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Antidiabetic potential of *Andrographis echioides* Nees. leaf extract on high fat diet-fed C57BL/6J diabetic mice

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Abstract: The present study validates the antidiabetic potential of *Andrographis echioides* leaf extract (AeLE) on high fat diet-fed diabetic C57BL/6J mice. The male C57BL/6J mouse (age 6-8 weeks) were divided into 2 groups (lean control group and diabetic group). The lean control group (6 animals) was fed with standard diet pellets. The diabetic group animals (24 animals) were made diabetic by feeding a high-fat diet for 12 weeks. This group was then further divided into 4 groups of 6 animals each and treated orally (for 28 days) with vehicle (0.5%carboxymethyl cellulose), metformin 100mg/kg body weight and 2 different concentrations of test drug viz., 100mg/kg and 200mg/kg body weight. The results show a significant reduction in blood glucose and other biochemical parameters. After 28 days, the metformin and AeLE (200 mg/kg b.w) treated animals had an average serum glucose value of 129.69±1.97 mg/dl and 109.6±3.92 mg/dl, respectively. Also, the liver markers were positively affected by AeLE. In conclusion, *A. echioides* leaf extract was found to reduce hyperglycemia and significantly improve the biochemical profile of the mice.

Keywords: *Andrographis echioides*, antihyperglycemic, high-fat diet, C57BL/6J

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood sugar levels over a prolonged period. It is associated with several conditions namely, obesity, insulin resistance, hyperglycemia, etc. There is an increased incidence of type 2 diabetes due to diet patterns rich in saturated fatty acids that cause obesity (Mann, 2002). Regardless of fewer incidences of overweight and obesity rates in India, the prevalence of diabetes is higher when compared to the western nations (Mohan and Deepa, 2006; Rao *et al*., 2011). Recent exploration of the use of herbs against a wide range of communicable and non-communicable diseases has widened the scientific platform to develop new drugs (Mohanasundaram *et al*., 2019). Achieving complete glycemic control using oral antidiabetic agents is not possible and hence alternative therapies with more efficacy and lesser side effects are the need of the hour. The Indian systems of medicine greatly involve the use of herbal plants such as *Gymnema sylvestre*, *Trigonella foenum-graecum*, *Withania somnifera*, *Aegle marmelos*, *Momordica charantia*, *Senna alata*, etc., for their hypoglycemic potential (Grover *et al*., 2002; Mohanasundaram *et al*., 2016; Rajendran *et al*., 2018). Such antidiabetic medicinal plants are not only used in monotherapy but also used in combination as polyherbal formulations (Riaz *et al.,* 2020). The glucose-lowering effect of these plants is attributed to the presence of phytocompounds such as polyphenols, flavonoids, saponins, alkaloids, and essential oils (Hamed, 2018). One such medicinally potent plant is *Andrographis echioides* (family: Acanthaceae) commonly known as false water willow which is reported in Indian materia medica for its use in the treatment of fevers. Notably, *Andrographis paniculata*, a medicinally important plant from the same family, is widely used for its antidiabetic potential (Sarma, 2020). Phytocompounds such as oxygenated flavonoids and phenyl glycosides were isolated from the whole plant extract (Shen *et al*., 2013). We have previously reported the glucose uptake enhancing potential of *A. echioides* leaf extract in 3T3 cell lines. The GC-MS profile of leaves revealed the presence of an antidiabetic agent 5-ethyl, 2-imino thiazolidine-4-one (Sindhu *et al*., 2016). The current study evaluates the *in vivo* antidiabetic potential of *A. echioides* leaves using high-fat diet-fed C57BL/6J mice model.

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MATERIALS AND METHODS

Preparation of plant extract

The leaves of *A. echioides* were collected from Thandarai forest, Chengalpet, Tamilnadu and taxonomically identified by Prof P. Jayaraman, Plant Anatomy Research Centre, Chennai, and a voucher specimen was submitted there (PARC/2015/3179). The leaves were subjected to solvent extraction using methanol following the standard soxhlet method (Redfern *et al*., 2014). The dry, solvent-free extract was used to evaluate its antihyperglycemic potential *in vivo*.

Test system and environmental conditions

The C57BL/6J male mice of age 6-8 weeks were procured from CPCSEA approved breeder and experiments were carried out at Central Animal House, Karpaga Vinayaga Institute of Medical Sciences (1818/GO/ERE/S/15/CPCSEA), following the regulations of CPCSEA. The present study is approved by the Institutional animal ethics committee with ethical clearance number 007/IAEC/KIMS/2016. The animals were housed in polypropylene cages and maintained in standard laboratory conditions of 19-23°C, 30-70% humidity, light 12L: 12D and 12-15 air changes per hour.

Induction of diabetes and Experimental Design

After 3 days of acclimatization in the laboratory, the animals were divided into 2 groups of 6 and 24 animals, respectively. The group 1 animal served as lean control and received a normal fat diet, in the form of standard diet pellets. Group 2 animals were subjected to induction of diabetes according to the method stated by Surwit and his co-workers (1988). Diabetes was induced by feeding the animals with a commercially procured High-fat diet (HFD) containing 47.7Kcal% fat for 12 weeks. After 12 weeks, animals with serum glucose level ≥ 180 mg/dl were considered as diabetic mice and were further separated into 4 groups (group 2-5) of 6 animals each. Then, all the groups were treated (oral administration) for 28 days as given below.

Group 1: Normal fat diet
Group 2: HFD + vehicle (0.5% CMC)
Group 3: HFD + Metformin (100 mg/kg b.w)
Group 4: HFD + *A. echioides* leaf extract (100mg/kg b.w)
Group 5: HFD + *A. echioides* leaf extract (200mg/kg b.w)

Evaluation of antihyperglycemic potential

The animals were observed for mortality, morbidity and signs of toxicity throughout the study. The bodyweight of animals was recorded weekly till the day of necropsy. For the evaluation of hypoglycemic potential, blood was collected from the retro-orbital plexus of 4 h fast mice before and after 28 days of treatment under anesthetic condition. The collected blood was centrifuged and the serum was stored at -80°C and subjected to biochemical estimations such as serum glucose, lipid profile, cholesterol (Cho), triglycerides (TG), Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST). All the estimations were done using commercial kits according to the manufacturer's instructions, using an automatic biochemical analyzer.

STATISTICAL ANALYSIS

The obtained data were subjected to statistical analysis for a significant difference between the variables using student's t-test and one-way ANOVA followed by DMRT's performed using SPSS 21.0 ver. The data are represented as Mean ± Standard Error of Mean (SEM).

RESULTS

Clinical Signs

No morbidity or mortality of animals was observed during the experimental period.

Evaluation of antihyperglycemic potential

Table 1 depicts the effect of HFD (fed to groups 2, 3, 4 and 5 for diabetes induction) before starting of drug treatment. The mean body weight has increased significantly ($p<0.05$) among the HFD fed groups (table 1). Also, variation in the weight gain was observed among the different groups which might be due to variation in animal behavior. The highest weight gain of 38.4 ± 3.18g was recorded in group 2 animals.

The effect of metformin and AeLE treatment on serum biochemical parameters of C57BL/6J mice are listed in table 2. The mean serum glucose levels of groups after treatment for 28 days with metformin and AeLE showed statistically significant ($p<0.05$) reduction when compared to the diabetic control group (group 2). Final serum glucose values of 129.69±1.97 mg/dl, 134.57±3.57 mg/dl and 109.6 ± 3.92 mg/dl was obtained for groups 3, 4 and 5, respectively. Furthermore, the treatment of the animals with AeLE has resulted in statistically significant ($p< 0.05$) reduction of serum cholesterol levels. The final mean cholesterol level of diabetic control mice was 151.11±1.78 mg/dl and that of treated groups 3, 4 and 5 were 124.91±3.15 mg/dl, 131.46±2.17mg/dl and 122.56 ± 2.31 mg/dl, respectively. However, it is to be noted that the reduction in serum triglyceride levels was not statistically significant when compared to the diabetic control group. In addition, the treatment with metformin and AeLE had varied effects on liver markers such as ALT and AST. The reduction in AST levels was found to be statistically non-significant whereas, statistically significant reduction in ALT levels was observed in groups treated with AeLE.

Glucose and cholesterol levels of groups 3, 4 and 5, show statistically significant reduction when compared to diabetic control (group 2). The reduction in TG and AST levels were not statistically significant during metformin and AeLE treatment. The ALT level of groups 4 and 5 showed a statistically significant reduction when compared to diabetic control (group 2) (table 2).

DISCUSSION

The diet-induced C57BL/6J diabetic model has unveiled marked fasting and basal hyperglycemia and also demonstrates several characteristics of genetic predisposition in human subjects to develop type 2 diabetes when they become obese (Winzell and Ahren, 2004). This obesity-induced hyperglycemic mice model also exhibits hyperlipidemia, sedentary behavior and disturbance in temperature regulation (King, 2012). The practice of feeding the mice with a high-fat diet has resulted in body weight gain, stable hyperglycemia, insulin resistance evident from the resulting increased body weight and serum lipid levels (He *et al*., 2018; Winzell and Ahren, 2004).

Table 1: Effect of High Fat Diet on body weight of C57BL/6J mice

Groups	Body weight (g)	
	Initial	After HFD
Group 1	24.20 ± 1.05 [a]	30.30 ± 1.83 [a]
Group2	23.36 ± 0.97 [a]	38.40 ± 3.18 [b]
Group3	23.46 ± 1.24 [a]	30.70 ± 2.87 [ab]
Group4	20.20 ± 2.20 [a]	30.20 ± 2.23 [ab]
Group5	22.30 ± 0.69 [a]	36.50 ± 3.45 [ab]

Table 2: Effect of *A. echioides* leaf extract on serum biochemical parameters

Parameters	Glucose(mg/dl)	TG (mg/dl)	Cho (mg/dl)	ALT (U/l)	AST (U/l)
Group 1	89.03 ± 6.02[a]	72.20 ± 7.06[a]	72.08 ± 2.61 [a]	50.94 ± 1.01 [ab]	45.5 ± 2.21 [a]
Group 2	254.03 ± 1.36[c]	77.73 ± 1.53 [a]	151.11 ± 1.78 [d]	80.69 ± 0.07 [ab]	46.83 ± 1.42 [a]
Group 3	129.69 ± 1.97[ab]	64.64 ± 5.77 [a]	124.91 ± 3.15 [c]	68.87 ± 1.04 [ab]	43.33 ± 1.89 [a]
Group 4	134.57 ± 3.57[ab]	73.53 ± 1.27 [a]	131.46 ± 2.17[c]	65.74 ± 4.01 [b]	42.83 ± 2.41 [a]
Group 5	109.6 ± 3.92[ab]	66.76 ± 6.57 [a]	122.56 ± 2.31[b]	61.44 ± 3.06 [a]	38.66 ± 1.85 [a]

The values are represented as Mean ± SEM; Different superscripts in the same column showed significant difference with $p < 0.05$.

In our study, metformin-treated animals showed a reduction in body weight the reason for which, as explained by various researchers, is the enhancement of leptin sensitivity (Al-Barazanji *et al*., 2015; Masud *et al*., 2016; Seifarth *et al*., 2013). The blood glucose levels were significantly reduced by AeLE which could be attributed to its ability to enhance glucose uptake, which was proven in our previous *in vitro* study (Sindhu *et al*., 2016). However, this proposed mechanism of action *in vivo* is yet to be elucidated. This hypothesis can be understood by studying the mechanism of action of metformin which inhibits intestinal absorption and hepatic production of glucose. It also enhances the ability of skeletal, smooth, and adipose tissues to uptake glucose effectively by insulin stimulation (Borst *et al*., 2000). It is also said that metformin enhances glucose uptake by glucose oxidation and glycogen synthesis (Klip and Leiter, 1990). Another relevant study on the anti-diabetic effect of *Anvillea radiata* extract on HFD fed C57BL/6J mice shows a significant reduction in blood glucose levels by the aqueous extract of *A. radiata* when administered either alone or in combination with metformin. Also, the *A. radiata* leaf extract exerted lipolytic and anti-adipogenic activity (Kandouli *et al*., 2017). The data obtained in our study show an increase in the blood cholesterol and triglyceride levels in high-fat diet-fed animals. A similarly elevated blood lipid level was recorded in streptozotocin-induced diabetic rats that were given high fat and high sucrose diet (Wang *et al*., 2020). However, AeLE significantly reduced lipid levels thereby proving its hyperlipidemic potential. The levels of liver markers such as ALT and AST play vital roles in the pathogenesis of diabetes. A high incidence of elevated ALT and AST levels was seen in newly diagnosed type 2 diabetic patients. This correlation, found in various studies, suggest that liver injury might be a cause for obesity-related type 2 diabetes (Saligram *et al*., 2012; Villegas *et al*., 2011). However, in some studies, AST levels remained unaffected by the metabolic disorder. Metformin treatment for a shorter duration of up to 2 months has also resulted in increased ALT and unaffected AST levels (Al-Mola and Ahmed, 2006). These findings could be well correlated to the data obtained in the current study.

CONCLUSION

In conclusion, *A. echioides* leaf extract reduces hyperglycemia with positive regulation of serum lipids and liver biomarkers. However, further studies are required to derive its effect on the metabolic pathways to explain this hypoglycemic action.

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