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# Hypoglycaemic and Hypolipidaemic Effects of an Ethylacetate Fraction of *Artocarpus heterophyllus* Leaves

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Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. Pharmacognosy of Diabetes Mellitus

Diabetes mellitus type II (T2DM) is a global public health crisis that threatens the economies of all nations, particularly developing countries. Fueled by rapid urbanization, nutrition transition, and increasingly sedentary lifestyles, the epidemic has grown in parallel with the worldwide rise in obesity. According to the International Diabetes Federation [1], diabetes affects at least 285 million people worldwide, and that number is expected to reach 438 million by the year 2030, with two-thirds of all diabetes cases occurring in low- to middle-income countries. The number of adults with impaired glucose tolerance will rise from 344 million in 2010 to an estimated 472 million by 2030. Several factors contribute to accelerated diabetes epidemic, including the “normal-weight metabolically obese” phenotype; high prevalence of smoking and heavy alcohol use; high intake of refined carbohydrates (e.g., white rice); and dramatically decreased physical activity levels [2].

In spite of the tremendous progress in the management of diabetes using synthetic drugs, potential new and inexpensive treatments should be used to reduce the global morbidity and mortality, as most of the people with T2DM lives in areas of the world, where existing treatments are unavailable or are too expensive. It is well documented that insulin sensitivity and glucose tolerance can be modulated by use of traditional medicines that are mainly derived from plants [3-5]. Natural antidiabetic agents with fewer side effects from readily available medicinal plants offer great potential in the discovery of new antidiabetic drugs.

Pharmacognosy (the study of the medicinal properties of materials of natural origin) has played an important role in the management of diabetes mellitus since ancient times.

Indeed, it has been estimated that more than 800 herbal or plant-derived products have been used for the management of T2DM across geographically and culturally diverse populations worldwide [6-9].

World Health Organization (WHO) recommendations [10] on the use of alternative medicines for treating diabetes mellitus provide an impetus for research in this area. Currently, the focus of research includes discovering newer antidiabetic agents as well as isolating the active compounds from herbal sources that have been documented to have antidiabetic properties as have been described in ancient texts. The active components of a number of plant-derived antidiabetic compounds have been identified, and amongst these are flavonoids, alkaloids, glycosides, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids and amino acids. Potentially beneficial effects on the rate of food digestion, glucose transport, potentiation of insulin release, inhibition of insulin clearance, insulin-mimetic effects, reduced gluconeogenesis, and  $\beta$ -cell protection have been attributed to these agents [11].

Type 2 diabetes (T2DM) is a disease characterized by a dual defect: 1) by insulin resistance which prevents cells from using insulin properly and 2) degrees of reduced pancreatic insulin secretion. It is a progressive disease that shows a consistent deterioration in glycemic control over time. While the pathophysiology and pathogenesis of the T2DM is not fully understood, it is clear that impaired glucose tolerance (IGT) often develops into T2DM. Interventions that may delay or prevent the progression of IGT to T2DM are desperately needed. Hyperlipidaemia is a secondary complication in diabetes and there is a growing interest in plants with both hypoglycaemic and hypolipidemic properties since they have a potential to be developed further for effective treatment for diabetes specially associated with a hyperlipidaemic state. Since *Artocarpus heterophyllus* is traditionally used for management of diabetes mellitus, it may hold promise in this regard and warrants pharmacognostical, pharmacological and clinical studies to investigate the therapeutic potential of this plant in the treatment of T2DM.

## 1.2. Overview of *Artocarpus heterophyllus*

*Artocarpus heterophyllus* Lam (family Moraceae), commonly known as jakfruit is one of the most significant trees in tropical homegardens and perhaps the most widespread tree in the genus *Artocarpus*. It is a medium-size evergreen tree typically reaching 8–25 m (26–82 ft) in height with evergreen, alternate, glossy and leathery leaves to 22.5 cm (9 in) in length. Jackfruit's place of origin is believed to be indigenous to the rainforests of the Western Ghats. Today, it is cultivated at low elevations throughout India, Sri Lanka, Myanmar, southern China, Malaya, East Indies, Queensland, Mauritius, Kenya, Uganda and former Zanzibar, Pacific islands and Brazil [12].

Many parts of the plant including the bark, roots, leaves, and fruit are attributed with medicinal properties. It is reported in Ayurveda (a traditional medicine system in Sri Lanka and India) to possess antibacterial, anti-inflammatory, antidiabetic, antioxidant and

immunomodulatory properties. It is an important source of compounds like morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone, betulinic acid, artocarpanone and heterophyllol which have therapeutic properties [13].

The root is a remedy for skin diseases and asthma and the extract is taken in cases of fever and diarrhea. The ashes of the leaves, burned together with corn and coconut shells are used alone or mixed with coconut oil to heal ulcers. Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swellings. Heated leaves alone are placed on wounds and the bark is made into poultices. The seed starch is given to relieve biliousness and the roasted seeds are regarded as aphrodisiac. In Chinese medicine the pulp and seeds are considered tonic and nutritious [12].



**Figure 1.** Mature leaves of *A. heterophyllus*

### 1.3. Aim of the chapter

Alternative systems of medicine such as Ayurveda is widely used in Sri Lanka and India.

In Ayurveda diabetes falls under the term *Madhumeha* [14]. Various types of herbal preparations such as decoctions (boiled extracts), *Swaras* (expressed juices), *Asav-Arisht* (fermented juices), and powders have been used for the treatment of *Madhumeha* [14].

Hot water extracts of *A. heterophyllus* leaves are used in the treatment of T2DM by traditional medical practitioners in Sri Lanka and India [15,16]. This traditional claim was first scientifically validated by the investigations carried out by Fernando *et. al* [17] which demonstrated the hypoglycaemic potential of the leaf extract. This preliminary work has been followed by many other studies which have provided insights into the efficacy, safety, mechanisms of action and the presence of other bioactivities of therapeutic potential in *A. heterophyllus* leaves. This chapter is based on the investigations carried out by Chackrewarthy *et al* [18] to evaluate the hypoglycaemic and hypolipidaemic potential of an ethylacetate fraction of *A. heterophyllus* leaves using a normal and streptozotocin induced diabetic rat models.

## 2. Phytochemical screening and standardized extraction

Plant extracts used in traditional medicine are chemically complex and may contain one or more structurally related active compounds that produce a combined effect. Standardization and phytochemical screening are essential measurements of ensuring quality control of herbal drugs.

Phytochemical screening of the aqueous extract of the leaves has revealed the presence of a range of polyphenols such as flavanoids, anthocyanins, tannins and polysaccharides as constituents [19]. In a more recent study of the aqueous extract of leaves, the presence of proteins, saponins, sterols, glycosides and lipids have been revealed in addition to the compounds mentioned [20]. A total ash value of 0.84%, acid insoluble ash value of 0.12% and water soluble ash value of 0.35% on dry weight basis have been reported for the aqueous extract of the leaves.

The purpose of standardized extraction procedures of plant crude extracts is to attain the therapeutically desired fractions and to eliminate unwanted material by treatment with selective solvents. In general, the solvent system used for the extraction plays a significant role in the solubility of the active principles of plant materials which in turn influence the bioactivities of the fractions [21].

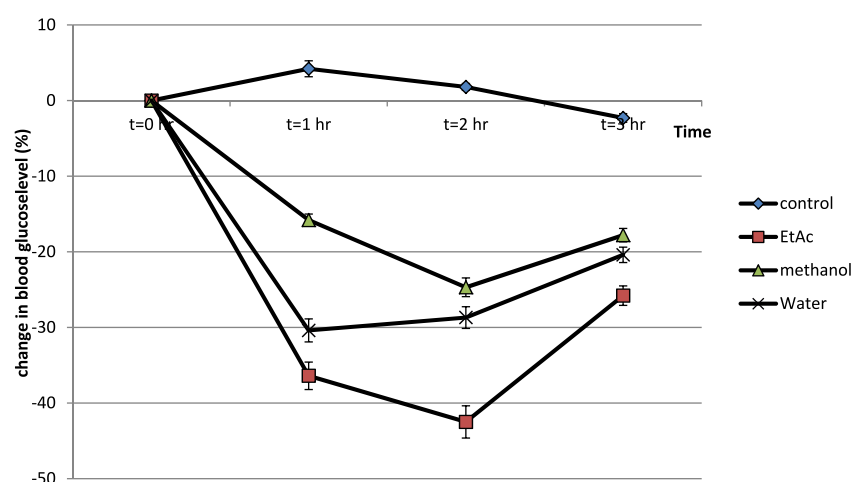
Plant crude extracts usually contain large amounts of carbohydrates and/or lipoidal material and the concentration of the phenolics in the crude extract may be low [22]. To concentrate and obtain polyphenol rich fractions before analysis, strategies including sequential extraction is commonly used. Sequential extraction with solvents of increasing polarity; eg: hexane, dichloromethane (DCM), ethyl acetate, methanol and water leads to initial fractionation of constituents of plant materials based mainly on their nature of polarity and with minimal chemical damage [23]. Based on this procedure an initial fractionation of the constituents of *A. heterophyllum* leaves was carried out in the present study. Generally, waxy and lipid substances are extracted into hexane and DCM and polyphenols are extracted into more polar solvents such as ethylacetate, methanol and water. In particular, methanol has been generally found to be more efficient in extraction of sugars, organic acids and lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with ethylacetate [24-27]. In recent studies, the phytochemical screening of the ethylacetate fraction of *A. heterophyllum* leaves and bark has revealed the presence of flavanoids in high content. [28,29]. All fractions were evaporated to dryness and the residues stored at -4°C until use.

## 3. Hypoglycaemic activity of *A.heterophyllum* leaves

### 3.1. Effects on fasting blood glucose levels

The hypoglycemic potential of fractions separated from *A. heterophyllum* leaves by sequential fractionation, were tested using a normoglycaemic rat model. Male Wistar rats were fasted overnight, divided into groups and each group was treated with a different fraction by oral

administration at a dose equivalent to 50 mg/kg body weight. The ethylacetate (EA) fraction and the water fraction were found to exert the highest hypoglycemic effects compared to the controls treated with distilled water (Fig 2). The reduction in fasting blood glucose levels at +2 hr mediated by ethylacetate and water fractions were 42.5% and 28.7% respectively. This demonstrates that both these fractions contain the active principals mediating the hypoglycaemic effect in varying proportions. Methanol fraction also had significant activity similar to the aqueous fraction, but the initial hypoglycaemic effect (+1 hr) was more prominent in the aqueous fraction (30% vs 16%). In experiments carried out by Fernando *et al* [17] with the crude extract of *A. heterophyllus* leaves, the fasting blood glucose levels of normoglycaemic rats were reduced by 24% at +3hr. However, this reduction was obtained with a higher dose of 10g/kg body weight of *A. heterophyllus* starting material. Further, purification and fractionation results in an enhancement of bioactivity by eliminating the unwanted material which could exert inhibitory effects on the bioactivity of interest. Since the ethylacetate fraction has been shown to contain a high content of flavanoids by phytochemical screening [19,28,29], the hypoglycaemic activity of this fraction could be attributed to a high molecular weight flavanoid which has a higher solubility in ethylacetate than in water.



**Figure 2.** The effects of ethylacetate (EtAc), methanol and water fractions of *A.heterophyllus* leaves on fasting blood glucose levels of normoglycaemic rats. Data expressed as % change in blood glucose level. Each point is the mean of six determinations.

### 3.2. Effects on glucose tolerance

Impaired glucose tolerance is a pre-diabetic state which may precede T2DM. The oral glucose tolerance test (OGTT) was used as a screening method for acute antihyperglycemic activity since the results give the overall effect of the test material on the handling of an external glucose load [30].

Glucose tolerance studies with normoglycaemic rats, receiving the ethylacetate fraction showed a significant improvement in their ability to utilize the external glucose load compared to the control group (Fig. 3). This was dose dependant, and a dosage of 20mg/kg



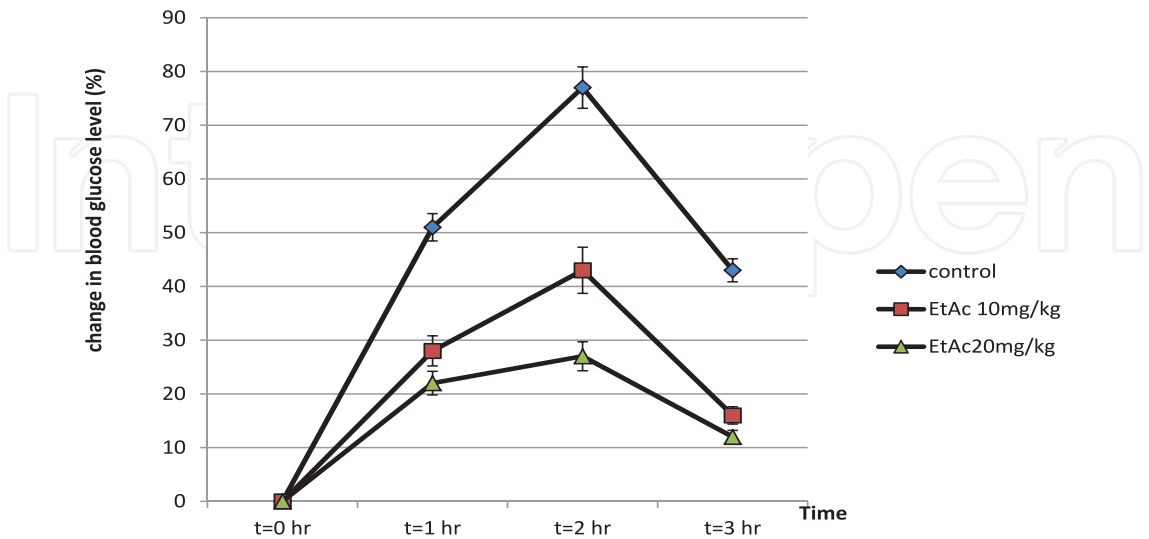
was found to be more effective than 10 mg/kg. Compared with the controls, the reduction in blood glucose concentration at 2 hr post glucose administration was 18.9% and 26.4% for dosages of 10 mg/kg and 20 mg/kg respectively (Table 1). Reductions in blood glucose levels were statistically significant between the control curve and test curves at all time intervals. In similar experiments a higher dose of 150mg/kg of the ethylacetate fraction had resulted in a 36.9% reduction in blood glucose at 2 hr post glucose administration [28].

Effects of the hot water extract of *A. heterophyllus* leaves on glucose tolerance of healthy individuals and maturity onset diabetic patients have been investigated by Fernando *et al* [31]. Oral administration of the hot water extract equivalent to a dose of 20g/kg starting material, one hour before the glucose load of 50 g, resulted in a significant improvement in the glucose tolerance of normal subjects and in the diabetic patients compared to the controls receiving distilled water. These data provide confirmatory evidence for the presence of antidiabetic principals in the leaf extract and validate its use in the treatment of diabetes by traditional medical practitioners.

	Mean serum glucose concentration (mg/dl)		% reduction in serum glucose compared to control
	Fasting	2 hrs post glucose	
Control	95.1 ± 4.4	169.5 ± 3.6	
EA fraction (10 mg/kg)	96.5 ± 2.3	137.3 ± 2.7	18.9*
EA fraction (20 mg/kg)	98.6 ± 3.5	124.8 ± 4.2	26.4*
Gibenclamide (0.6 mg/kg)	98.3 ± 1.9	108.5 ± 2.9	35.9*

\*Significantly different (p<0.05) compared to control

**Table 1.** Effect of ethylacetate fraction on glucose tolerance



**Figure 3.** The effects of ethylacetate (EtAc) fraction of *A.heterophyllus* leaves at doses of 10 mg/kg and 20 mg/kg bodyweight, on glucose tolerance of normoglycaemic rats. Data expressed as % change in blood glucose level. Each point is the mean of six determinations.

### 3.3. Animal models of diabetes mellitus

Non-insulin-dependent forms of diabetes can be produced by administration of a low dose of Streptozotocin (STZ) or alloxan [32]. These kinds of models of diabetes are considered a screening step in the search for drugs for the treatment of diabetes [33].

Streptozotocin (STZ) is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Its structural similarity to glucose allows it to enter the pancreatic beta-cell via a glucose transporter-GLUT-2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore, STZ induces activation of poly-adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic beta-cells are destroyed by necrosis [34]. The diabetes induced by STZ is associated with polydipsia and loss in body weight [35]. Although high-dose STZ severely impairs insulin secretion mimicking type 1 diabetes, low-dose STZ has been known to induce a mild impairment of insulin secretion which generate an impairment in glucose metabolism leading to glucose intolerance and mild, moderate or severe hyperglycaemia [36, 37]. Further, it also affects the lipid metabolism which leads to hyperlipidaemia which closely mimic the natural course of pathogenesis of T2DM [36-39]. The potential problem with STZ is that its toxic effects are not restricted to pancreatic beta-cells since it may cause renal injury [40], oxidative stress, inflammation and endothelial dysfunction [41]. Despite its widespread use, there is a wide variability in the extent of diabetes depending on species, strain and age limiting the predictability of its effects and precautions should be taken when trying to extrapolate the findings to the clinical practice [42,43].

### 3.4. Effect of chronic administration of ethylacetate extract on fasting blood glucose levels of streptozotocin-induced diabetic rats

Diabetes was induced in male Wistar rats by a single intravenous injection of freshly prepared STZ solution (in 0.1 M citrate buffer pH = 4.5) at a dose of 60 mg/kg body weight. The efficacy of antidiabetic plant materials may vary with time and investigating the effects of sub chronic or chronic administration is a necessary requirement in evaluating the profile of long term effects of herbal extracts [44].

Chronic administration (Table 2) of the EA fraction to rats at dose of 20 mg/kg for five consecutive weeks caused a significant fall ( $P < 0.05$ ) in the fasting blood sugar levels of diabetic rats when compared with diabetic controls which did not receive the ethylacetate fraction. This is evident in the second week itself and the reduction in the fasting blood sugar in the ethylacetate fraction treated rats (22.8%) was fairly comparable to that produced by the reference drug glibenclamide (32.2%) during the same time period. As in the glibenclamide-treated rats, in rats receiving the ethylacetate fraction also, the fall in the blood sugar level continued progressively till the end of the fifth week. A maximum fall of 39.1% and 55.7% were seen for the ethylacetate fraction and glibenclamide respectively at the end of the fifth week. In a more recent study, chronic administration of the aqueous extract of *A. heterophyllus* leaves to streptozotocin induced diabetic rats has shown similar results [20]. There was a 51% decrease in fasting blood sugar level for a dose of 250 mg/kg in



diabetic rats at the end of the first week when compared with diabetic controls. The fall in fasting blood sugar has continued progressively during the period of treatment with the extract. In another study conducted by Mohana Priya *et al* [29] using a similar protocol, chronic administration of an ethylacetate fraction of the *A. heterophyllum* bark extract has resulted in a maximum fall of fasting blood glucose level by 27.5% for a dose of 400mg/kg body weight.

Glibenclamide being a standard drug for the treatment of T2DM, stimulates insulin secretion from pancreatic  $\beta$ -cells.[45] Therefore, it may be suggested that stimulation of insulin release from the still functioning  $\beta$ -cells by active principles in ethylacetate fraction may be one of the mechanisms by which this fraction mediates its hypoglycemic effect, as proposed for some other plant extracts.[46,47] Further, components of the ethylacetate fraction as proposed by Gomes *et al.*[48] with reference to *Camellia sinensis* (black tea), may be able to generate  $\beta$ -cells of the pancreas or protect the intact  $\beta$ -cells from further deterioration so that they may remain active and continue to produce insulin. Histopathological analysis of pancreas of streptozotocin induced diabetic rats chronically treated with an ethylacetate fraction of *A. heterophyllum* bark extract has revealed initial stages of regenerating islet cells with loss of degenerative features when compared with diabetic controls in which progressive  $\beta$  cell vacuolation, occasional apoptotic cells with vascular congestion and fibrous tissue infiltration was observed in the pancreas. All the above findings provide the biochemical basis for the use of *A. heterophyllum* leaves in the management of patients with diabetes and confirms its role as a traditional antidiabetic remedy.

Groups	Post STZ	2 weeks	4 weeks	5 weeks
Normal control	98.4 $\pm$ 2.9	90.6 $\pm$ 5.7	91.6 $\pm$ 4.8	92.7 $\pm$ 6.6
Diabetic control	254.3 $\pm$ 2.5	245.5 $\pm$ 6.6 <sup>a</sup>	250.0 $\pm$ 3.9 <sup>a</sup>	253.4 $\pm$ 6.3 <sup>a</sup>
Diabetic + EA fraction (20 mg kg <sup>-1</sup> bw)	252.8 $\pm$ 11.9	189.4 $\pm$ 5.1 <sup>b</sup>	160.1 $\pm$ 11.0 <sup>b</sup>	154.2 $\pm$ 11.1 <sup>b</sup>
Diabetic + Glibenclamide (0.6 mg kg <sup>-1</sup> bw)	235.2 $\pm$ 8.3	166.4 $\pm$ 4.8 <sup>c</sup>	126.6 $\pm$ 9.2 <sup>c</sup>	112.3 $\pm$ 7.5 <sup>c</sup>

Serum glucose levels in mg dl<sup>-1</sup>. Significantly different (p<0.05) compared to normal controls<sup>a</sup>, compared to diabetic controls<sup>b</sup>, EA treated diabetic rats<sup>c</sup>

**Table 2.** Serum glucose levels in STZ-induced diabetic rats after prolonged treatment with ethylacetate fraction

#### 4. Effect of chronic administration of ethylacetate extract on body weights and hyperlipidaemia associated with diabetes

Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes.[49] Insulin plays a major role in lipid metabolism apart from its regulation of carbohydrate metabolism. Insulin increases the receptor mediated removal of LDL cholesterol and

activates lipoprotein lipase for the hydrolysis of triacylglycerols in lipoproteins. Therefore, reduced activity of insulin in diabetes causes hypercholesterolaemia and hypertriglyceridaemia. This is clearly demonstrated in the diabetic controls of the present study by the increased levels of serum total cholesterol (TC) and triacylglycerols (TG) in diabetic rats when compared to normal control rats (Table 3).

Groups	Total Cholesterol(mg/dl)	Triglycerides (mg/dl)	Body weight (g)
Normal control	78.2 ± 3.8	102.2 ± 2.3	201.0 ± 8.5
Diabetic control	127.9 ± 3.2 <sup>a</sup>	199.8 ± 6.2 <sup>a</sup>	172.1 ± 5.5
Diabetic + EA fraction (20 mg kg <sup>-1</sup> bw)	98.5 ± 4.3 <sup>b</sup>	119.0 ± 2.5 <sup>b</sup>	190.4 ± 1.2
Diabetic + Glibenclamide (0.6 mg kg <sup>-1</sup> bw)	87.4 ± 3.2 <sup>c</sup>	116.8 ± 1.3 <sup>c</sup>	196.5 ± 3.3

Significantly different ( $p < 0.05$ ) compared to normal controls <sup>a</sup>, compared to diabetic controls <sup>b</sup>, EA treated diabetic rats<sup>c</sup>

**Table 3.** Serum total cholesterol, triglyceride levels and body weight in STZ-induced diabetic rats after five weeks of treatment with ethylacetate fraction

Alterations in the lipid profiles and body weights of STZ-induced diabetic rats are summarized in Table 3. Treatment of diabetic rats with the ethylacetate fraction resulted in a significant fall ( $P < 0.05$ ) in the levels of both total cholesterol (TC) and TGs compared to diabetic controls. The fall in serum TG levels (40%) was more marked than that of cholesterol levels (23%). This effect on diabetic hypertriglyceridemia in ethylacetate fraction treated rats could be due to improved glycemic control. The improved glycemic control by sulfonylureas accompanied by decreased serum very low density lipoprotein (VLDL) and TG levels has already been reported.[50] The reduction in cholesterol levels in diabetic test rats could be due to an inhibitory effect of the active principles on enzymes of cholesterol biosynthesis[51,52] and/or due to the enhanced activity of enzymes involved in bile acid synthesis and its excretion [53]. Further, stimulation of insulin secretion in response to the treatment with the extract could increase the uptake of low density lipoprotein (LDL) cholesterol by extrahepatic tissues contributing to the cholesterol lowering effects of the ethylacetate fraction. However, to obtain confirmatory evidence of these views, further studies on the effects of the ethylacetate fraction on LDL and high density lipoprotein (HDL) cholesterol levels and their clearance need to be investigated. A high fat intake and increased levels of free fatty acids in circulation have been implicated in the development of insulin resistance.[54,55] Based on these observations, it could be speculated that the EA fraction mediated reduction in circulating levels of TGs may also help to ameliorate insulin resistance in diabetic rats and thereby stimulate glucose utilization by peripheral tissues.[56] In a more recent study the chronic administration of aqueous extract of *A. heterophyllus* leaves have been shown to reduce the serum total cholesterol level and improve the HDL

levels and the body weight in streptozotocin induced diabetic rats [20]. Similar effects have been shown for the ethylacetate fraction of *A. heterophyllum* bark on diabetic rats [29].

Loss of body weight in diabetic rats is due to increased muscle wasting and loss of tissue proteins [57]. As evident from Table 3, the ethylacetate fraction had an improving effect on the BW (11%) of diabetic rats, which was restored to near normal levels. This may be a reflection of improved health resulting from the effects of the ethylacetate fraction on insulin release.

## 5. Toxicity

The use of herbal medicines for the treatment or prevention of a variety of diseases has increased markedly. Herbal medicines are believed to be benign and not cause severe toxicity. This coupled with lower costs compared with conventional medications is the major attraction to these treatments. The active ingredients of plant extracts are chemicals similar to those in purified medications, and they have the same potential to cause serious side effects. The usefulness of any drug depends not only on its therapeutic efficacy but also on its lack of toxicity or adverse side effects. Therefore, toxicological investigations have to be carried out before any drug can be considered as safe. This is specially important in the case of antidiabetic drugs, which have to be administered over a relatively long period of time to obtain their pharmacological potency.

Hot water extracts of *A. heterophyllum* leaves have been used from ancient times as a treatment for diabetes in traditional medicine in Sri Lanka and no evidence has been reported of any toxicity or adverse side effects. However, this requires scientific validation with toxicological studies. An in vivo study using Sprague Dawley rats carried out by Fernando and Thabrew [58] has demonstrated that an aqueous extract of mature leaves of *A. heterophyllum* exerted no adverse effects even after daily administration for 30 days, as assessed by effects on (a) liver function, (b) haematological parameters such as haemoglobin concentration, red blood cell count, white blood cell count and packed cell volume, (c) reproductive ability of experimental animals and (d) histology of body organs (heart, liver, lung, kidney, intestine and pancreas). Investigations carried out by Chandrika *et al* [28] also have revealed that the chronic administration of the ethylacetate fraction of *A. heterophyllum* leaves to normoglycaemic rats over a period of twelve weeks at a dose of 50 mg/kg body weight failed to bring any overt signs of toxicity such as salivation, diarrhea, lacrymation, postural abnormalities or behavioural changes. No significant differences had been observed in the liver function tests and the histology of various body organs between the test and the control groups. *A. heterophyllum* leaf extract can therefore be considered to be free of any major toxic compounds or adverse effects.

## 6. Mechanism of action

Antidiabetic activity of plant extracts is mainly due to their ability to restore the function of the pancreatic tissue by causing an increase in insulin secretion or inhibit the intestinal absorption of glucose or by facilitation of the insulin dependant process.

In investigations carried out by Fernando & Thabrew [59] to investigate the mechanism of action, the hypoglycaemic activity of the aqueous extract of *A. heterophyllus* leaves has been attributed to an extra pancreatic effect, resulting from the inhibition or destruction of insulinase by the constituents of the leaf extract. Improved glycaemic control is achieved by prolonging the half life of insulin. Further, no effects of the extract have been observed on the intestinal glucose absorption in experimental rats.

*In vitro* investigations carried out by Kotowaroo *et al* [60], on the mechanism of action have revealed that aqueous leaf extract of *A. heterophyllus* significantly ( $p < 0.05$ ) inhibited  $\alpha$ -amylase activity in rat plasma. Further, enzyme kinetic studies using the Michaelis-Menten and Lineweaver-Burk equations have established that the aqueous leaf extract of *A. heterophyllus* behaved as a competitive inhibitor. Results from this study indicated that *A. heterophyllus* could act as a 'starch blocker' thereby reducing post-prandial glucose peaks. However, this finding does not agree with the fact that the leaf extract significantly improves the glucose tolerance by improving the ability to handle an external glucose load, which has been consistently demonstrated in both animal and human studies conducted so far. However, the possibility exists, that the leaf extract could exert its hypoglycaemic effects through more than one mechanism.

Furthermore, available evidence indicates that the active principals mediating the antidiabetic effect in *A. heterophyllus* leaves to be flavanoids. There is evidence that flavonoids, can increase the viability of beta-cells exposed to STZ or other oxidative stress conditions and improve beta-cell function [61-64]. Increased oxidative stress due either to fasting or postprandial hyperglycemia is accepted as a participant in increased beta-cell damage contributing to the development and progression of diabetes [65]. There is also evidence that the antioxidant defense system is under-expressed in pancreatic cells [66] and flavonoid-rich extracts administration to diabetic animal models has been shown to increase expression of enzymes like catalase and superoxide dismutase as well as glutathione peroxidase system [67, 68]. Therefore, there is a strong possibility of a pancreatic mechanism either by induction of insulin secretion or by recovery of beta-cell mass through which the hypoglycaemic activity of the leaf extract could be exerted. However, further research is necessary to elucidate the mechanism(s) of action.

## 7. Conclusions

An ethylacetate fraction of *A. heterophyllus* leaf extract exerts strong hypoglycaemic activity in both normoglycaemic and diabetic rats. Chronic administration of the ethylacetate fraction to STZ-induced diabetic rats resulted in a significant improvement in the hyperlipidaemia associated with diabetes leading to a significant lowering of serum total cholesterol and triglycerides. The effects of the improved glycaemic control was evident in the improvement of body weights in diabetic rats under chronic treatment with the ethylacetate fraction. Isolation and characterization of the active principals in the ethylacetate fraction followed by further pharmacological and clinical studies would provide with a novel herbal drug for T2DM therapy.

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