Exploring the therapeutic potential of *Adathoda vasica* and *Andrographis paniculata* in alleviating diabetic cardiopathy and neuropathy: A study on high-fat diet and low-dose STZ-induced rat model

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Abstract

Diabetes mellitus is the metabolic illness, if it is left untreated it leads to secondary complications especially in patients suffering from T2DM. Herbal drugs are in medical use since ages. It is found that both Adathoda vasica and Andrographis paniculata exhibited anti-diabetic activity. Therefore, this study is aimed to explore their therapeutic effects in alleviating the secondary complications like neuropathy, cardiopathy using HFD-low dose STZ experimental model. Present study is designed to explore the therapeutic potential of an aqueous ethanolic extract of Adathoda vasica and Andrographis paniculata in alleviating diabetic cardiopathy and neuropathy against HFD-low dose of STZ induced diabetic model.

Diabetes was induced by treating the animals with HFD-low dose of STZ injection. Diabetic animals were selected for further investigation on cardiopathy and neuropathy where untreated and treated (Pioglitazone, increasing doses of AEEAV, AEEAP) diabetic animals were subjected to Eddy's hot plate test, Tail immersion test and Charcoal meal test. Oxidative parameters (MDA, GSH, SOD, CAT) were measured from sciatic nerve and heart homogenate, serum CK-MB, LDH, TC, TG, LDL and HDL were also measured. AEEAP showed improvement in intestinal transit time ($P<0.001$), restored antioxidants and reduced MDA levels in comparison with untreated diabetic animals ($P<0.001$). Cardiac biomarkers like CK-MB, LDH, TC, TG, LDL were significantly reduced in dose dependent manner ($P<0.001$) whereas HDL was found to be increased in treated diabetic animals. In addition, histopathology studies were well co-related with bio chemical improvement.

Therefore, aqueous ethanolic extract of Adathoda vasica and Andrographis paniculata alleviates diabetic cardiopathy and neuropathy in HFD- STZ model of diabetes.

Keywords: High fat diet, Intestinal transit, Creatinine kinase.

Introduction

Diabetes mellitus (DM) is a metabolic illness that has long-term repercussion with complications like nephropathy, retinopathy, neuropathy, cardiopathy etc.[2] and affects people's health, life expectancy and quality of life as well as burden on healthcare systems as a result of the fast rising incidences of DM around the world.[21] According to estimates, 285 million people worldwide have diabetes and by 2030, that number is expected to rise to 366 million with type 2 diabetes accounting for 90% of cases.[28]

Feeding an experimental animal with high fat diet (HFD) is associated with hyperinsulinemia.[10] Further exposure to low dose streptozotocin (STZ) therapy causes destruction of the β-cells, which reduces insulin production and results in the emergence of overt hyperglycemia.[22]

The evolution of antidiabetic drug discovery has changed its focus towards natural plant sources with low side effects because it has been reported that long term drug therapy produces variety of adverse effects[27]. Currently, there is a lot of interest in the benefits of phytoconstituents, which are frequently used as dietary ingredients and seem to be linked to lower the risk of developing certain chronic diseases[7].

Literature review revealed that numerous scientific studies have been carried out, some based on its ethnomedical use to investigate pharmacological effects of *Adathoda vasica* and *Andrographis paniculata* including its antioxidant, anti-inflammatory, anti-cancer, anti-HIV, immunomodulation, antipyretic, anticarcinogenic, antibacterial and an antispasmodic properties involving variety of animal models.[3,11,15,23] Anti-diabetic activity and metabolic changes induced by *Andrographis paniculata* have been reported in obese and obese diabetic (ob-db) rats. Aqueous extract treatment to these rats effectively restored the metabolic profile to normal[4]. Similarly, ethanolic extract of *Adathoda vasica* and its fraction has been reported to possess anti-diabetic activity in *in vitro* models by inhibiting α-glucosidase and α- amylase enzymes[26].

In the current study, both *A. vasica and A. paniculata* were evaluated for their therapeutic potential in alleviating diabetic cardiopathy and neuropathy in HFD-low dose STZ induced diabetic rat model.

Material and Methods

Extracts: The aqueous ethanolic extract of aerial parts of *Adathoda vasica* (AEEAV) and *Andrographis paniculata* (AEEAP) was obtained as gift samples from Green Chem®, Herbal Extracts and Formulations, Bengaluru.

Animals: For the study, adult, healthy male albino Wistar rats (180–200 g) were used. All the experimental animals were maintained as per standard laboratory conditions (temperature 25±2°C with 12/12 hr dark/ light cycle) in clean polypropylene cages. Water *ad libitum* and standard pellet diet were provided. Animals were acclimatized to experimental condition prior to experiment. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC). Test animals were procured from a commercial source (Sri Raghavendra Enterprises, Bengaluru, #541/PO/Bt/04/CPCSEA).

Acute toxicity study[17]: Acute oral toxicity studies of AEEAV and AEEAP were carried out according to OECD guidelines 425 (TG 425) in adult female albino Wistar rats (150-180g).

Experimental design HFD-STZ induced diabetic model[12,18]: The study consists of 09 different groups, each containing 12 male albino Wistar rats in the weight range of 180-200grams. All the animals except the normal control group were fed a high-fat diet (HFD) for 4 weeks and after 4 weeks of dietary manipulation, test animals were injected intraperitonially low dose (35 mg/kg) of STZ and normal control animals received vehicle. Animals with fasting blood glucose levels greater than 200 mg/dl were selected for further study after 72hr of STZ injection. Selected diabetic animals were subjected to a treatment protocol as follows:

Group 1: Normal control (saline treatment)
Group 2: Positive control (HFD-STZ)
Group 3: Standard group; Diabetic rats treated with Pioglitazone (10mg/kg, b.w. p.o.)
Group 4: Test group; Diabetic rats treated with AEEAV (200mg/ kg, b.w, p.o.)
Group 5: Test group; Diabetic rats treated with AEEAV (300mg/ kg, b.w, p.o.)
Group 6: Test group; Diabetic rats treated with AEEAV (400mg/ kg, b.w, p.o.)
Group 7: Test group; Diabetic rats treated with AEEAP (200mg/ kg, b.w, p.o.)
Group 8: Test group; Diabetic rats treated with AEEAP (300mg/ kg, b.w, p.o.)
Group 9: Test group; Diabetic rats treated with AEEAP (400mg/ kg, b.w, p.o.)

At the end of the study, animals were overnight fasted and partially anesthetized to collect blood from retro-orbital puncture. Serum and plasma samples were stored at -70°C for biochemical assays. Anti-diabetic activity in diabetic animals was established through OGTT, serum insulin and HbA1c using commercial test kits following suggested guidelines (Unpublished data). Further to explore the potential of AEEAV and AEEAP against neuropathy and cardiopathy, following assessments were carried out.

Eddy's hot plate test[14]: The Eddy's hot plate test was used to evaluate the thermal hyperalgesia. As a part of investigation, animals were placed on a hot plate that was kept at 55 ± 0.5°C. The amount of time needed for the initial response (such as licking, jumping, or flickering of the back paw) was measured as response latency.

Tail immersion test[24]: Effect of AEEAV and AEEAP on the nociceptive threshold to thermal stimuli in diabetic rats was examined by immersing the tail in hot water maintained at 55 ± 0.5° C. The latency of the response which refers to the time it took for the animal to remove its tail from the hot water, was recorded. A cut-off duration was set to 15 seconds to prevent injury to test animals, was adhered to while performing both the tests.

Charcoal meal test [6,8]: Charcoal meal test was adopted to evaluate gastroparesis of untreated and treated diabetic rats. Briefly, charcoal meal was prepared (10% charcoal and 5% gum acacia) and administered to animals under study at 2ml/animal orally. After 15 minutes of administration, rats were sacrificed by cervical dislocation method, their small intestine was isolated (from pyloric sphincter to ilioceacal junction). The distance travelled by charcoal was measured and percentage of intestinal transit was calculated using the formula:

$$\% \text{ of Intestinal transit} = \frac{\text{Distance travelled by charcoal meal}}{\text{Total length of small intestine}} \times 100$$

Oxidative stress parameters[8,14,24]: Upon completion of the study, the animals were euthanized and their sciatic nerve and heart were extracted. Subsequently, the isolated tissues were homogenised with a phosphate buffer and fractions of the homogenised tissue preparations were centrifuged and the levels of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were measured.

Cardiac diagnostic serum marker analysis[1,19,30]: The serum levels of creatinine kinase-MB (CK-MB), lactate dehydrogenase (LDH), total cholesterol (TC), triglycerides (TG) and HDL were measured using commercially available kits (M/s AGAPPE Diagnostics Ltd.).

Statistical analysis: Statistical analysis was performed using GraphPad Prism Version 6.0. One-way ANOVA followed by the Newman-Keuls multiple comparison test chosen to assess the significance of mean differences. Results with a p-value less than 0.05 were considered statistically significant.

Results

Effect of Pioglitazone, increasing doses of AEEAV and AEEAP on Behavioural Parameters: Untreated diabetic rats showed significant reduction in response time on Eddy's hot plate ($P<0.001$) whereas diabetic rats treated with pioglitazone showed significant increase in response latency ($P<0.01$). Diabetic rats treated with AEEAV and AEEAP at higher dose (400mg/kg, b.w.) have shown significant change in the reaction time when placed on Eddy's hot plate ($P<0.001$) but animals treated with AEEAV and AEEAP at 200, 300 mg/kg, b.w. showed insignificant response when compared to untreated diabetic rats.

In tail immersion test, treated diabetic rats showed significantly increased response latency at dose of pioglitazone 10 mg/kg ($P<0.001$), AEEAV, AEEAP 200, 300 mg/kg ($P<0.05$), AEEAV, AEEAP 400 mg/kg ($P<0.001$). (Table 1)

Effect of Pioglitazone, increasing doses of AEEAV and AEEAP on Functional Biomarker of Neuropathy (Charcoal meal test): Untreated diabetic rats showed significant reduction in intestinal transit time of charcoal agar meal in comparison with normal control animals ($P<0.001$). Whereas diabetic rats treated with pioglitazone, AEEAV and AEEAP at experimental doses showed significant increase in intestinal transit time of charcoal meal ($P<0.001$) when compared to untreated diabetic rats (Table 2).

Effect of Pioglitazone, increasing doses of AEEAV and AEEAP on Biomarkers of Oxidative stress (Neuropathy): Untreated diabetic rats' brain homogenized product showed significant reduction in antioxidant levels like GSH, CAT, SOD. However, significant raise in LPO ($P<0.001$) level was observed when compared to normal control animals whereas diabetic rats treated with pioglitazone, AEEAV and AEEAP at experimental doses showed significant increase in GSH, CAT, SOD and significant reduction in LPO in comparison to diabetic control animals ($P<0.001$) (Table 3).

Table 1
Effect of Pioglitazone, increasing doses of AEEAV and AEEAP on Behavioural Parameters

Group	Treatment and Dose (/kg, b.w.)	Change in response time (sec) (Eddy's hot plate test)	Change in response time (sec) (Tail immersion test)
I	Normal saline	7.5±0.84	7.16±0.6
II	HFD-low dose of STZ	3.0±0.36#	2.66±0.3#
III	Pioglitazone, 10mg	6.3±0.88 *	5.83±0.6*
IV	AEEAV, 200mg	4.5±0.42 ^{ns}	4.66±0.4 (a)
V	AEEAV, 300mg	4.8±0.54 ^{ns}	4.83±0.4 (a)
VI	AEEAV, 400mg	6.8±0.60 *	5.83±0.4*
VII	AEEAP, 200mg	5.0±0.36 ^{ns}	4.50±0.3 (a)
VIII	AEEAP, 300mg	4.8±0.47 ^{ns}	4.16±0.4 (a)
IX	AEEAP, 400mg	7.6±0.42 *	6.66±0.3*

All the values are expressed as mean ± SEM (n = 6). # $P<0.001$ compared with normal control, * $P<0.001$, a $P<0.05$, ns $P>0.05$ compared with diabetic control.

Table 2
Effect of Pioglitazone, increasing doses of AEEAV and AEEAP on Functional biomarker of neuropathy

Group	Treatment and Dose (/kg, b.w.)	Intestinal Transit (%)
I	Normal saline	62±0.93
II	HFD-low dose of STZ	30.33±0.71#
III	Pioglitazone, 10mg	57.33±1.3*
IV	AEEAV, 200mg	43.17±1.3*
V	AEEAV, 300mg	49.17±0.6*
VI	AEEAV, 400mg	53.83±1.1*
VII	AEEAP, 200mg	40.0±0.9*
VIII	AEEAP, 300mg	47.50±1.0*
IX	AEEAP, 400mg	51.50±0.7*

All the values are expressed as mean ± SEM (n = 6). # $P<0.001$ compared with normal control, * $P<0.001$ compared with diabetic control.

Effect of Pioglitazone, increasing doses of AEEAV and AEEAP on Change in serum TC, TG, HDL-C, LDL-C levels: Untreated diabetic rats showed significant elevation in serum lipid profile like total cholesterol, triglycerides and LDL-c, CK-MB, LDH but HDL-c was significantly reduced in comparison with normal control animals (P<0.001). Diabetic rats treated with pioglitazone, increasing doses of AEEAV, AEEAP showed significant reduction in TC, TG, LDL-c, CK-MB, LDH and significant increase in HDL-c (P<0.001). However, diabetic rats treated with AEEAV at 200mg/kg, b.w. did not show any significant changes with HDL-c (P>0.05) (Figure 1).

Table 3
Effect of Pioglitazone, increasing doses of AEEAV and AEEAP on Biomarkers of Oxidative stress (Neuropathy):

Group	Treatment and Dose (/kg, b.w.)	GSH (mmol/mg of protein)	LPO (µmol/mg of protein)	CAT (units of absorbance)	SOD (units of absorbance)
I	Normal saline	24.24±1.01	2.1±0.09073	1.19±0.01	0.87±0.03
II	HFD-low dose of STZ (35mg)	10.62±0.3#	15.49±0.17#	0.08±0.004#	0.24±0.03#
III	Pioglitazone, 10mg	19.28±0.7*	5.37±0.1*	1.13±0.0186*	0.75±0.02*
IV	AEEAV, 200mg	15.45±0.9*	9.13±0.1*	1.11±0.007*	0.45±0.01*
V	AEEAV, 300mg	15.65±0.4*	8.24±0.2*	1.11±0.010*	0.50±0.01*
VI	AEEAV, 400mg	17.30±0.3*	7.15±0.08*	1.13±0.01*	0.59±0.01*
VII	AEEAP, 200mg	14.68±0.3*	8.33±0.1*	1.12±0.01*	0.48±0.01*
VIII	AEEAP, 300mg	14.72±0.4*	7.7±0.1*	1.12±0.01*	0.56±0.03*
IX	AEEAP, 400mg	17.10±0.6*	6.3±0.1*	1.27±0.1*	0.61±0.01*

All the values are expressed as mean ± SEM (n = 6). # P<0.001 compared with normal control, * P<0.001 compared with diabetic control

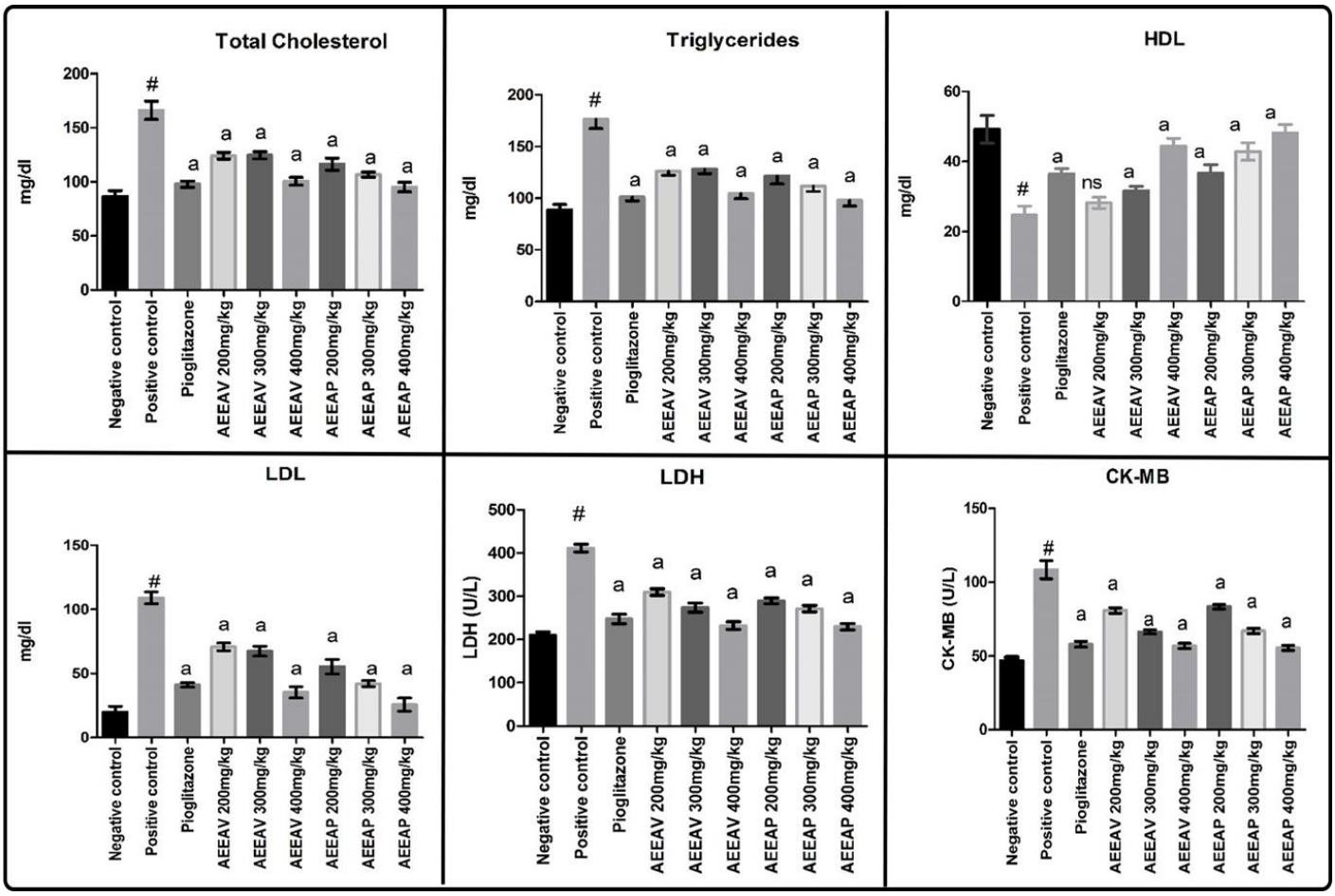

Figure 1: Change in serum TC, TG, HDL-C, LDL-C, CK-MB, LDH levels in diabetic rats treated with Pioglitazone, increasing doses of AEEAV and AEEAP

All the values are expressed as mean ± SEM (n = 6). # P<0.001 compared with normal control, (a) P<0.001 compared with diabetic control, ns P>0.05 compared with diabetic control.

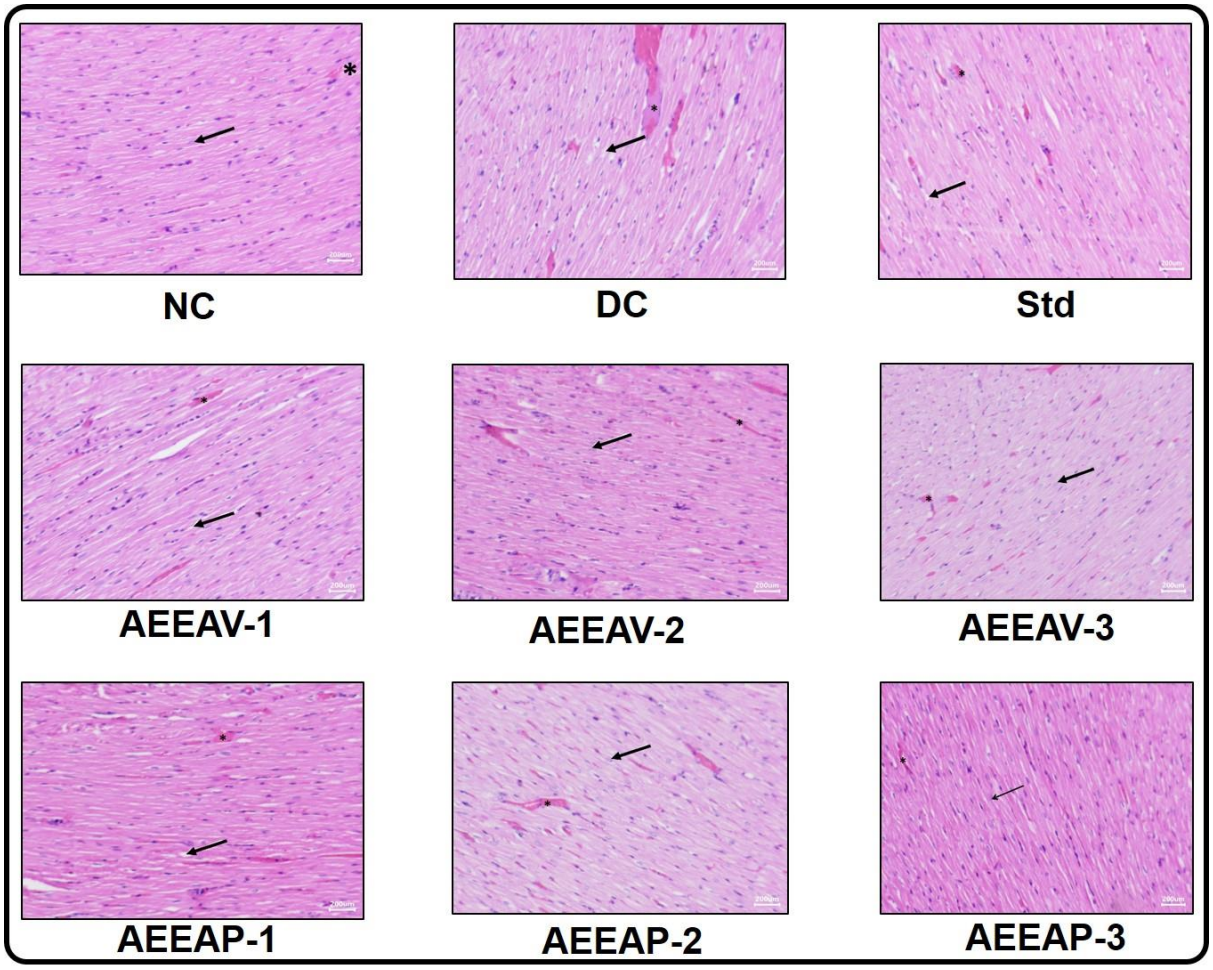

Figure 2: Representative images of H and E-stained sections of myocardium of untreated and treated with Pioglitazone, increasing doses of AEEAV and AEEAP diabetic rats:
NC- Normal Control, **DC**- Diabetic Control, **Std**- Diabetic rats treated with Pioglitazone (10mg/kg, b.w.) **AEEAV-1, 2, 3:** Diabetic rats treated with AEEAV (200mg/kg, b.w), (300mg/kg, b.w), (400mg/kg, b.w) respectively. **AEEAP-1, 2,3:** Diabetic rats treated with AEEAP (200mg/kg, b.w), (300mg/kg, b.w), (400mg/kg, b.w) respectively

Histopathology studies: Histopathological changes in the myocardium of diabetic rats and extract treated rats were evaluated by a competent pathologist blind to treatment protocol. Significant pathological changes were observed in the cardiac muscle sample of untreated diabetic animals and changes in the same were observed in cardiac muscle samples of extract treated animals. (Figure 2).

Discussion

Herbs with folklore history of ethnomedical uses and combination of herbs are available for the management of several metabolic disorders. India and several Asia pacific regions have strong cultural background of using herbs /herbal preparation in the health care system of these countries. This alternate / complimentary medicine has attracted the attention of the world and is now currently acceptable form disease management.

Herbs chosen for our investigations have been in folklore use and are reported to possess pleiotropic benefits when evaluated in lab animals and contemporary methodologies. Unfortunately, several metabolic disorders like diabetes etc. over a period lead to several secondary complications despite medication indicating limitation of drugs in effectively suppressing pathological process. Consequently, herbs / extracts ideally must also be screened / evaluated for preventing / protecting the patient from such secondary complications. Diabetes mellitus both type 1 and type 2 with complex interplay of several pathways led to several complications. Eid et al[9] in their review article while discussing the role of hyperglycemia, dyslipidemia etc. have thrown new insight on the mechanism of diabetic secondary complications like diabetic neuropathy, retinopathy.

However, from literature review, we found that these chosen herbs have not been investigated for number of secondary complications of diabetes. Sangeetha et al[20] have reported antidiabetic property and (3β)-stigmast-5-en-3-ol possess significant insulin mimetic effects *in vitro* model mimicking skeletal muscle cell and glucose uptake through activation of important insulin signalling intermediate. The study was limited and this promoted us to investigate the effect on the secondary complications.

Reported antidiabetic properties of *A. paniculata* are: the Andrographolide and Andrographolide-enriched extract of *A. paniculata* possessed significant blood glucose lowering effect and improved β cell function when tested using neonatal STZ diabetic animals[16], crude ethanolic extract possessing significant lowering property and lipid lowering in STZ diabetic animals [32] and anti-hyperglycemic anti-hyperlipidemic effects of combination of *Andrographis paniculata and Caesalpinia sappan* against STZ- HFD diabetic animals[13].

A water-soluble polysaccharide was investigated for its beneficial effect on the kidney function and histological features in a diabetic kidney[29]. This investigation involved recording the effect of extract in high fat diet – STZ animal model that mimics the human type 2 diabetes. Diabetic animals were treated with increasing dose of extract and at the study, biochemical parameters like lipid profile and endogenous oxidant and anti-oxidant profile, behavioural profile to assess diabetic neuropathy and histopathological changes were observed in cardiac muscle to assess whether the treatment alleviates diabetic cardiopathy and neuropathy.

Results of behavioural studies involving diabetic animals yielded interesting results. Extract treatment resulted in reversed hyperalgesia in diabetic animals in a dose dependent manner and effects were significant. Antioxidant biomarker levels further suggested possible antioxidant mechanism in relieving the symptoms of secondary complications. It is well established that free radical generation oxidative stress has been considered as risk factor / associated with initiation / progress of secondary complications[5,31].

Diabetic cardiomyopathy are the diabetes-related changes in myocardial structure and function, different from cardiomyopathy that is secondary to typical cardiovascular disease and it includes left ventricular hypertrophy, myocardial fibrosis and significant impaired diastolic function. Diabetic cardiomyopathy is likely to result in cardiac dysfunction in a diabetic patient. Although the pathogenesis is complex, oxidative stress (OS), inflammatory response, glucolipid metabolism disorder etc. are possible pathological basis of cardiac myopathy. Therefore, studies strongly propose regulating the oxidative stress signaling pathways and reducing the generation and accumulation of reactive oxidative stress in an ideal direction for the treatment of cardiac myopathy in diabetic patients[25]. Significant changes in antioxidant profile, lipid profile and other biomarkers of free radical damage in diabetic animals treated with extract chosen for the investifgation are confirmation of definite role played by extracts in reversing the progress of diabetic complications.

Conclusion

Aqueous ethanolic extract of *Adathoda vasica* and *Andrographis paniculata* alleviates diabetic cardiopathy and neuropathy in HFD- STZ model of diabetes.

Acknowledgement

Our sincere thanks go to the KLE College of Pharmacy, Bengaluru for their help and support in carrying out this research work.

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(Received 20th December 2023, accepted 18th February 2024)