postoperative day 18, 500 mg/kg also significantly increased SFI on day 21. The 750 mg/kg extract resulted in a significantly shorter PWL on postoperative day 18 and 21 ($p<0.05$). Further, MDA levels were also significantly reduced in groups treated with either of the concentrations.	[53]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
Effect on carbohydrate digestion enzymes								
in vitro	Swiss male mice	Leaf	Chloroform, methanol, aqueous	0.125 mg/mL single dose	Acarbose (1.9 mM)	Not defined	A. indica extracts did not inhibit porcine α-amylase and murine pancreatic glucosidase. Aqueous and methanolic extracts of A. indica inhibited the murine small intestinal enzyme by 62.44 and 41.07%, murine liver glucosidase by 52.11% and 69.29%.. Methanolic (IC_{50}: 2.60 and 1.80 µg/mL) and aqueous extract (IC_{50}: 3.17 and 6.21 µg/mL) showed inhibition with murine liver and intestinal glucosidases, respectively, significant than acarbose.	[54]
in vivo	Alloxan induced Wistar rats	Leaf	Aqueous	400 mg/kg	Placebo treatment of 0.5 mL distilled water	A. indica does not inhibit the α-amylase directly. Instead, it is believed to enhance the number of β-islet and acini cells, which in turn stimulates insulin, ultimately reducing the α-amylase activity.	About 48.65% of reduction observed in the serum amylase activity upon the consumption of A. indica extract ($p<0.05$).	[55]
in vitro	Not defined	Aerial part	Meliacinolin	20 mg/kg	Acarbose (6.5–32.8 µg/mL), glibenclamide (4 mg/kg)	Meliacinolin is believed to reduce hyperglycemia by inhibiting α-amylase and α-glucosidase activities.	Meliacinolin significantly inhibited α-amylase at IC_{50}: 46.74 µg/mL in comparison with acarbose at IC_{50}: 12.23 µg/mL. While α-glucosidase was inhibited at IC_{50}: 32.18 µg/mL compared to acarbose at IC_{50}: 78.54 µg/mL. Addition of meliacinolin also increased the insulin production significantly ($p<0.01$) compared to glibenclamide.	[56]
in vitro	Not defined	Leaf	Acetone, ethanol, and water	250 µL	Not defined	Possible inhibitory activity by flavonoids and tannins	The aqueous extract inhibited the activity of α-amylase significantly (IC_{50}: 9.15 mg/mL) compared to acetone (IC_{50}: 10.62 mg/mL), and ethanol (IC_{50}: 9.5 mg/mL) extracts. Conversely, acetone extract showed higher inhibitory effect on α-glucosidase (IC_{50}: 5.0 mg/mL), in comparison with ethanol (IC_{50}: 8.70 mg/mL), and aqueous (IC_{50}: 7.25 mg/mL) extracts.	[46]
in vitro	Not defined	Root, stem-bark	Aqueous, methanol	10 mL	Acarbose, voglibose, salacinol, kotalanol, mangiferin, and 1-deoxynojirinmycin	Possible inhibition by nimbidiol, which has efficiently inhibited maltase-glucoamylase, sucrase-isomaltase, lactase and trehalase.	Carbohydrate digestion enzymes including 6 intestinal and 2 fungal enzymes were used where, methanolic extract of root (IC_{50}: 6.0-83.0 µg/mL), aqueous extract of root (IC_{50}: 11.5-88.0 µg/mL), methanolic stem-bark extract (IC_{50}: 10.5-85.0 µg/mL), and aqueous stem-bark extract (IC_{50}: 14.5-89.0 µg/mL) showed the varied inhibition. Nimbidiol (IC_{50}: 0.85-30.0 µg/mL) showed greater inhibitory activity than the controls used.	[57]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
in vitro	Not defined	Leaves	Aqueous extract + Milk	500 µL of yogurt water extract	Plain yogurt	Not defined	Fresh A. indica-yogurt inhibited α-amylase by 44.4% 0 greater thanv plain yogurt (29.8%). However, refrigerated storage increased α-amylase inhibition activity of A. indica-yogurt to 55.0% by day 21, which reduced to 42.0% by day 28. Both yogurts showed similar inhibition (15%) on α-glucosidase on day 14.	[58]
in vitro, in silico	Not defined	Not defined	Neem limonoids (gedunin and azadiradione)	Azadiradione (22.2–133.1 µM) and gedunin (20.7–124.3 µM)	Acarbose	Efficient binding of both gedunin and azadiradione leads to the reduction in the activity of human pancreatic α-amylase, thus could be lead candidates in reducing hyperglycemia.	Azadiradione and gedunin exhibited in vitro potential inhibition with an IC_{50} value of 74.17 and 68.38 µM, respectively. Further screening on AR42J α-amylase secretory cell line for cytotoxicity and bioactivity revealed that azadiradione and gedunin exhibited cytotoxicity with IC_{50} of 11.1 and 13.4µM, respectively.	[59]
in vitro	Not defined	Leaf	ZnO nanoparticles from aqueous extract	1.56-100.0 µg/mL	Not defined	Not defined	ZnO nanoparticles synthesized from A. indica leaf aqueous showed significant inhibition both over α-amylase and α-glucosidase ($p<0.05$).	[60]
in vitro	Not defined	Leaf	Ethyl acetate soluble fraction	Not defined	Acarbose	Not defined	Nimbandiolactone-23 showed the most potent α-glucosidase inhibitory activity, with an IC_{50} value of 38.7 µM.	[61]
in vitro	Not defined	Stem bark	Hexane, dichloromethane, ethyl acetate, butanol, aqueous	15–240 µg/mL	Acarbose	Not defined	Significant inhibition of both α-amylase and α-glucosidase was achieved by all the extracts within the feasible range ($p<0.05$). The binding efficiency of the molecules reveals that the phytochemicals sitosterol, stigmasterol and squalene (from ethyl acetate and butanol extract) have inhibitory potential against both the enzymes.	[41]
in vitro and in silico	Not defined	Not defined	Not defined	5 µM, 10 µM and 20 µM	Acarbose	Molecular chaperone Hsp90, a promoter of tissue damage in diabetics, is said to be inhibited by gedunin. Gedunin has sequence similarity with acarbose and thus, inhibition of both the enzymes by gedunin is based on the interaction of gedunin with important amino acid residues (Trp58, Trp59, His201, Asp197, and Asp300) of the enzymes.	The inhibitory activity of gedunin on pancreatic α-amylase varies from 12.66% to 49.64% in the concentration range of 5 µM to 20 µM. IC_{50} values of gedunin and acarbose were found to be 20.25 µM and 31.12 µM, respectively. Gedunin inhibited the salivary α-amylase by 27.48%, 32.6% and 38.27% by 5 µM,10µM and 20 µM concentrations, respectively. IC_{50} values of gedunin and acarbose were 36.34 µM. And 15.74 µM, respectively.	[62]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
Effect on oral glucose tolerance								
in vivo	STZ induced male Wistar rats	Leaf	Aqueous and Methanol	100 mg/kg in single dose	Diabetic untreated rats given with saline	Not defined	$AUC_{glucose}$ was normalized by Dihar, in comparison with non-diabetic and diabetic controls (about 51.98%). Also, there was a decrease of 26.32% in serum glucose concentration, yet elevation in the insulin level by about 39.46% ($p<0.05$).	[37]
in vivo	STZ induced Swiss mice	Leaf	Aqueous, methanol, and chloroform	100 µg/200 µL	Diabetic untreated rats given with distilled water	Not defined	The glucose tolerance test showed that both methanol and aqueous extracts of A. indica have the potential to reduce the glycemic concentration after the treatment. In fact, the extracts could tolerate the glucose level after 120 min of treatment ($p<0.05$).	[63]
in vivo	STZ induced Wistar male albino rats	Leaf	Hexane, chloroform, and methanol	300 mg/kg	Diabetic untreated rats	Possible increase of peripheral utilization of glucose by the extract, thus reducing the insulin resistance.	A.indica extracts significantly reduced glucose level at 60, 90, and 120 min intervals, in comparison with the control, which decreased after 60 min ($p<0.05$). The glucose level tends to decrease quicker after A. indica treatment compared to the control used.	[38]
in vivo	STZ induced Swiss albino mice	Leaf	Not defined	250 mg/kg and 500 mg/kg	Glibenclamide (2 g/kg)	All the plants used in the preparation of Dianex (Momordica charantia, Cassia auriculata, Azadirachta indica and Aegle marmelose) have been proven to increase the glucose tolerance	Both the concentrations of Dianex (250 and 500 mg/kg) reduced sugar level over 120 minutes, thus increasing the glucose tolerance significantly ($p<0.05$). The results were in accordance with the control used.	[64]
in vivo	Alloxan induced Wistar albino rats (male and female)	Root	Ethanol	200, 400, and 800 mg/kg double dose	Glibenclamide 0.5 mg/kg single dose, distil water 2 ml/day	Possible hypoglycemic activity by nimbidin.	A. indica extract reduced blood sugar level and increased the glucose tolerance at 200 and 400 mg/kg concentration, yet insignificant in comparison with glibenclamide. However, at 800 mg/kg increased glucose tolerance.	[28]
in vivo	STZ induced male Sprague-Dawley rats	Leaf	Aqueous	250 mg/kg and 500 mg/kg single doses	Glipizide (5 mg/kg)	Possible anti-hyperglycemic activity of a flavonoid-quercetin. Possible regeneration of islet-b cells to enhance insulin secretion.	A dose of 250 mg/kg reduced the glucose level after 1 hour. This was significant compared to glipizide. A. indica extract alone reduced the glycemic content at 120 min significantly ($p<0.001$).	[42]
Effect on glucose uptake								
in vitro	Rat muscle cell line L6 myotubules	Flowers	Aqueous	10, 25, and 50 µg/mL single doses	Not defined	Not defined	A. indica flower aqueous extract at 10, 25, and 50 µg/mL concentration shows the glucose uptake of 1.17, 1.19, 1.02 fold over basal, respectively in case of rat muscle L6 cell line in vitro.	[65]

Table 1 Continued

Type of study	Models	Plant part/ material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
in vivo	STZ induced male Wistar rats	Leaves	Aqueous	400 mg/kg single dose	Metformin (50 mg/kg)	Possible restoration of fatty acids from elevated level to normal level in diabetic animals may ameliorate the GLUT4 mRNA expression.	The aqueous extract of A. indica increased the expression of GLUT4 protein by 1.6 fold in both cytosolic and plasma membrane fractions. The extract also maintained the protein level at 1.6-1.8 fold.	[66]
in vivo	STZ induced male albino Wistar rats	Leaves	Chloroform	300 mg/kg single dose	2% Tween-80	Not defined	Chloroform extract of A. indica reported to reduce the expression of G6Pase activity. The activity of G6Pase enzyme was increased by A. indica extract, which in turn increases the hepatic glycogen as well as decreases the hepatic glucose levels.	[38]
in vitro and ex vivo	Male Sprague-Dawley rats	Stem bark	Hexane, dichloromethane, ethylacetate, butanol,aqueous.	15-240 µg/mL	Gallic acid	Possible activity of β- sitosterol	Out of all the fractions, butanol fraction showed significant glucose uptake (GU_{50} of 6.22 µg/mL), whereas the aqueous extract showed 43.98.	[23]
in vitro and in silico	Yeast cells	Not defined	Not defined	5 µM, 10 µM and 20 µM	Metformin (10 µM)	Gedunin inhibits glucose uptake, which has been regarded as a debacle in the process of glucose uptake. As GLUT4 acts as a principle glucose transporter in the cell, relation between gedunin and GLUT4 needs to be elucidated.	The glucose uptake in the presence of 10 and 20 µM gedunin was found to be 30.18% and 21.76%. The results were reported to be significant and in accordance with the control used.	[47]

between 1971–1976 and 1977–1982. The trend has been increasing with respect to parameters like carbohydrate digestion enzymes, blood glucose level and diabetic complications. The detailed analysis of the year-wise publications with respect to these parameters as depicted in Figure 3. Further, out of 63 pharmacological investigations, 17 dealt with blood glucose and cholesterol level (27%), 11 on oxidative stress (17%), 13 on diabetic complications (21%), 11 on carbohydrate digestive enzymes (17%), 6 on glucose tolerance (10%) and 5 on glucose uptake (8%) (Figure 4). Furthermore, classification based on the plant parts used for antidiabetic pharmacological evaluation was carried out resulting in a total of 48 studies that used leaves (76%), 4 used seeds (6%), 4 used stem bark (6%), 3 used root (2%), 2 used flowers (3%), 1 used fruit (2%) and 1 used complete aerial part (2%) (Figure 5). We further proceeded with reviewing and commenting based on the information available in the studies. The article was primarily divided into different sections based on the previous work of authors on phytochemistry and pharmacological review articles.

Phytochemistry

The phytochemical profile of *A. indica* shows the presence of diverse chemical compounds with different pharmacological activities related to DM. These phytochemicals are the secondary metabolites either available in pure or associated form. One of the earliest studies on the antidiabetic activity of *A. indica* by Chattopadhyay[21] revealed the presence of different classes of novel flavonoid glucosides named quercetin-3-O-β-D-glucoside, myricetin-3-O-rutinoside, quercetin-3-O-rutinoside (also known as rutin), kaempferol-3-O-rutinoside, kaempferol-3-O-β-D-glucoside and quercetin-3-O-α-L-rhamnoside (Figure 6a) extracted from leaf ethanol extract. These flavonoids were surmised to lower the blood glucose level by enhancing insulin secretion.[21] However, no chemical structures of these compounds were deduced. Chemical structure and characterisation are important to analyse the effectivity of a chemical compound in pharmacological evaluations. These findings could be of a great help in *in silico* studies. In support of this, Akinola *et al.*[26] showed

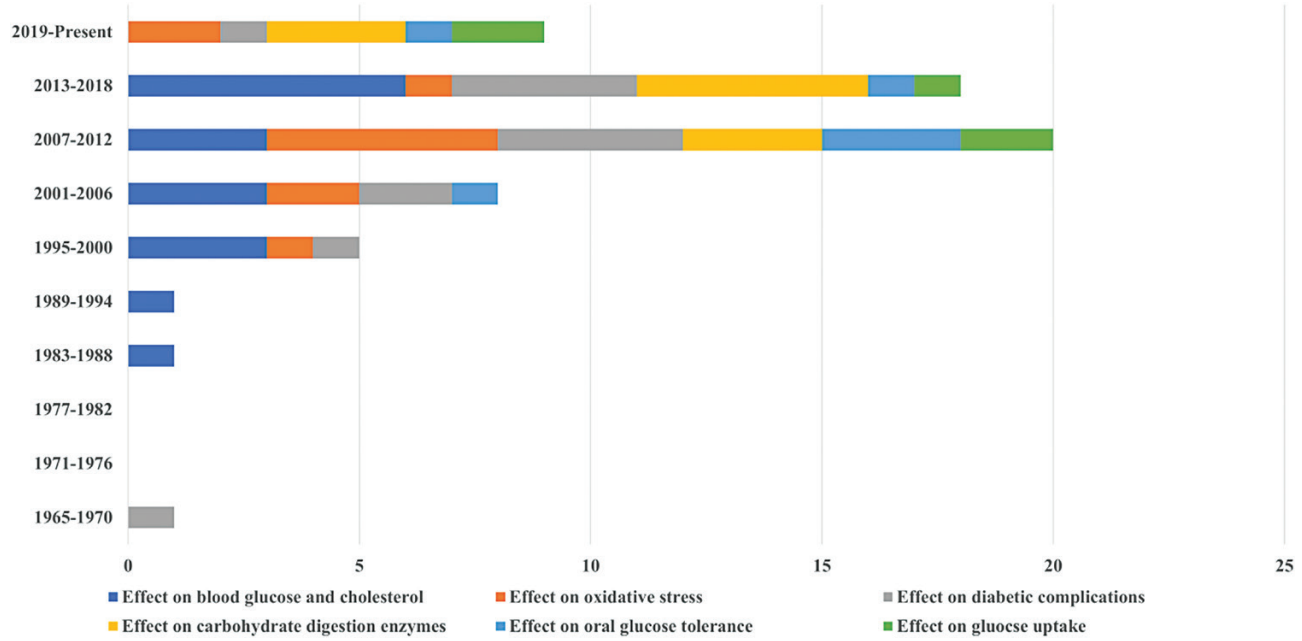

Figure 3 Year-wise analysis of number of pharmacological investigations conducted.

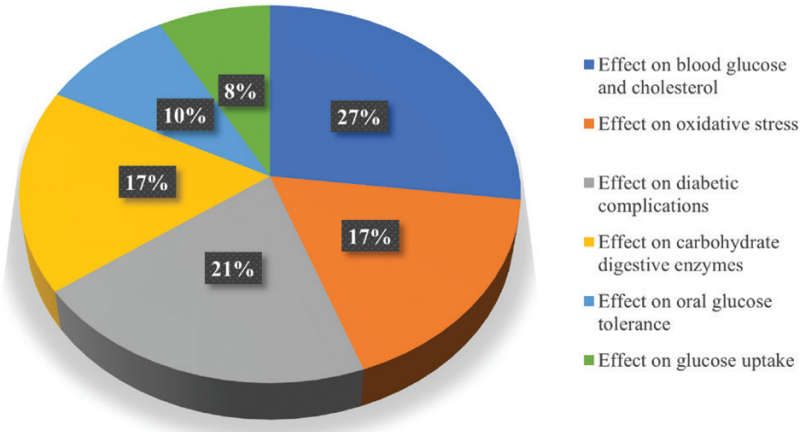

Figure 4 Types of pharmacological evaluations reviewed.

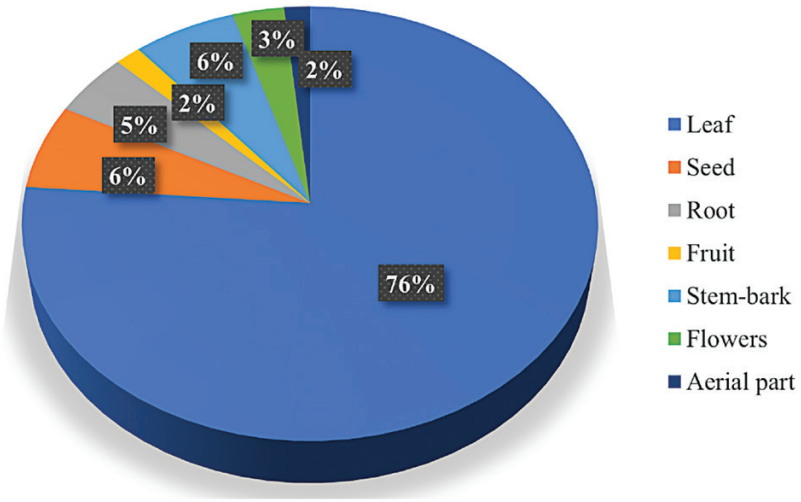

Figure 5 Types of plant parts used in pharmacological evaluations reviewed.

that flavonoids including quercetin, myricetin, kaempferol, rutin (Figure 6b) including their glycosides extracted from leaf ethanol extract have improved the morphology of pancreatic islet, hence insulin secretion. Wound healing activity of *A. indica* through promoting collagen synthesis is also attributed to rutin, which is also extracted from leaf ethanol extract.[50] The flavonol glycosides from leaf ethanol extract can reduce oxidative enzyme activity. These can also act as cardiac-depressants and produce a hypotensive effect.[44] As flavonoids like quercetin and its glycosides have been attributed to ameliorate the oxidative stress, they could be used to treat diabetic nephropathy.[42, 49, 53] Quercetin is reported to suppress cytochrome P450 mediated ROS generation.[42] Also, modulatory effect on leptin production or activity was hypothesised, where high leptin acts as a biomarker of obesity, which may further turn into DM.[26] Antidiabetic property of rutin was identified when the flavonoid was attributed to improving the morphological features of the islets of Langerhans and β cells by inhibiting lipid peroxidation. As most of the studies were focused on the evaluation of antidiabetic potential, it becomes difficult to decipher the role of individual compound. However, in future, we can expect meticulous analysis of these compounds *in vitro*, *in vivo*, and *in silico*.

In addition to flavonoids and their glycosides, some phytosterols are reported with antidiabetic activity as well. Role of β-sitosterol from the butanol fraction in the enhancement of glucose uptake has been hypothesised. Yet it is to be confirmed by advanced studies.[67] Similarly, wound healing mechanism of phytosterols including sitosterols, sigmasterol and campesterol from leaf ethanol extract is also surmised (Figure 7). These compounds are believed to possess antioxidant, anti-inflammatory and cytoprotective activities. Yet they need concrete evidence to be attributed with antidiabetic potential.[50] Further, the role of phytosterols in binding to the carbohydrate digestive enzymes, thereby reducing the glucose load in the body has been depicted. Significant inhibition of both α-amylase and α-glucosidase was achieved using sitosterol, stigmasterol and squalene (Figure 7) from butanol fraction.[67] Advances in technology including molecular docking, these hypotheses could be proved.

Apart from flavonoids, terpenoids are the 2nd major class of phytochemicals reported with an antidiabetic profile. In-depth chromatographic investigations by Perez-Gutierrez and Damian-Guzman[56] revealed the presence of a novel tetranortriterpenoid called 24,25,26,27-tetranor-apotirucalla-(apoeupha)-1α-senecioyloxy-3α,7α-dihydroxy-14,20,22-trien-21,2-epoxy. This was named as meliacinolin (Figure 8a). Meliacinolin, extracted from combined aerial parts of *A.* indica, is believed to reduce the hyperglycaemia by inhibiting the α-amylase and α-glucosidase activities.[56] In case of triterpenoids, antihyperglycaemic activity shown by the triterpenoids nimbin and nimbidin extracted from root ethanol extract has been surmised. These are hypothesised to lower the blood sugar level as well as improvise the glucose tolerance. However, these results are yet to be confirmed after detailed analysis.[28] Antioxidant and anti-inflammatory activities of terpenoids like azadirachtin, azadiradione, nimbin, nimbolin, nimbolide, nimbinene, desacetylnimbin and salanin (Figure 8b and c) from leaf aqueous extract have been predicted. These properties are essential to reverse the oxidative stress and the possible diabetic complications.[16] Among these, nimbidiol from root and stem-bark extracts is also reported with efficient inhibition of carbohydrate digestive enzymes like maltase-glucoamylase, sucrase-isomaltase, lactase and trehalase.[57] Although hypothesised with probable antidiabetic potential, these compounds lack concrete evidence.

Further, limonoids from whole-plant extract have been reported with antidiabetic potential. Among them, a tetranortriterpenoid known as gedunin (Figure 9) was reported with amelioration of tissue damage initiated by DM. As the amino acid sequence of the gedunin and acarbose are matchable in inhibiting a pathogenic molecular chaperone Hsp90, a promoter of tissue damage.[62] Apart from gedunin, another limonid known as azadiradione (Figure 9) is reported to bind with human pancreatic α-amylase resulting in a reduction in the enzymatic activity. Thus, the limonoidsazadiradione and gedunin could be lead candidates in reducing hyperglycaemia.[59] Besides, from ethyl acetate-soluble fraction of *A. indica*, 3 novel limonoids were isolated, including a new lactam 28-norlimonoid named nimbandiolactam-21 along with 2 other limonoids, odoratone, and nimbandiolactone-23 (Figure 9) with potent α-glucosidase inhibitory activity.[61] This study resembles that of Chattopadhyay *et al.*,[21] where

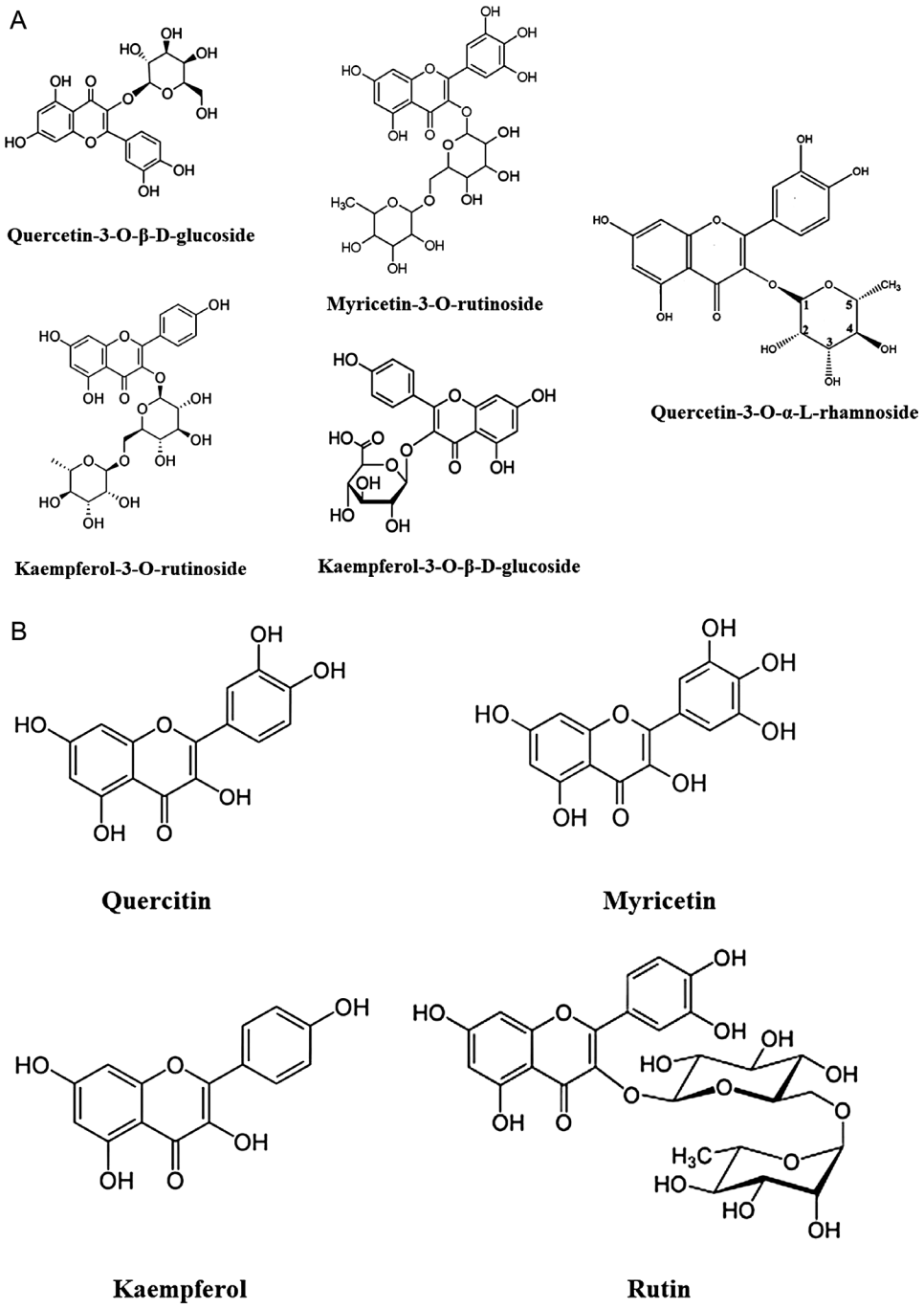

Figure 6 (a) Flavonoid glucosides reported with antidiabetic potential. (b) Flavonoids reported with antidiabetic potential.

most of the compounds were not provided with chemical structure and characterisation. However, we could find only one study in our literature survey that could depict the pharmacological significance of tannins in DM treatment. Patel *et al.*[37] surmised the role of tannins from root ethanol extract in carbohydrate digestive enzyme inhibitory activity. Including this, there are several studies that have predicted the role of phytochemicals in the effective DM treatment. Up to the beginning of 21st century, several studies have reported the role of plant extracts in DM treatment. From now on, it could be a better approach if we divert our focus towards phytochemicals rather than plant extracts because with suitable modifications with respect to minimising the toxicity and enhancing bioavailability, phytochemicals could be used as potent medicines. We also suggest that future studies need to focus on biosynthesis, bioavailability, and dose-dependent clinical trials of these compounds. After careful review of the toxicity aspects of these compounds, it could be synthesised as chemotherapeutic agents that are safe and efficient.

Pharmacology

Effects on blood glucose and cholesterol levels

A substantial amount of research has been done on regulation of the blood glucose level using *A. indica* extracts and compounds with little information on its individual phytochemicals. The pioneer studies made to evaluate the

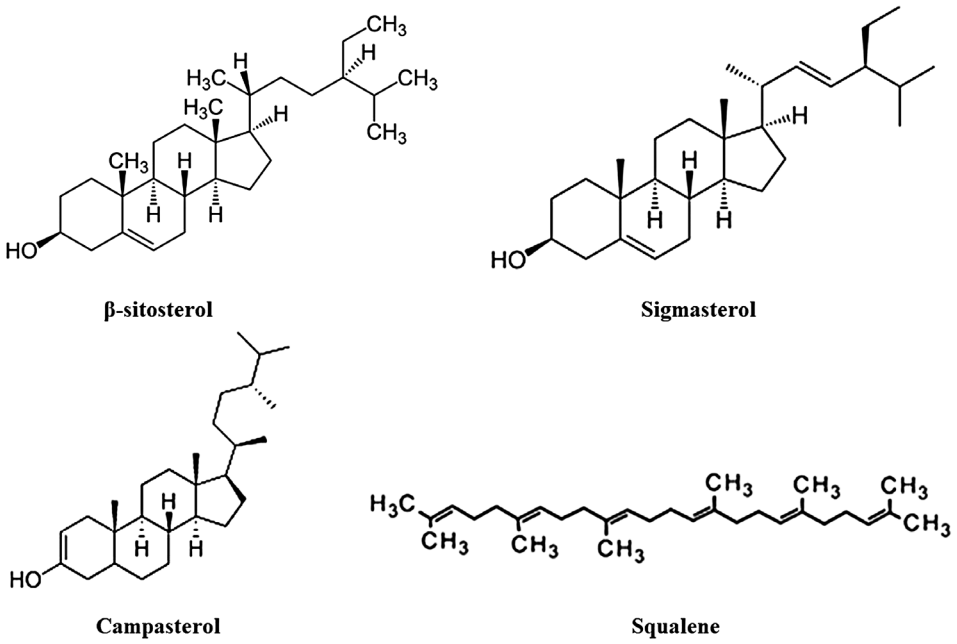

Figure 7 Phytosterols reported with antidiabetic potential.

antihyperglycaemic potential of *A. indica* revealed the efficacy of leaves over the seeds. In an *in vivo* study, oral administration of leaf aqueous extract reduced the blood glucose in the range of 3.3% to 11.7% at 200 mg/kg and 38.7% at 300 mg/kg concentration in case of alloxan-induced diabetic rats ($P < 0.05$),[19] whereas the seed aqueous extract at 200 mg/rat showed a reduction in the range 20 to 33.8% ($P < 0.05$), in comparison with that of the untreated control used.[18] In another *in vivo* study using alloxan-induced rats, the leaf aqueous increased the cholesterol content by 609 % and 670.3 % compared with that of the normoglycaemic control group ($P < 0.05$), with toxic effects like loss of appetite, weight loss and mortality were reported at high dose. Clotting time of diabetic blood was higher than that of the normal, indicating platelet dysfunction resulting in delayed wound healing.[19] However, the hypoglycaemic activity of *A. indica* leaf aqueous extract injected to male Wistar rats was found 45.4%in T1DM at 1mg/kg concentration in streptozotocin (STZ) induced diabetic rats.[20] But in the case of T2DM rats, the extract showed a greater reduction (60%) than glibenclamide control (0.025 mg/kg, 53.95%) ($P < 0.05$) at the same concentration.

Similarly, in an *in vivo* study, a fall of 34% and 32% was observed after 4 weeks with leaf aqueous extract (500 mg/kg) and oil (5 ml/kg) respectively, in normoglycaemic rabbits. Reduction was at 47% and 44% in the case of alloxan-induced diabetic rabbits, respectively, in comparison with initial readings. Pre-treatment with the extract and oil (2 weeks prior to the type II diabetic induction using alloxan) resulted in partial reduction of blood glucose (35.5% and 41.5%, respectively) ($P < 0.05$).[22] In support of this, with the treatment led to the reduction of blood glucose level by about 50 % on an average in the same rats, controls were able to reduce it only by 18.71% (glibenclamide) and 4.43% (distilled water) ($P < 0.05$) with a lethal dose (LD_{50}) of 4.8 g/kg. Oral pre-treatment with the extract (100 mg/kg) on 3 and 14 days before the DM induction showed more significant 39% on the 3rd day.[30] Furthermore, another *in vivo* study evaluated management of hyperglycaemia in pregnant rats where blood glucose reduction was observed in non-pregnant rats(29.52%), while pregnant rats showed a fall of blood glucose (25.07%) in comparison with that of the control upon administration of 600 mg/kg leaf aqueous extract given orally ($P < 0.05$). Packed cell volume (PCV) in normal and pregnant rats decreased by 11.53 and 15.50% along with the reduction in the levels of haemoglobin (14.25 and 17.29%), RBC (22.6 and 26.86%), WBC (37.42 and 31.11%), platelets (39.86 and 44.41%) compared with that of the control ($P < 0.05$), indicating the effects on haematological parameters in diabetic conditions.[29]

In case of leaf ethanol extract, Akinola *et al.*[26] showed that oral administration of 500 mg/kg double dose concentration of leaf extract reduced blood glucose levels during initial 11-hour period (16.6%), and metformin (8.7%) in streptozotocin (STZ)- induced type II diabetic rats. No significant reductions were reported on weekly studies, except the conversion of hyperglycaemic animals to normoglycaemic ($P > 0.05$).[26] But a concentration 800 mg/kg reduced the glucose level by 76% ($P < 0.05$) compared to the control group in alloxan rats by the end of 15th day. However, reduction at 200 and 400 mg/kg was statistically not significant in this study.[28] But in 2018, highly significant blood glucose reduction was observed in rats treated with 500 mg/kg leaf ethanol extract (51.07%) in comparison with seed ethanol extract (47%) in alloxan diabetic rats ($P < 0.001$),[32] in accordance with El-Hawary and Kholief.[19] Thus, leaf ethanol extract was found to be effective at only >500 mg/kg concentration. Meanwhile the fruit methanol extract also prevented protein glycation *in vitro*; a major activity seen in diabetic patients. Inhibition at IC_{50} = 18.02% observed at 20 µL (2 mg/mL) concentration of fruit extract compared to the DMSO control.[31]

Serotonin in blood is reported to inhibit insulin production by activating 5-HT_{1F} receptors, in turn increasing the blood sugar levels. Serotonin is reported to be prevalent in

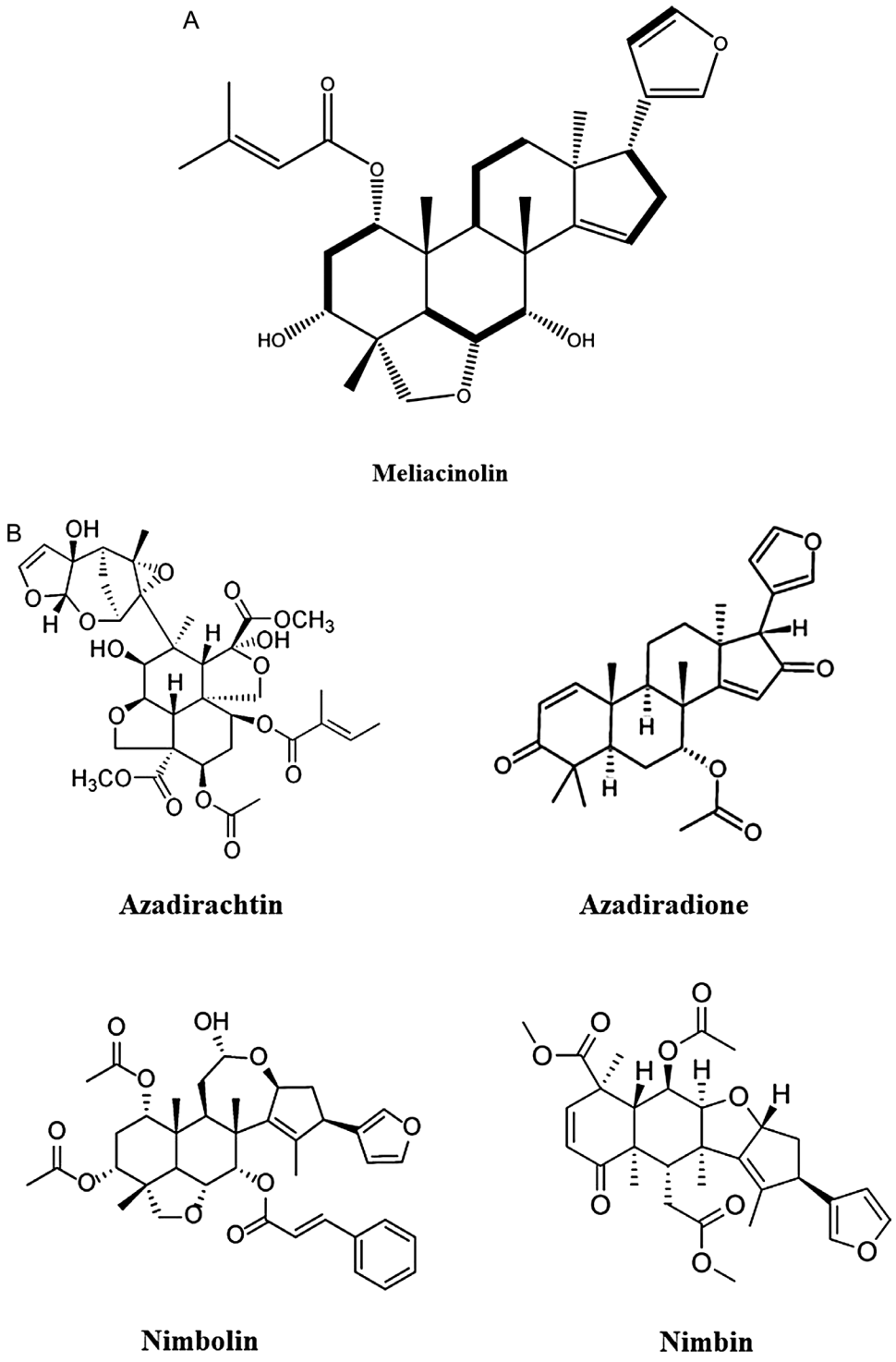

Figure 8 (a) Structure of meliacinolin. (b) Terpenoids with antidiabetic potential. (c) Terpenoids reported with antidiabetic potential.

obese individuals as well.[68, 69] In case of ethanol extracts, Chattopadhyay[21] reported the *in vitro* inhibition of serotonin by the leaf extract at 25 mg/mL concentration ($P < 0.05$) with no mention of the concordant blood glucose levels. But the study reported that a preparation of *A. indica* extract and glucose can trigger good amount of insulin (>12.0 µU/mg/15 min) secretion from isolated and cultured cells of the pancreas.[21] The leaf ethanol extract at 250 mg/kg double doses also reduced the urine sugar levels from 1 mg/dl to 0 mg/dl (100% reduction) and blood glucose level by 50% in alloxan-induced diabetic Charles Foster rats.[23]

A significant reduction was observed in case of STZ-induced diabetic rats oral administration of 200 mg/kg leaf ethanol extracts of *Veronina amygdalina* + *A. indica* (61%), *A. indica* (63.82%), and insulin control (66.0%) ($P < 0.05$).[27] In case of alloxan rats orally administered with 400 mg/kg of single and 200 mg/kg double doses, reduction more significant with *V. amygdalina* + *A. indica* (71.05%), *A. indica* (44.95%), and

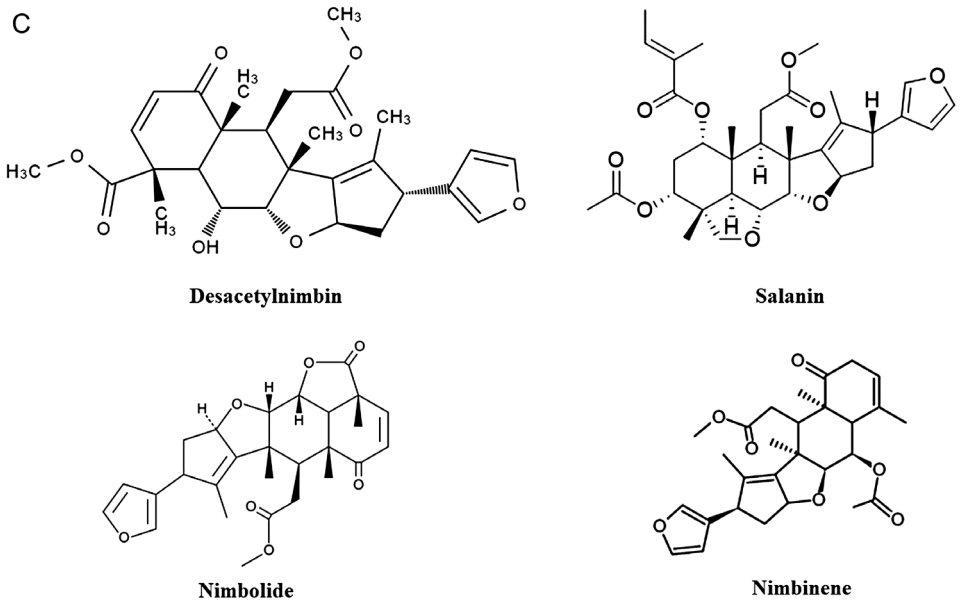

Figure 8 Continued

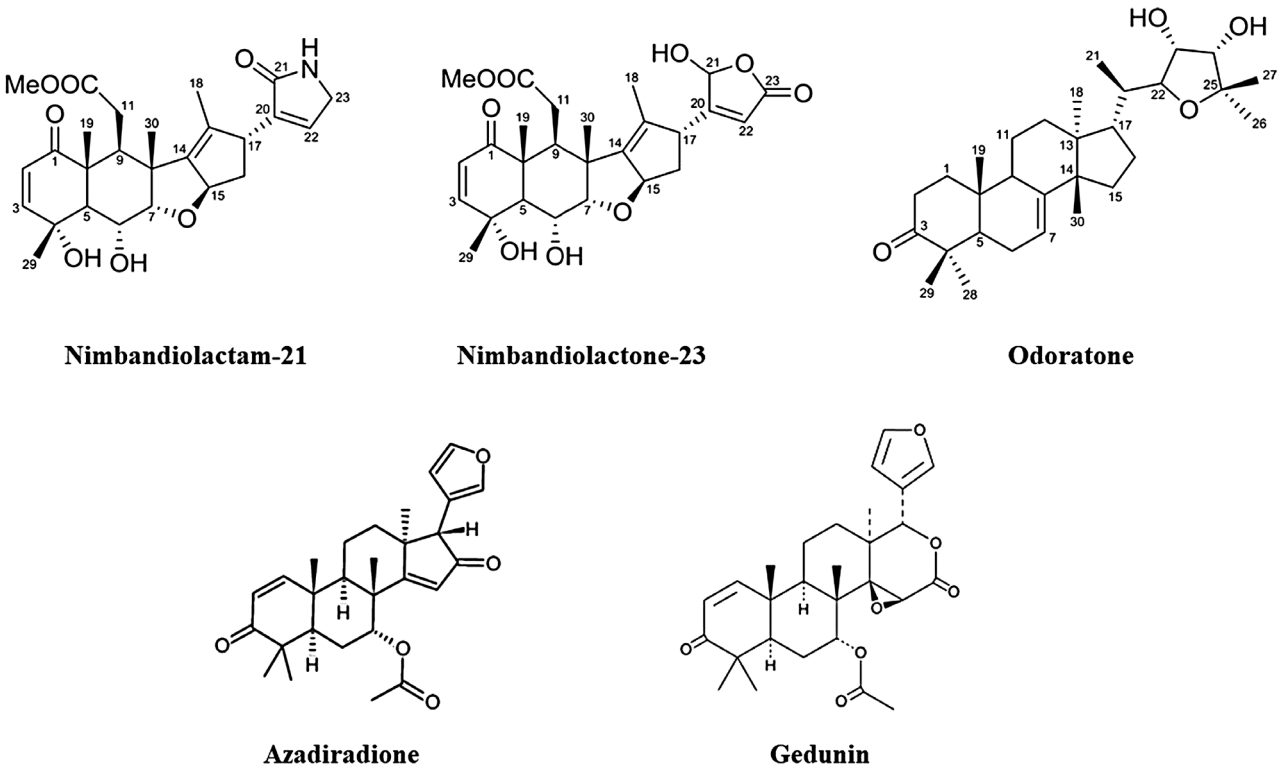

Figure 9 Limonoids reported with antidiabetic potential.

chlorpropamide control (75.83%) ($P < 0.01$). Liver glutamic pyruvic transaminase (GPT) and liver glutamic oxaloacetic transaminase (GOT) enzymes that increase the glucose content were decreased by 2.4, 2.2 and 3.5 times. Increasing protein breakdown also reported with this combination.[25] In contradiction, Sunarwidhi *et al.*[70] showed that combination of *A. indica* + *Gynura procumbens* (50 + 112.5 mg/kg, respectively) injected intraperitoneal reduced the blood glucose by 68.74% in pre-prandial and by 73.91% post-prandial conditions ($P < 0.05$). Results were in accordance with the glibenclamide control (0.45 mg/kg) that showed 70% blood glucose reduction activity in both pre- and post-prandial conditions.[70]

Extracts of *A. indica* have been reported to possess anti-hyperlipidemic properties. As per the epidemiological data, subjects with high fat diet are more prone to impairments in glucose metabolism, resulting in T2DM, insulin resistance or impaired glucose tolerance. The complete biological network of this mechanism has not yet to be elucidated.[71] A few of the studies have also deliberated on cholesterol and lipid

levels. In an *in vivo* study conducted by Zuraini *et al.*,[24] oral administration of leaf ethanol extract at 50 and 300 mg/kg reduced total cholesterol (TC, 38.96 and 51.41%), low-density lipoproteins (LDL, 68.33 and 71.3%), and triglycerides (TG, 36.64 and 53.39 %, respectively) levels except high density lipoproteins (HDL) in comparison with cholesterol control group in Sprague-Dawley male rats. The study reported no significant changes in C-reactive protein (CRP) concentrations, as the latter is involved in hyperglycaemic conditions.[24] Meanwhile, upon consumption of the same extract at 500 mg/kg concentration, STZ-induced diabetic rats showed the reduction in total cholesterol (TC, 26%), total lipids (TL, 16.0%), triglycerides (TG, 22.69%), low-density lipoproteins (LDL, 38.98%), very-low-density lipoproteins (VLDL, 27.5%) levels in diabetic rats treated with the extract compared to diabetic untreated control. Contrastingly, increasing level of HDL (12.5%) was found, indicating hypolipidemic efficiency of *A. indica* extracts in accordance with Kar *et al.*[23] Thus, it becomes important to investigate the molecular basis of hypolipidemic properties using dose-dependent, placebo-controlled studies either *in vivo*, *in vitro* and *in silico*, as hyperlipidaemia is directly associated with T2DM

Although various extracts at different concentrations have shown to be effective, the effects differ in many cases because of the discrepancies in the study design, formulations, and animal handling. Most of the studies share a single probable mechanism of antihyperglycaemic activity. Chattopadhyay[21] proposed that administration of leaf extract led to the possible of enhancement of insulin by inhibiting serotonin. Khosla *et al.*[22] reported the same but upon pre-treatment with the extract. However, Akinola *et al.*[26] came up with the histological evidence to prove that the plant extract improves the morphology of pancreatic islet that could possibly be damaged due to oxidative stress, hence escalating the insulin levels. This was supported by Sunarwidhi *et al.*,[70] who proposed that the presence of rutin protects the islets of Langerhans and β cells by inhibiting lipid peroxidation, hence decreasing the damage caused by reactive oxygen species (ROS) and escalating the antioxidant enzymes in the kidney of diabetic rats. This in turn increases the insulin production and hence the blood glucose levels. The same mechanism with no attribution to any compound was deduced by Siddiqui *et al.* (2016). Along with rutin, the possible action of phytochemicals including quercetin, myricetin, kaempferol, rutin and other glycosides has also been reported,[21, 26] along with nimbin and nimbidin.[28] In addition, mechanism of possible increased peripheral glucose utilisation has been put forth by Dixit *et al.*,[18] Bajaj and Srinivasan,[20] Khosla *et al.*,[22] where the glucose is efficiently absorbed in the gut with the help of the plant extract. But no practical evidence has been recovered on these reports, except the study by Bajaj and Srinivasan[20] who reported the possible effect on increasing the sensitivity of insulin receptor. It is noteworthy to mention the role of hematological parameters in blood glucose levels. There is a considerable relation between the hematological parameters including PVC, RBC count, haemoglobin, and platelet count. Chronic inflammatory state observed in DM is mainly due to the action of insulin on muscle, hepatic, and adipose tissues, which may promote differentiation and maturation of WBC through proinflammatory cytokines.[72] Diabetic complications like platelet dysfunction and increased clotting time in diabetic blood was addressed by El-Hawary and Kholief[19] who demonstrated the effect of leaf extract on the possible improvisation of platelet function. Meanwhile, it was also found to improve the chronic inflammatory condition by decreasing all the hematological parameters stated above.[32]

In general, future studies to use appropriate positive controls is suggested, which have been neglected in most of the studies selected for this review. Apart from the flaws in basic experimental design, there are conceptual drawbacks that are needed to be answered. For example, despite a colossal amount of research with the plant extracts, only a few of the studies could attribute the hypoglycaemic activities to the individual compounds and lack information on the mechanism of action. For example, the reported phytochemicals namely nimbin and nimbidin have no proven evidence to be declared as antidiabetic agents.[21, 26, 28] Thus, it becomes essential to conduct individual assessments of these phytochemicals to prove their antihyperglycaemic nature both *in vivo* and *in vitro* with respect to their interaction with agents like serotonin, advanced glycation end products (AGEs), and oxidative radicals. Ion scavenging activity also needs to be evaluated in this regard. In addition, two of the studies shed light on the role of hematological parameters but were not able to provide enough proof on underlying mechansisms.[19, 29] *In silico* methods for the identification of molecular mechanism behind the action of individual compounds are trending.

Furthermore, the studies which used double doses of *A. indica* in a day resulted in greater activity than single doses. For example, Studies conducted by Ebong *et al.*,[25] Akinola *et al.*,[26] Patil *et al.*,[28] Sunarwidhi *et al.*[70] have resulted in better reduction in the blood glucose levels in comparison with that of the single dose studies. Although it appears that higher doses result in better pharmacological activity, adverse effects including mortality with 300 mg/kg single dose should also be considered.[19] A lethal dose (LD_{50}) of 4.8 g/kg was reported by Ononamadu*et al.*[30] in case of leaf extract, where further consumption resulted in mortality. A detailed examination of the experimental design could reveal the minimum usage of antidiabetic drugs as controls.

Effects on oxidative stress

The amelioration of oxidative stress has been evaluated using various parameters, though the determination of antioxidant and oxidative enzyme levels has been carried out frequently. Most of the studies were performed using liver enzymes and reported the elevation or reduction in the activity of antioxidant/oxidative enzymes after treatment with *A. indica* extracts. The earliest study in this regard was conducted by Bopanna *et al.*,[33] in which seed kernel powder of *A. indica* was evaluated for its antioxidant activity in alloxan-induced diabetic rats. A significant reduction ($P < 0.001$) in the levels of serum alkaline phosphatase (ALP) and acid phosphatise (AP) was observed in case of seed kernel powder (250 mg/kg) and glibenclamide (0.25 mg/kg) supplementation (about 50.44%), seed kernel powder (500 mg/kg) alone (38.93%, 15.51%), glibenclamide (43.36%, 34.48%), and insulin (44.24%, 24.13%), in comparison with that of the diabetic control.[33]

However, a majority of the studies focused on the leaf aqueous extract which showed significant results regarding amelioration of oxidative stress. For example, in an *in vivo* study, Mahdi *et al.*[34] showed that leaf aqueous extract injected intraperitoneally at 500 mg/kg concentration increased about 20.16% of superoxide dismutase (SOD) in STZ albino rats,

compared to their diabetic control. But this was comparatively low where insulin (114.78%), and glibenclamide (70.58%) showed higher enzyme enhancement.[34] In another *in vivo study,* the same extract administered orally at 200 mg/kg concentration was found to enhance SOD by about 8.48%, catalase (CAT) by 6.04%, glutathione peroxidase (GPx) by 34.05%, and glutathione s-transferase (GST) by 121.33% in alloxan-induced diabetic rats, in comparison with diabetic untreated rats.[35] Chandra *et al.*[36] conceptualised the role of plasma metal ions in the elevation of antioxidant enzyme activity. Treatment with *A. indica* leaf aqueous extract orally on diabetic Sprague-Dawley rats induced using STZ restored the reduced levels of plasma metal-like copper (128%), and iron (36.0%). Significant increase in the levels of antioxidant enzymes like SOD (20.78%), CAT (15.45%), and glutathione (GSH, 11.53%) was also observed in comparison with diabetic untreated rats ($P < 0.05$).[36]

In an uncommon *in vitro* study by Shrivastava *et al.*,[39] the leaf aqueous extract further reduced the O_2^- anions in xanthine-xanthine oxidase enzymatic system by 26.8% at 200 µg concentration. In addition, OH ions in both enzymatic (hypoxanthine, $FeSO_4.7H_2O$, sodium salicylate, and xanthine oxidase) and non-enzymatic systems ($FeSO_4$, sodium ascorbate, H_2O_2, deoxyribose), were neutralised up to 19.3% and 36.0%, respectively. Serum lipid peroxide and GSH levels were lowered by 28.0% and 31% with the oral administration of 400 mg/kg concentration *in vivo*.[39] The oral administration of chloroform leaves extract at 300 mg/kg significantly increased the activity of enzymes like SOD (75.19%), CAT (17.82%), GSH (28.64%), glutathione disulphide/GSSG (36.76%), in comparison with STZ-induced diabetic control rats. 39.19% reduction in thiobarbituric acid reactive substances (TBARS) was also detected, in accordance with the glibenclamide standard used ($P < 0.05$).[38] In a completely different *in vivo* approach, *A. Indica* leaf aqueous extract administered orally at a single dose of (100 µg) concentration induced the activity of CYP3A, a drug metabolising enzyme ($P < 0.001$) in high fat diet induced rats. However, at both 250 and 300 mg/kg concentrations, the extract reduced ALP and aspartate transaminase (AST) levels in STZ Sprague-Dawley rats ($P < 0.05$).[27]

Contrastingly, the stem-bark aqueous extract could not enhance the enzyme levels of SOD, CAT, and LPO in comparison with hexane, dichloromethane, ethyl acetate, and butanol. Although feeble, the extract was able to show an IC_{50} value of 16.98 µg/mL against DPPH radicals *in vitro* against the gallic acid control.[41] The antioxidant potential of *A. indica* has been deduced even with the multi-herbal experiments. For example, Patel *et al.*[37]used a multi-herbal preparation consisting 10% of *A. indica* leaves known as 'Dihar', which increased the activity of antioxidant enzymes including GSH after the oral administration of Dihar at 100 mg/kg, along with reduction in lipid peroxidation detected by TBARS in STZ-induced type II diabetic rats ($P < 0.05$).[37] Administration of *A. indica* and *V. amygdalina*, which reduced the activity of ALT (by 22.70%), AST (65.91%), and GPx (49.18%). Further, in another *in vivo* study, a combined therapy using vanadate and *A. indica* leaf aqueous extract (500 mg/kg) given orally significantly decreased the level of lipid peroxidation and increased SOD levels ($P < 0.05$) in STZ-induced type II diabetic rats compared with control rats. Vanadate alone reduced lipid peroxidation and a corresponding weight loss. However, combined therapy resulted in higher antioxidant activity with minimal toxic effects.[40]

The multifactorial studies have resulted in a varying number of findings, principally in terms of enzyme levels with little knowledge on its mechanism owing to the lack of a single known mechanism for the management of oxidative stress. Though some of the studies have mentioned a few facts, they appear to be too far from the experimental proof. For example, Mahdi *et al.*[34] surmised the possible synthesis of antioxidant molecules by the extract hence the reduction of oxidative stress. To this date, there has been no concrete evidence to prove the ameliorative effects of *A. indica* extracts on oxidative stress.[34] Halim *et al.*[35] reported the synergistic effects of plant extracts in reverting lipid peroxidation and provided the evidence for reduction of diabetic retinopathy, the study fails to deliver the molecular inflammatory pathway for the same. Out of the 11 studies considered, only 1 study established the mechanism of action exerted by the herbal drugs. Chandra *et al.*[36] reported the role of herbal drugs in the modulation of an interstitial drug receptor pump p-glycoprotein, in turn regulating the hydraulic permeability in cells, resulting in the uptake of glucose and a possible activation of GSH synthase. Studies have demonstrated the role of metal ions in the activation of antioxidant enzymes and a study on this interaction could reveal the underlying mechanism.[36] Presence of flavonoids and tannins in Dihar is believed to produce antioxidant effects with an undefined mechanism of action.[37] Similarly, a possible effect of polyphenols in *A. indica* on lipid peroxidation and enhancement of antioxidant enzyme activity is reported with lack reasoning on its mode of action.[40] Overall, the studies conducted to evaluate the ameliorative effects of *A. indica* were principally based on the activities of liver enzymes. Most of the studies deliberated on the evaluation of either the antioxidant enzyme levels or that of the oxidative enzymes. Apart from discrepancies found in the results that might be a result of variations in study designs and experimentation, there are a few limitations that could be addressed. Besides, studies reviewed in this section lack proper use of positive controls and flaws in the basic experimental design including dose-dependent assessments. In conceptual limitations, most of the studies have not performed the TBARS and MDA tests that could result in the efficient elucidation of lipid peroxidation levels.[36, 38, 73] Except a few,[36, 38, 73] none of the studies highlighted the ROS level before and after the consumption of *A. indica* preparations. In addition, a few studies have not represented the statistical significance in numbers, which could have been helped evaluating the accurate antioxidant potential of the extract.[28, 40–42] Also, appropriate positive controls (chemotherapeutic agents) and negative controls (diabetic untreated or saline-treated animals) need to be used in the studies. Owing to a great deal of damage caused by ROS, it is one of the main targets while carrying out studies on the management of diabetic complications.

Studies need to focus on deducing the molecular mechanisms of drug action using *in silico* methods like molecular docking. Thus, isolation, characterisation, and *in silico* evaluation of antioxidant phytochemicals from *A. indica* could be a breakthrough. Although a few studies have reported the possible role of phytochemicals like flavonoids and tannins,[28] polyphenols,[38] flavanol glycosides, and quercetin,[42] their interactions with the liver enzymes along with their cytotoxic effects are yet to be elucidated. Considering these limitations, it could be a better approach to develop more plant-based drugs like Dihar, which could be evaluated in dose-dependent clinical trials. Though it appears that the combined extracts

of different plants may outrun *A. indica* extracts in terms of pharmacological efficiency, it is noteworthy to mention the other studies which have assessed the extracts of *A. indica* alone. Therefore, considering these recommendations, it could be feasible to develop *A. indica* -based herbal drug for DM.

Effects on diabetic complications

Diabetic complications primarily arise because of the generation of ROS that in turn damage the tissues and organs. However, there has been no deduction of a specific mechanism to edify these broad-spectrum aberrations. Majority of the studies followed the cardioprotective property of *A. indica*. The earliest of them was conducted in 1967 where, increasing oral doses of leaf aqueous extract were able to reduce the heartbeat rate in anesthetised rabbits. A dose of 200 mg/kg greatly reduced the heartbeat rate from both pre-drug and post-drug administration conditions. However, there was no effect on the heartbeat of guinea pigs upon the consumption of the extract. The extract also decreased arterial blood pressure in anaesthetised rabbits and was more effective on diastolic pressure than systolic. However, 5 and 10 mg/kg extract increased the blood pressure in guinea pigs, whereas 40 mg/kg induced mortality. A double dose of 20 mg/kg normalised ouabain-induced cardiac dysrhythmia within 8 min.[74]

Conversely, cats and frogs intravenously treated with 200 mg/kg of leaf ethanol extract failed to alter the sensitivity of α- and β-adrenergic, cholinergic, or histaminergic receptors, yet reducing the blood pressure. No significant changes in the amplitude of cat respiration were observed. The extract resulted in cardiac arrest in diastole at 1–10 mg concentration in case of frogs.[44] The cardioprotective activity was proved again *in vivo*, when *A. indica* leaf aqueous extract significantly elevated the cardiac content of reduced GSH in STZ-induced type II diabetic rats by about 38% ($P < 0.001$) while the lipid per oxidation (LPO) was reduced by about 27% ($P < 0.001$). Histological examination revealed the absence of inflammation and fibrosis, restoring the deformed muscles. Transmission electron microscope (TEM) analysis showed the rearrangement of myofibrils, reformation of swollen mitochondria, and absence of glycogen.[74] Omóbòwálé *et al.*[51] showed the efficacy of orally administered *A. indica* leaf methanol extract in reducing xanthine oxidase levels by 16.07% and 12.05% at 100 and 200 mg/kg, respectively in rats that underwent ischaemia-reperfusion injury. Also, about a reduction of 51.82% and 67.41% myeloperoxidase was observed in case of 100 and 200 mg/kg of the extract, respectively ($P < 0.05$).[51]

However, the literature survey could map only a few studies related to the other diabetic complications. Apart from cardioprotective activity, *A. indica* has also proved its efficiency in treating diabetic retinopathy *in vivo*. Upon oral consumption of 250 mg/kg of leaf aqueous extract, reversal of diabetic retinopathy observed with normalisation of dilated vessels of STZ-induced type II diabetic rats.[45] In case of diabetic nephropathy, *A. indica* leaf ethanol extract at 500 mg/kg administered orally reduced the glomerular lesions including nodular glomerulosclerosis, also known as Kimmelstiel-Wilson disease, in STZ male Wistar rats.[49] In an exceptional *in vivo* study conducted by Gautam *et al.*,[50] leaf ethanol extract of *A. indica* (500 mg/kg) given orally to STZ-induced type II mild diabetic rats showed an increase in the vascular endothelial growth factor (VEGF) level along with a decrease in tumour necrosis factor-α (TNF-α) and interleukin-Iβ (IL-1β) ($P < 0.1$ to $P < 0.01$) levels in comparison with diabetic rats and in accordance with glibenclamide standard. The extract also levelled up the granular tissue weight, which was decreased in case of diabetic rats in accordance with that of the controls through collagen synthesis ($P < 0.05$ to $P < 0.001$). In addition, the extract induced the formation of new capillaries, eosinophilic collagen tissue, and neovascularisation with few inflammatory cells, indicative of healing by fibrosis and newly formed blood vessels, along with the reduction in the number of mononuclear inflammatory cells.[50] In support of this, in another *in vivo* study, a significant decrease in WBC, neutrophils, CD4, lymphocyte levels was observed in rats oral administration of *A. indica* leaf ethanol extract (400 mg/kg), in accordance with that of the control used ($P < 0.05$).[48]

The efficacy of *A. indica* in ameliorating intestinal aberrations *in vivo* came to the light when Akinola *et al.*[47] showed that the intestinal epithelium was found to be intact and well-functioning in STZ-induced type II diabetic rats treated orally with both *A. indica* leaf ethanol extract of 500 mg/kg and glibenclamide control during histological examination. Whereas, the STZ untreated control showed intestinal cell necrosis, reduced mucin secretion, poor secretion of goblet cells, erosion of surface epithelium, absence of mucus lining on the lumen.[47] In case of pancreas, the same extract given to Wistar rats through gavaging significantly reduced the fibrosis and necrosis of the islet-b cells in STZ type II diabetic rats at the end of the experiment (50 d). Rats treated with the extract showed highest viability of islet-β cells (50%), in accordance with glibenclamide control used ($P < 0.05$).[49]

Furthermore, *A. indica* was evaluated for its neuroprotective effects in two *in vivo* studies. In one study, *A. indica* leaf aqueous extract given orally at 200 and 400 mg/kg concentration increased the paw withdrawal threshold, whereas 100, 200 and 400 mg/kg concentration increased the paw and tail withdrawal latency in rats with a concomitant increase in motor nerve conduction velocity. After the administration of 200 and 400 mg/kg, neural calcium level and neural TNF-α, IL-1β and NF-κB were reduced, along with the downregulation of neural Bax, Caspase-3, and iNOs mRNA expression. Yet again, ROS was significantly decreased, induced axonal degeneration and histopathological alterations were reverted. All the results were significant in comparison with partial sciatic nerve ligation (PSNL) induced rats ($P < 0.05$).[52] In another study, 750 mg/kg of *A. indica* dried flower powder given orally significantly increased the sciatic function index (SFI) on postoperative day 18 ($P < 0.05$) and 21 ($P < 0.00l$), which was found to be better than surgical intervention group of STZ-induced type II diabetic rats. 500 mg/kg treatment also significantly increased SFI on day 21 compared with the diabetic untreated rats ($P < 0.01$). The extract at 750 mg/kg treatment, a significantly shorter paw withdrawal latency (PWL) on postoperative day 18 and 21 ($P < 0.05$) was observed in accordance with the control used. However, all the doses exhibited an increase in the average falling latency, yet with no significant difference. Further, MDA levels were also significantly reduced in rats treated with both the concentrations along with the increased axon density.[53]

A diverse nature of complications is addressed in these studies and unlike other pharmacological investigations most

of them have provided satisfactory mechanisms to decipher the actual role of *A. indica* in ameliorating these complications. Although a majority of the studies indicate the role of oxidative stress, there are a few studies that have listed the role of other pathological mechanisms. For example, the earliest study by Ulrich and Bräunlich[43] surmised the possible effect of the extract on vascular smooth muscle, giving rise to vasodilation due to the reduction in arteriolar tone, inotropic and chronotropic effects suggesting a direct effect on vasoconstriction. Although Chattopadhyay[44] stated the possible cardiac-depressant and hypotensive effect of flavonol-O-glycosides, the study failed to reveal the actual underlying mechanisms of cardio-protectivity.[44] Yet another study by Gupta *et al.*[74] attributed the antioxidant and anti-inflammatory activities of terpenoids which includes azadirachtin, azadiradione, nimbin, nimbolin, nimbolide, nimbinene, desacetylnimbin, azadirone and salanin, that are present in *A. indica*.[74] However, a precise explanation was given by Omóbòwálé *et al.*,[51] where *A. indica* phytochemicals are reported to activate the extracellular signal-regulated protein kinases 1 and 2 (ERK 1/2) signalling pathway which in turn activates the reperfusion injury salvage kinase (RISK) pathway, ultimately healing the intestinal ischaemia-reperfusion injury (IIRI). *A. indica* phytochemicals prevented IIRI-induced cardio-renal dysfunction via reducing oxidative stress, improving the antioxidant defense system and increasing ERK1/2 expressions.[51]

In case of diabetic retinopathy, though Eshrat and Hussain[45] showed the efficacy of *A. indica* in normalisation of retinopathic eye, the study did not provide information about either the source of the complication or the mechanism of normalisation by *A. indica*. Though Ekaidem *et al.*[48] supported the concept that flavonoids and triterpenoids possess remarkable antioxidant, anti-inflammatory and cytoprotective properties by showing the ability of *A. indica* in decreasing immune cells, a concrete evidence is yet to be provided. Further, a series of studies proved the gastroprotective activity of *A. indica*. Among them, Dorababu *et al.*[46] reported the possible involvement of flavonoids in the restoration of mucin secretion activity through the reduction of acid-pepsin secretion and output in H^+/K^+ -ATPase which pumps protons in exchange for potassium ions across the apical membrane.[46] In addition, role of ROS- and AGE-mediated mechanisms in the intestinal aberrations, causing arteriosclerosis of vessels of the small bowel, with thickening of vessel wall, narrowing of their lumen, and the resultant mucosal injury was surmised by Akinola *et al.*[46] Similarly, possible enhancement of islet-b cells by the phytochemicals through the upregulation of glucose-6-phosphate dehydrogenase (G6PD) produced in islet-b cells was reported. The enzyme is reported to enhance DNA synthesis by activating ribose-5-phosphate (R-5-P), which in turn aids in nucleotide synthesis.[49] Furthermore, along with neuroprotective effects the possible antioxidant, anti-inflammatory, anti-apoptotic potential of *A. indica* were reported.[52] Likewise, the second study reported the possible antioxidant potential of quercetin.[53] However, no specific mechanism was deduced by either of the studies.

The detailed analysis of the experimental design pointed towards a few gaps in research. In case of basic experimental design, we still emphasise on suggesting the use of appropriate positive controls as well as conducting dose-dependent evaluations of extracts. Even with the provision of suitable underlying mechanisms to support their theory, a few studies fall back owing to the lack of appropriate controls.[43] For instance, Chattopadhyay[44] was able to prove the null effect of *A. indica* extract on α- and β-adrenergic, cholinergic, or histaminergic receptors, which in turn reduces the blood pressure. Though the experiment was designed well, study of blocking these receptors *in vitro* and *in silico* could use a beneficial aspect.[44] In these pioneer efforts, usage of antidiabetic therapeutics as control, and DM-induced animal models has been neglected. Further, while proving the protective ability of *A. indica* on diabetic nephropathy, Eshrat and Hussain[45] did not provide any mechanism behind the protective activity with lack of statistical data. Though histological examination has been done, there is no provision of statistical data primarily because this is a qualitative examination. However, the author could have examined the rat behaviour/movements towards a target with or without healthy eye.[45] Similarly, Akinola *et al.*[47] demonstrated the gastroprotective ability of the extract with no description on its mechanism although the protection against ROS mediated pathway was expected to play a role. The study could have used the diagnosis of ROS and AGEs to track down their amelioration by phytochemicals along with the aid of *in silico* studies. Upon treatment with *A. indica* extracts, cell density in pancreas, stomach and intestine are expected to grow up. In contrast, islet b-cell density in the diabetic and glibenclamide-treated diabetic rats remained relatively low. However, it has been a laudable approach by the investigators that they have performed *in vitro* cell assessment to analyse the effect of *A. indica* extract on islet-b cells. The study has also given the statistical inference where 50% viability of islet-b cells has been recorded.[49] Furthermore, a study conducted by Omóbòwálé *et al.*[51] has revealed the role of ERK 1/2 and RISK pathways in the amelioration of IIRI, a complication related to heart cell injury. Although histological evidence has been provided, the study underperforms to give a concrete proof for the involvement of ERK 1/2 and RISK pathways. We suggest that an *in silico* proof would be better to prove the role of phytochemicals in the enhancement of both pathways.[51]

Effects on carbohydrate digestion enzymes

Several studies have been conducted on the effect of *A. indica* on carbohydrate digestive enzymes that are present in different organs present in alimentary canal including pancreas, liver and intestine. The earliest report by Bhat *et al.*[54] suggests that *A. indica* extracts (0.125 mg/mL) did not inhibit porcine α-amylase and murine pancreatic glucosidase. However, in case of murine small intestinal glucosidase, aqueous and methanolic extracts of *A. indica* inhibited the enzyme by 62.44 and 41.07%, respectively. In case of murine liver glucosidase, methanolic and aqueous extract of *A. indica* showed an inhibition of 52.11% and 69.29%, respectively. Methanolic (IC_{50}: 2.60 and 1.80 µg/mL) and aqueous extract (IC_{50}: 3.17 and 6.21 µg/mL) showed inhibition with murine liver and intestinal glucosidases, respectively, significant than acarbose.[54] In another study, about 48.65% of reduction was observed in the serum amylase(extracted from rat) activity upon consumption of 400 mg/kg *A. indica* leaf aqueous extract orally ($P < 0.05$).[55] In addition, acetone, ethanol, and aqueous extracts (250 µL each) were assessed for their effect on carbohydrate digestive enzymes by Kazeem *et al.*[75] The aqueous extract inhibited the activity of α-amylase significantly (IC_{50}: 9.15 mg/mL) compared with that of the acetone (IC_{50}: 10.62 mg/mL), and ethanol (IC_{50}: 9.5 mg/mL) extracts. Conversely, acetone extract showed higher inhibitory effect on

α-glucosidase (IC_{50}: 5.0 mg/mL), in comparison with that of the ethanol (IC_{50}: 8.70 mg/mL), and aqueous (IC_{50}: 7.25 mg/mL) extracts. In a different approach, *A. indica* yogurt was prepared from leaf aqueous extract and milk. It was analysed for its effect on the carbohydrate digestive enzymes. Fresh *A. indica*-yogurt formulation (500 µL) inhibited α-amylase by 44.4% on day 0 yet had higher (29.8%) and significant ($P < 0.05$) inhibitory effect than plain yogurt. However, refrigerated storage increased α-amylase inhibition activity of *A. indica*-yogurt to 55.0% by day 21, which reduced to 42.0% by day 28. Both yogurts showed similar inhibition (15%) on α-glucosidase on day 14.[58] Similarly, ZnO nanoparticles synthesised from aqueous extract of *A. indica* (1.56–100.0 µg/mL) significantly inhibited α-amylase and α-glucosidase enzymes ($P < 0.05$).[60]

Apart from leaves, root, stem-bark extracts have also been assessed for their effects on carbohydrate digestive enzymes. Around 10 types of carbohydrate digestion enzymes including 6 intestinal and 2 fungal enzymes were evaluated with 10 mL of methanolic extract of root (IC_{50}: 6.0–83.0 µg/mL), aqueous extract of root (IC_{50}: 11.5–88.0 µg/mL), methanolic stem-bark extract (IC_{50}: 10.5–85.0 µg/mL), and aqueous stem-bark extract (IC_{50}: 14.5–89.0 µg/mL) showing varied inhibition. IC_{50} values of pancreatic amylase were not significant in both types of the extracts (IC_{50}: >100 µg/mL). Nimbidiol (IC_{50}: 0.85–30.0 µg/mL) showed a greater inhibitory activity than the controls used.[57] The study did focus on only one phytochemical, i.e., nimbidiol. Similarly, a novel tetranortriterpenoid named meliacinolin was identified and isolated from the aerial parts of *A. indica*. Meliacinolin was reported with a significant inhibition of α-amylase at IC_{50}: 46.74 µg/mL in comparison with acarbose at IC_{50}: 12.23 µg/mL. While α-glucosidase was inhibited at IC_{50}: 32.18 µg/mL the values were compared with acarbose at IC_{50}: 78.54 µg/mL. Addition of meliacinolin also increased the insulin production significantly ($P < 0.01$) in comparison with that of glibenclamide.[56]

Focus on the assessment of phytochemical composition of the extracts provides promising evidence in identification of lead drug targets and therefore herein, a few studies that have focused on various phytochemical compounds present in *A. indica* are listed. For example, Ponnuswamy *et al.*[59] isolated 9 novel limonoids from *A. indica*. Out of the 9 limonoids isolated/semi-synthesised and screened for human pancreatic α-amylase inhibition, azadiradione (22.2–133.1 µM) and gedunin (20.7–124.3 µM) exhibited *in vitro* inhibition with an IC_{50} value of 74.17 and 68.38 µM, respectively. Further screening on AR42J α-amylase secretory cell line for cytotoxicity and bioactivity revealed that azadiradione and gedunin exhibited cytotoxicity with IC_{50} of 11.1 and 13.4µM, respectively.[59] Similarly, from ethyl acetate-soluble fraction of *A. indica* leaves, 3 novel limonoids were isolated, including a new lactam 28-norlimonoid, nimbandiolactam-21, along with 2 other limonoids, odoratone, and nimbandiolactone-23. Nimbandiolactone-23 showed the most potent α-glucosidase inhibitory activity, with an IC_{50} value of 38.7 µM.[61] Use of *in silico* approach to prove the antidiabetic efficacy of phytochemicals could be a laudable approach in phytotherapy. We could find only a few studies that have performed molecular docking of the reported phytochemicals with the targets. For example, Sanni *et al.*[41] isolated a few compounds from different extracts of *A. indica* that showed significant inhibition of both α-amylase and α-glucosidase ($P < 0.05$). The *in silico* binding efficiency of the molecules reveals that the phytochemicals sitosterol, stigmasterol and squalene (from ethyl acetate and butanol extract) have inhibitory potential against both the enzymes.[41] However, a near perfect approach was given by Mazumdar *et al.*,[62] where the α-amylase inhibitory activity was studied *in silico*. The inhibitory activity of gedunin on pancreatic α-amylase varies from 12.66% to 49.64% in the concentration range of 5 µM to 20 µM. Yet there was inhibition of 18.75% to 36.5% of α-amylase by acarbose. However, IC_{50} values of gedunin and acarbose were found to be 20.25 µM and 31.12 µM, respectively. Gedunin inhibited salivary α-amylase by 27.48%, 32.6% and 38.27% after the treatment of 5, 10 and 20 µM concentrations, respectively. However, the IC_{50} values of gedunin and acarbose were 36.34 µM and 15.74 µM, respectively. Gedunin also showed significant binding efficiency in docking studies.[62]

The studies on the inhibition of carbohydrate digestive enzymes have depicted the efficiency of *A. indica* over several chemotherapeutics used conventionally. Most of them used acarbose as a positive control, where it was proved that *A. indica* either outwitted it or was in accordance with it, in terms of extent of enzyme inhibition. The pioneer studies focused only on pure extracts and they did not reveal the mechanism behind the antidiabetic effect. Akpan *et al.*[55] revealed that *A. indica* extract does not inhibit α-amylase directly. Instead, it is believed to enhance the number of β-islet and acini cells, which in turn stimulates insulin, ultimately reducing the α-amylase activity. Earlier, Bhat *et al.*[54] also reported the inefficiency of *A. indica* extracts to inhibit murine pancreatic glucosidase. However, advanced studies that have used purified phytochemicals have reported an effective inhibition of the pancreatic amylase.[59, 62]

Most of the studies used commercially available enzymes for the study, which could be supported by a parallel study on the enzymes from animal models. It is a laudable approach by many of the studies that they have pointed the efficiency of phytochemicals in the inhibition of carbohydrate digestive enzymes. For example, meliacinolin is believed to reduce the hyperglycaemia by inhibiting the α-amylase and α-glucosidase activities.[56] There were a few assumptions about the possible inhibitory activity by flavonoids and tannins, and by nimbidiol, which has efficiently inhibited maltase-glucoamylase, sucrase-isomaltase, lactase and trehalase.[57, 75] Further, it was reported that efficient binding of both gedunin and azadiradione leads to the reduction in the activity of human pancreatic α-amylase. Thus, the two limonoids could be lead candidates in reducing hyperglycaemia.[59] Furthermore, a study by Mazumdar *et al.* (2020) reported the efficacy of gedunin. A molecular chaperone termed Hsp90, which is said to be a promoter of tissue damage in diabetics, is inhibited by gedunin as well as acarbose. However, amino acid sequence of acarbose and gedunin are matchable. This implies that gedunin can recognise and generate a stable conformation near the binding site of the enzyme therefore presenting similar benefits as acarbose. The interaction with amino acids such as Trp58, Trp59, His201, Asp197, and Asp300 are involved in diverse pathogenic mechanisms.[62]

A dose-dependent evaluation for the efficiency of these extracts is lacking. Although several antidiabetic drugs like acarbose, voglibose, miglitol, glibenclamide, salacinol,

kotalanol, mangiferin, and 1-deoxynojirinmycin are available, a few studies have used distilled water, and some others did not use the controls in their experiments.[58, 60, 75] In addition, studies have concentrated on α-amylase more than any other enzymes. Further, it becomes important to analyse the level of insulin secretion upon inhibition of these enzymes. Studies have not focused on insulin secretion, which is regarded as one of the key ingredients in antidiabetic aspects. With studies reporting the ameliorative activity of *A. indica* extract on islet-b cells, it is important to analyse the secretion of insulin.[55] Furthermore, a few studies have reported the efficiency of phytochemicals in the inhibition of carbohydrate digestive enzymes.[56, 57, 75] With the suitable and concrete evidence on binding of the reported phytochemicals with targets, it could be easier to define the antidiabetic potential of the reported phytochemicals.

Effects on oral glucose tolerance

Studies on the effect of *A. indica* on oral glucose tolerance have been linked with the assessment of hypoglycaemic effect of the *A. indica*. In this review we look forward to highlighting the effects on oral glucose tolerance in a separate section, as the experimental designs for blood glucose analysis and oral glucose tolerance analysis have been completely different. However, compared to all the pharmacological evaluations in DM, oral glucose tolerance test has been evaluated in a negligible number of studies.

In an *in vivo* case of glucose tolerance test, both methanol and aqueous extracts of *A. indica* leaves (100 µg/200 µL) given orally have the potential to reduce the glycaemic concentration after the treatment. In fact, the extracts can tolerate the glucose level after 120 min of treatment in STZ-induced type II diabetic rats. All the results were in accordance with controls used ($P < 0.05$).[64] Yet another study with leaf aqueous by Chaudhari *et al.*[42] showed that a dose of 250 mg/kg reduced the glucose level after 1 hour. This was significant compared to glipizide. *A. indica* extract alone reduced the glycaemic level at 120 min significantly ($P < 0.001$).[42] Furthermore, in another *in vivo* study, root ethanol extract of *A. indica* given orally reduced the blood sugar level and increased glucose tolerance at 200 and 400 mg/kg concentration, yet insignificant in comparison with glibenclamide in alloxan diabetic rats. However, 800 mg/kg administration led to a significant increase in the glucose tolerance. This was also considered as insignificant in comparison with glibenclamide.[28]

The mechanisms exhibited by the extracts on oral glucose tolerance are like those from blood glucose regulation. A few studies have not reported the underlying mechanisms for oral glucose tolerance of *A. indica* extracts, whereas a few other studies have attributed the activity to the phytochemicals present. In case of Dianex, a polyherbal drug prepared from the extracts of *Momordica charantia, Cassia auriculata, Azadirachta indica* and *Aegle marmelose*, the increased glucose tolerance has been attributed to the antidiabetic potential of these plants with no further studies to substantiate these findings.[64] Further, Gutierrez *et al.*[38] hypothesised the role of increasing of peripheral utilisation of glucose by the extract, thus reducing insulin resistance. This leads to the elevation of glucose tolerance in animals.[38] Furthermore, a triterpenoid named nimbidin was reported to reduce the glycaemic content, which was extracted from the root ethanol extract of *A. indica*.[28] In the same context, a flavonoid known as quercetin is also attributed for its antihyperglycaemic property. The study also indicates the possible regeneration of islet-b cells to enhance insulin secretion.[42] However, the discrepancies in the experimental design have been different in both approaches.

Despite achieving a significant control on insulin resistance and hyperglycaemic conditions, these studies lack a few research elements that could be dealt in future. Like we suggested the flaws in the basic experiment design, it becomes important to include positive controls and dose-dependent assessments during the evaluation. Assessment of the phytochemicals present and their mode of action could be a concrete proof for the herbal products in order to replace the conventional antidiabetic chemotherapeutics.[37, 64] In addition to these studies, another study conducted by Bhat *et al.*[63] depicted the effect of aqueous extract on glucose tolerance without mentioning the mechanism behind the activity.[63] Although a study conducted by Gutierrez *et al.*[38] attributed the effect on glucose tolerance to increasing of peripheral utilisation of glucose by the extract, the study did not indicate the actual mechanism behind the activity. Also, these studies need to provide the evidence with reference to the enhancement of insulin synthesis and secretion.[38] Furthermore, a few recent studies have been successful in providing significant evidence. For example, Patil *et al.*[28] have indicated the role of nimbidin in hypoglycaemic effect. Yet, it also becomes important to give more proof on its mode of action. Therefore, *in silico* approaches would be beneficial in proving the involvement of nimbidin. This applies to the study conducted by Chaudhari *et al.*[42] as well, for its projection of the possible role of quercetin in hypoglycaemic activity.[28, 42] In the end, we also suggest the researchers to inspect the release as well as the level of insulin after the end of the oral glucose tolerance test. With these suggestions, one can effectively represent the antidiabetic studies conveniently in future.

Effects on glucose uptake

Although a colossal amount of literature is available on other pharmacological evaluations on of *A. indica* in the treatment of DM, we could get only a few studies that have performed oral glucose tolerance test and glucose uptake. Both the sections contain minimal number of studies that have been reviewed and represented. During the literature survey, a few multi-dimensional studies involving various experiments regarding the antidiabetic potential of the plant were observed. However, herein we review and represent the studies that have performed glucose uptake as a part of their complete experimental design. Noipha *et al.*[65] showed that *A. indica* flower aqueous extract at 10, 25 and 50 µg/mL concentrations increased the glucose uptake by 1.17, 1.19 and 1.02 fold over basal levels, respectively in case of rat muscle L6 cell line *in vitro*.[65] In addition, in an *in vivo* study, chloroform extract of *A. indica* (300 mg/kg) given orally was reported to reduce the expression of G6Pase activity in STZ-induced type II diabetic rats. The activity of G6Pase enzyme was increased, which in turn led to the increase of hepatic glycogen as well as decrease the hepatic glucose levels.[38] Further, in an *in vivo* study, aqueous extract of *A. indica* (400 mg/kg) given orally to STZ-induced type II diabetic rats increased the expression of GLUT4 protein by 1.6-fold in both cytosolic and plasma membrane fractions. The extract also maintained the protein level at 1.6-1.8-fold.[66] Furthermore, in an *ex vivo* study conducted by Sanni *et al.*,[41] psoas muscle sample of the rat was taken for thw analysis of glucose uptake. Out of 5 difference fractions studied, butanol fraction showed significant

glucose uptake (GU_{50} of 6.22 µg/mL). Whereas the aqueous extract showed 43.98.[41] In another *in vitro* study, with an antidiabetic tetranortriterpenoid named gedunin, Mazumdar *et al.*[62] showed that glucose uptake in the presence of 10 and 20 µM gedunin was found to be 30.18% and 21.76% in yeast cells. The results were reported to be significant and in accordance with the control used.[62]

The studies conducted on glucose uptake have focused on different factors that are responsible for glucose reabsorption and utilisation in the body. Therefore, a plethora of mechanisms could be expected from this aspect. However, due to the unavailability of fine and diverse literature, we could only represent few of the studies in this review. Noipha *et al.*[65] and Gutierrez *et al.*[38] have not produced any of the mechanism behind the increasing of glucose uptake. However, Satyanarayana *et al.*[66] hypothesised the fact that restoration of fatty acids from elevated level to normal level in diabetic animals could have been the reason behind the amelioration of glucose transporter type 4 (GLUT4) mRNA expression. Increased expression of GLUT4 could be one of the prominent ways to deal with the increased hyperglycaemic conditions and a corresponding increase in glucose reabsorption.[66] Sanni *et al* suggests the role of β sitosterol in the enhancement of GLUT4 expression.[41] Conversely, gedunin has been declared as the antagonistic molecule, as it was found to inhibit the glucose uptake process. Mazumdar*et al.*[62] reported that gedunin inhibits glucose uptake, which has been regarded as a debacle in the process of glucose uptake. As GLUT4 acts as a principle glucose transporter in the cell, its relation with gedunin needs to be elucidated.[62]

However, it becomes essential to highlight some elements in these studies that need to be refined. Most of the studies did not use proper positive control and dose-dependent methods for the evaluation of glucose uptake. This refinement chiefly involves improvisation in experimental design and data interpretation. For example, in the end of the study researchers need to provide substantial evidence supporting their hypothesis. Yet, many of them fail to deliver it in a proper way. In addition, the researchers advised to use pancreatic or intestinal cell lines, instead of others because DM occurs because of the depletion of pancreatic islet-b cells. Therefore, it could be a better approach if *in vitro* studies use either pancreatic or intestinal cell lines. Also, only a few studies were able to mention the role of GLUT4, a principle protein involved in glucose uptake. In addition to GLUT4, other glucose transporters like SLC2A12, SLC2A6, GLUT3, etc. must be exploited.

Toxicology

To advocate any phytomedical formulation, the suitability of it needs to be evaluated. The toxic effects of the therapy need to be understood using cell lines and animal models. In this regard, the leaves, seeds and stem of *A. indica* are evaluated for their toxic effects. During the assessment of blood glucose level, El-Hawary and Kholief[19] reported that oral administration of leaf aqueous extract at high dose over 600 mg/kg in alloxan rats resulted in toxic effects such as loss of appetite, weight loss and mortality. Although the extract at 400 mg/kg normalised the blood glucose level, higher doses of the extract resulted in adverse effects.[19] Further, during the evaluation of antioxidant activity in a combined therapy of *A. indica* leaf aqueous extract with vanadate minimal toxic effects was observed in STZ male Wistar albino rats. Rats treated with 500 mg/kg have shown adverse effects, which have not been described by the authors.[40] Furthermore, terpenoids azadiradione and gedunin exhibited cytotoxicity with IC_{50} of 11.1 and 13.4µM, respectively during the *in vitro* assessment of carbohydrate digestive enzyme inhibition.[57]

However, it appears that consumption of *A. indica* could lead to health aberrations during pregnancy. Several studies have proved the contraceptive activity of *A. indica,* which has been popularly known for its spermicidal activities on several organisms. In fact, several studies have proven to be lethal for organisms like mice and snail.[76, 77] In pregnant rats, neem oil and azadirachtin had no hypoglycaemic and antihyperglycaemic effects. They also elevated the proportion of fetuses classified as small for pregnancy age (SPA) in all groups. Conversely, both neem oil (0.6 mL double dose) and azadirachtin (0.5 mg/mL) increased oxidative stress through increasing lipoperoxidation, which was characterised by increased MDA levels. However, *A. indica* extract is believed to play no role in the amelioration of reactive oxygen species (ROS) that are generated by increased metabolic activity in the placenta mitochondria. Further, consumption of stem-bark ethanol extract unexpectedly increased the weights of Wistar rat body organs like liver, kidney, lungs, and heart as well as their organ-body weight ratios.[78] Therefore, it is advised not to continue the *A. indica* treatment during pregnancy.

In addition, serum globulins, total and conjugated bilirubin, serum cholesterol, low-density lipoprotein cholesterol and computed atherogenic index increased significantly after the treatment. Apart from ALP, AST enzymes, metal ions like sodium, calcium and potassium as well as feed and water intake were also altered at specific doses. In total, these alterations have raised the chances of toxicity, which may have consequential effects on the normal functioning of metabolism. Thus, the ethanolic extract of *A. indica* stem bark at 50, 100, 200 and 300 mg/kg concentrations may result in adverse effects, if consumed incessantly in rodents.[79] But in case of adult humans, these doses may not be deleterious, as observed in an assessment of hypoglycaemic potential of *A. indica* aqueous extract using human blood cells without stating evidence of cytotoxicity.[80] Yet, some of the studies have also proved the adverse effects of the extracts in children such as neem oil poisoning causing vomiting, hepatic toxicity, metabolic acidosis, and encephalopathy.[81] Therefore, it is recommended that any form of *A. indica* should be consumed with regular intervals in between successive consumptions. We also suggest that a greater number of studies need to be conducted using human cell lines to evaluate the effect of *A. indica* on humans. Dose-dependent evaluations on different age groups and sexes need to be done. Regarding dosage, age-related dosage charts need to be prepared with special reference to pregnant women, who could be the victim of abortifacient activity of *A. indica*. It is better to use animal models other than rats and mice to evaluate the toxic effects, as most of the studies have already used rats and mice for their assessments. With these considerations on toxicological studies, an *A. indica*-based drug could be discovered. For this to happen, one must go ahead with clinical trials, after designing a novel drug consisting of relevant phytochemicals.

Perspectives and projections

Throughout the reviewing process we came across several advancements in pharmacological evaluations, which are

summarised in Table 1. Based on the available literature and gaps with respect to ethnopharmacology, pharmacology, and phytochemistry of *A. indica*, a few perspectives and future projections could be drawn out. Though a tremendous amount of pharmacological literature is present, it lacks specifications in terms of basic experimental design, advancements in research methodologies and product development. Herein we represent our views and comment on these aspects.

Experimental design

In the basic experimental design, researchers ought to look forward to nullifying basic errors via setting proper control groups. Most of the studies have not used controls in different pharmacological evaluations related to DM.[32, 37, 39, 40, 60, 63] However, using negative controls like diabetic untreated rats sounds comprehensive yet using no positive control becomes unrepresentative. Even with the availability of several chemotherapeutic agents to treat DM, it becomes essential to use them as a positive control. During *in vivo* studies, animals need to be fed with drugs like acarbose, miglitol, metformin etc. In addition, usage of diabetic animal models sounds accurate instead of inducing healthy rats with STZ and alloxan. Animal models like non-obese diabetic (NOD) mouse, diabetes-prone Bio-Breeding (BB) rat, the Long-Evans Tokushima Lean (LETL) rat, the Komeda diabetes-prone (KDP) rat and Lewis rat that represent various diabetic complications should be used to comprehensively evaluate the effect of *A. indica* extracts and phytochemicals.[82] Apart from usage of controls and animal models, we have noticed a plethora of discrepancies in terms of results. It may be due to the differences made in the experimental design. It is also speculated that involvement of different variants of trees present around the world may yield variable amount of phytoconstituents, which in turn may affect the results. In course of reviewing the pharmacological evaluations, we came across a few studies that have performed combined therapy that includes plant extracts from both *A. indica* and other plant.[29, 30, 58, 61] In fact, a combination therapies have performed better than *A. indica* alone in terms of outcomes. However, it may be attributed to the presence of diverse phytochemicals present in the polyherbal formulations. A few studies have shown that pre-treatment of *A. indica* could yield better results, in comparison with the freshly treated models.[22, 30] Thus, instead of using polyherbal medications, we could deal with complications using pre-treatment approach to get better results.

Mechanisms of inducing antidiabetic activity

Further, many of the studies were not able to deduce the mechanism underlying the respective pharmacological activities. Even after getting significant results from their experiments, the researchers failed to provide suitable evidences.[32, 37, 39, 40, 60, 63] In this context, many of the studies remain unacknowledged, especially at molecular level. With the availability of molecular evidence, it could be an easier approach to develop phytochemical-based therapeutics. It is expected that researchers should be focusing on biological pathways of DM to deliver target-based medicines synthesised from the plant extracts and phytochemicals. In addition, we noticed that most of the studies have focused on a single factor such as blood glucose level or lipid profile. One must focus on conducting multifactorial pharmacological evaluations, i.e., evaluating blood glucose level, lipid profile, effect on carbohydrate digestive enzymes, effect on oral glucose tolerance etc. in a single study, which could produce a massive and effective outcome.[41] In addition, interaction of *A. indica* phytochemicals with biological pathways related to diabetes like AMPK, p38-MAPK, and PTP-1B pathways must be elucidated. Although Sanni *et al.*[41] have demonstrated the interaction and activation of AMPK by some of the *A. indica* phytoconstituents, much needs to be known about PTP-1B and p38-MAPK pathways, as both of them pose essential targets for phytochemical-based drug discovery and design.

Future perspectives

As stated earlier, DM is a group of interrelated complications. Therefore, we emphasise that the treatment for DM should be done in a multifactorial approach. In this context, it becomes essential to focus on unexplored molecular targets to develop novel therapeutic options. Most of the studies have not highlighted insulin synthesis and secretion in their evaluations. In pharmacological evaluations related to insulin, it is essential to evaluate the effect of *A. indica* on a molecular network that ameliorates insulin synthesis and secretion. Alongside insulin, it also becomes important to focus on antihypertensive effect of *A. indica*, as it is chiefly related to DM. For this, angiotensin-converting enzyme 2 (ACE2) that plays a key role in hypertension-related aspects need to be evaluated after the treatment of *A. indica*. Further, we also noticed the scarcity of evaluations on diabetic complications related to wound healing. Although various tissues like hepatic, pancreatic and intestinal tissues have been focused, there is dearth of information on the analysis of adipose tissue, which plays an important role in DM related arthritis. Also, there could be a lot of research done on glucose uptake evaluation. Molecular targets like GLUT4 should be studied *in silico* upon the treatment of *A. indica*, from which one could be able to know the diverse role of phytochemicals in DM management. Moreover, interaction of *A. indica* compounds has been elucidated with few diabetic target proteins like α-amylase, α-amylase, GLUT1, GLUT2 etc. Discovery of new molecular targets at both proteomic and genomic level could lead to the identification and screening of potent phytochemicals from *A. indica*. However, in the current scenario, effect of *A. indica* phytoconstituents against proteomic targets has been gaining significance. In near future, study of interaction between phytochemicals and genes could be carried out. In addition to this, new dimensions could be provided for the phytochemistry with the usage of novel approaches like plant-based bioactive peptides and endophytes.[83] In addition, considering the toxicological profile of the extract and isolated phytochemicals of *A. indica*, the plant extracts >500 mg/kg have been proved toxic to the animal models. However, with 2 consecutive doses <500 mg/kg, this issue can be sorted out. These findings sound beneficial in comparison with plethora of adverse effects induced by conventional therapeutics. As the phytochemicals come from natural origin, phytomedicines derived from them would be less toxic. However, more emphasis should be put on bioaccessibility and bioavailability, which can make a drug even more effective in terms of its pharmacological efficiency. Moreover, effect of *A. indica* on human body organs has not been studied comprehensively. In total, careful evaluation of toxicological profile could provide a positive factor to the consideration of *A. indica* for human clinical trials.

Furthermore, when it comes to research outcomes, it is advised to design the experiments to get an outcome either as a

drug or functional nutraceutical. For example, Dianex, Dihar, or *A. indica*-yogurt can be designed and could be made into commercially available nutraceuticals with clearance to make it available around the globe.[37, 58, 64] Polyherbal drugs with an emphasis on their mechanism of action and molecular targets should be focused. Meanwhile, one must take care about the toxicity issues. Such a meticulous design would facilitate clinical trials on human candidates. Stability and performance of the developed plant-based products thus becomes essential.

Conclusion

A. indica has created a significant mark in the traditional as well as modern system of medicine. In course of deciphering the phytochemical profile, novel antidiabetic compounds are still being discovered. Therefore, a thorough phytochemical screening of various extracts needs to be conducted. Despite several discoveries that depict the unmatchable antidiabetic potential of *A. indica*, we could only find scarce amount of studies related to oral glucose tolerance and glucose uptake. The ameliorative effects of *A. indica* even extended on diabetic complications like cardiomyopathy, nephropathy, neuropathy, and wound healing capacity. These studies have successfully pointed out the interconnected links between obesity, hypertension, and DM. However, pharmacological evaluations need to consider insulin as an important factor in their experimental design, and studies need to be conducted in this regard. Furthermore, the toxicological evaluations depict a few adverse effects of *A. indica* overdoses in diabetic animal models with no evidence of adversities in humans. Therefore, dose-dependent clinical evaluations need to be conducted using human cell lines, followed by human candidates to completely understand the perspectives of plant-based drug development. This shall pave avenues for the development of many prominent therapeutic agents with diverse nature of applications to resolve health maladies related to DM.

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Author Contributions

R.R. conceived of the presented idea. S.M.P. and P.S.S. wrote the manuscript. All the authors discussed the results, critically revised the work and contributed to the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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