

ANTI-HYPER LIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *SMILAX WIGHTII* A.DC. IN STREPTOZOTOCIN INDUCED MALE WISTAR ALBINO RATS

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ABSTRACT

Smilax wightii, an endemic medicinal plant is found in the shola forests at high altitudes in Nilgiri Biosphere Reserve, the Western Ghats, Southern India. The present study, was undertaken to find out the effect of methanolic extract of this plant on the body weight, fasting blood glucose levels and lipid profiles in all the streptozotocin (STZ) induced rats. The extract exerted a significant ($P < 0.05$) effect in the body weight of the experimental animals when compared to the control group. Treatment with the extract and glibenclamide resulted in a significant ($P < 0.01$) reduction in the fasting blood glucose levels in diabetic rats when compared to the normal. The lipid profile such as TC, TG, LDL, and VLDL contents in the serum registered a significant ($P < 0.01$) hike and a decline in the HDL contents in diabetic control group, which were retrieved to near normalcy in the plant extract treated groups. The effect produced by this plant extract was comparable with that of glibenclamide. The decreased fasting blood glucose levels and lipid lowering properties clearly showed the anti-hyperlipidemic effect of *S.wightii*.

Key words: *Smilax wightii*, anti-hyperlipidemic, streptozotocin, glibenclamide

1. INTRODUCTION

Traditional medicinal herbs have served as a potential alternative source of medicine and different healthcare systems (Kamboj, 2000). Diabetes mellitus is a metabolic disorder, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins (Scheen, 1997). In modern medicine, no satisfactory effective therapy is still available to cure the diabetes mellitus (Piedrola *et al.*, 2001). Herbal treatments are becoming popular as the herbal preparations have no or least side effects and also they are of relatively low cost (Rajasekaran *et al.*, 2001).

The genus *Smilax* has more than 300 species, found on temperature zones, tropic and subtropics worldwide and belongs to Smilacaceae (Fnaec, 2000). The roots of *Smilax wightii* have been reported to cure dysentery, amoebiasis, venereal diseases, urinary complaints, fever, spermatorrhoea, antifertility, anaemia, rheumatic-arthritis, veterinary amoebiasis and gastric complaints (Adhikari *et al.*, 2010). The species of *Smilax* have been reported to contain several phytoconstituents such as dioscin, steroids smilagenin and sarsapogenin (Coimbatore BSI, 1989). Several species of *Smilax* are used in Chinese traditional system of medicines as anticancer, anti-inflammatory and analgesic agents (Ozoy *et al.*, 2008). Therefore, the present study was aimed to assess the anti-hyperlipidemic effects of

methanolic extract of *Smilax wightii* in animal models.

2. MATERIALS AND METHODS

2.1 Plant material

The plant materials were collected from Kodanadu, the Nilgiri Hills, Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist.

2.2 Preparation of extract

The whole plant materials were dried in shade after washing with cold water and then powdered using pulveriser and passed through sieve. About 100 g of dried plant powder was extracted with petroleum ether using soxhlet apparatus for 18 hours. The petroleum ether was evaporated from the extract and then the residue was re-extracted with methanol. This extract after evaporation of methanol, the filtered residue was stored at 4°C in refrigerator for further use.

2.3 Experimental animals

Male Wistar Albino rats weighing 180-250 g were obtained from Agricultural University, Animal house lab, Trissur, Kerala. The animals were fed on a standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. They were maintained in a controlled environment (12 h/12 h light/dark) and temperature ($25 \pm 2^\circ\text{C}$). The animals were acclimatized to the laboratory conditions for one week before starting the experiment. All the

procedures performed on animals were approved and conducted in accordance with the Institution of Animal Ethics committee and by the Regulatory body of the government (659/02/a/CPCSEA).

2.4 Toxicity studies

The acute toxicity studies were performed in adult male albino rats weighing 180-250g. The animals were fasted overnight and 100- 1000 mg/kg of the test extract was provided to various groups containing six animals in each group. The treated animals were monitored for 14 days, for behaviour, general health and mortality.

2.5 Induction of diabetes

Diabetes was induced in overnight fasted Wistar albino rats by intraperitoneal (i.p) injection of streptozotocin at a dose of 55 mg/kg b.wt. Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels were determined to confirm diabetes. The rats with blood glucose level above 200mg/kg were considered diabetic and used for the study.

2.6 Experimental design

The rats were divided into six groups comprising of six animals in each group as follows:

Group I : - Rats given normal saline daily for 14 days, orally (by using an intragastric catheter tube (IGC). (Normal control)

Group II:- Diabetic rats given normal saline daily for 14 days, orally by using IGC. (Diabetic control)

Group III: - Diabetic rats given methanolic extract of *Smilax wightii* (MESW) at the dose of 100 mg/ Kg b.wt. daily for 14 days , orally by IGC

.Group IV: - Diabetic rats given methanolic extract of *Smilax wightii* (MESW) at the dose of 200 mg/ Kg b.wt. for 14 days , orally by IGC

Group V: - Diabetic rats given methanolic extract of *Smilax wightii* (MESW) at the dose of 400 mg/ Kg b.wt. for 14 days , orally by IGC

Group VI : - Diabetic rats given Glibenclamide at the dose of 600 µg/kg/b.wt. daily for 14 days , orally by IGC .

2.7 Determination of body weight

The rats from all the groups were weighed prior to the experiment and their initial body weights were recorded. The final body weights of the rats were recorded on the last day of experiment.

2.8 Estimation of fasting blood glucose (FBG) levels

Fasting blood glucose levels were measured on 0, 7 and 14 days of treatment of methanolic extract of *Smilax wightii* (MESW) supplement from the animals of all the groups. Blood samples were obtained by nicking the tails with a sharp razor and glucose concentrations were determined using a one-touch glucometer (Johnson& Johnson medical Ltd., Mumbai). The glucose concentration was read and documented from the glucometer readings. The results were expressed in terms of (mg/dl) of blood.

2.9 Estimation of lipid profile

The experimental animals from all the groups were sacrificed under light ether anaesthesia at the end of the experimental period. The rats were sacrificed by decapitation and blood was collected with anti-coagulant and the serum was used to for the estimation of lipid profile-TC-Total cholesterol (Parekh and Jung,197), TG-Triglycerides (Rice, 1970), HDL-High Density Lipoprotein (Warnick *et al.*, 1985), LDL- Low Density Lipoprotein and VLDL - Very Low Density Lipoprotein (Friedwald *et al.*, 1972).

2.10 Statistical Analysis

All biochemical data are expressed as Mean ± SEM. Statistical analysis was performed using one-way ANOVA using SPSS statistical analysis programme. In all cases, p-value less than 0.05 was considered to be significant.

3. RESULTS

3.1 Toxicity studies

Acute toxicity studies revealed that the methanolic extract of *Smilax wightii* (MESW) was found safe to up to a dose of 1000 mg/kg body weight. No sign of toxicity was noticed on the general health of the animals, and no death was observed in the animals when exposed to the extract.

The activity of methanolic extract of *Smilax wightii* (MESW) on body weight in streptozotocin induced diabetic rats are indicated in Table 1. There was a significant weight loss in the final body weight in the diabetic control group (Group II). A significant improvement in the body weight was observed in the extract and glibenclamide treated groups when compared to that of the diabetic control group.

Table 1: Effect of methanolic extract of *S.wightii* on the body weight of normal, diabetic induced and drug treated rats.

| Treatment Groups (n=6) | Initial body weight (g) | Final body weight (g) | Body weight Gain (G↑) / loss(L↓) (g) |
|-----------------------------------|----------------------------|--------------------------|--|
| Group II (Diabetic control) | 208.16±6.13 | 192.13±5.84* | 16.06↓ |
| Group III (MESW 100mg/kg) | 202.84±7.84 | 212.17±4.36* | 9.33↑ |
| Group IV (MESW 200mg/kg) | 198.28±3.84 | 211.84±4.15* | 13.56↑ |
| Group V (MESW 400mg/kg) | 210.16±7.86 | 218.91±7.24 | 8.72↑ |
| Group VI (Glibenclamide 600µg/kg) | 206.82±5.91 | 215.05±5.16 | 8.23↑ |

Each Value is SEM of 6 animals *P <0.05 ; **P< 0.01 comparison with Normal control vs diabetic and drug treated. a , Comparison made between diabetic control to drug treated groups Level of significance a: P<0.05 , aa: P<0.01

Table 2: Effect of methanolic extract of *S. wightii* on fasting blood glucose levels of normal, diabetic induced and drug treated rats.

| Treatment Groups (n=6) | Fasting Blood glucose levels (mg/dl) | | |
|---------------------------|--------------------------------------|---------------------------|--------------------------|
| | 0- day | 7 th day | 14 th day |
| Group I | 78.13±2.41 | 82.18±1.93 | 73.93±3.54 |
| Group II | 216.13±9.13** | 226.31±8.16** | 229.75±9.15** |
| Group III | 193.16±5.84** | 131.28±7.24 ^a | 102.16±4.38 ^a |
| Group IV | 204.83±4.33** | 118.27±3.28 ^a | 93.83±2.84 ^{aa} |
| Group V | 212.48±5.83*** | 104.38±2.84 ^{aa} | 85.16±2.13 ^{aa} |
| Group VI | 218.93±6.27*** | 112.18±3.16 ^{aa} | 76.83±1.84 ^{aa} |

Each Value is SEM of 6 animals *P <0.05 ; **P< 0.01 comparison with Normal control vs diabetic and drug treated. a ,Comparison made between diabetic control to drug treated groups Level of significance a: P<0.05 , aa: P<0.01

Table 3: Effect of methanolic extract of *S. wightii* on the serum lipid profile of normal, diabetic induced and drug treated rats.

| Treatment Groups (n=6) | TC (mg/dl) | TG(mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|------------------------------|--------------------------|---------------------------|-------------------------|-------------------------|-------------------------|
| Group I | 126.84±4.16 | 113.88±2.14 | 43.18±1.84 | 60.89±2.12 | 22.77±1.02 |
| Group II | 198.15±3.16** | 165.65±3.18** | 23.15±1.28** | 141.87±2.67** | 33.13±1.33* |
| Group III | 173.89±2.88* | 141.80±3.67* | 35.18±1.16* | 110.03±3.62* | 28.36±1.87 |
| Group IV | 156.93±2.13* | 126.67±1.93 ^a | 39.26±1.93 | 92.34±1.89 | 25.33±1.56 |
| Group V | 134.16±2.14 ^a | 118.31±1.88 ^{aa} | 43.91±1.88 ^a | 61.59±2.02 ^a | 23.66±1.45 ^a |
| Group VI | 126.48±2.09 ^a | 135.16±2.16 ^a | 41.93±1.65 | 57.52±1.34 | 27.03±1.63 |

Each Value is SEM of 6 animals *P <0.05 ; **P< 0.01 comparison with Normal control vs diabetic and drug treated. a ,Comparison made between diabetic control to drug treated groups Level of significance a: P<0.05 , aa: P<0.

The fasting blood glucose levels of normal, diabetic and the extract treated diabetic rats are summarized in Table 2. There was a significant ($P<0.01$) elevation in FBG level in diabetic control group when

compared to normal control group. The administration of the plant extract in STZ induced diabetic rats at doses of 100,200 and 400 mg/kg b.wt produced significant ($P<0.01$) reduction in the

fasting blood glucose levels. The reducing effect of the plant extract at a dose of 400 mg/kg b.wt was found to be comparable to that of the reference drug Glibenclamide (600 mg/kg b.wt). The initial reduction in blood glucose was observed on the 7th day after the administration of the *Smilax wightii* extract. Meanwhile, on the 14th day, almost the FBG levels were reduced significantly at the dose of 400mg/kg b.wt in the extract and glibenclamide treated groups.

Table 3 depicts the changes in the levels of TC (Total Cholesterol), TG (Triglycerides), HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein) of the experimental animals. Diabetes induced by STZ in rats significantly ($P<0.01$) elevated the TC, TG, LDL, VLDL levels and decreased the HDL levels in diabetic control compared with normal control rats. In the present study, administration of MESW at dose of 100, 200 and 400 mg/kg b.wt to the diabetic rats showed significant ($P<0.05$, $P<0.01$) reduction in TC, TG, LDL, and VLDL levels and increase in HDL levels than diabetic control rats.

4. DISCUSSION

Diabetes is a major health problem affecting major populations worldwide. It is a chronic disorder in metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance. There are more than 30 million people with diabetes mellitus in India and the incidence is increasing. Decreased physical activity, increasing obesity, stress and changes in food consumption have lead to the cause of diabetes (Jarald *et al.*, 2008).

The present study was aimed to investigate the anti-hyperlipidemic activity of methanoic extract of *S. wightii* (MESW) in STZ-induced diabetic rats. The results of the study revealed that MESW at the doses of 400 mg/kg b.wt. significantly normalized elevated blood glucose level and restored serum lipid profiles towards normal values.

Streptozotocin STZ, a highly cytotoxic agent of pancreatic β -cells induces diabetes by damaging the cells and causes reduction in insulin secretion (Esmaeli and Yazdanparas, 2004). The increased glucose level in the diabetic control animals may be due to the destruction of the pancreatic cells caused by the STZ induction. The increased levels of plasma glucose in STZ-induced diabetic rats were lowered by the administration of *Smilax wightii* extract.

Diabetes affects both glucose and lipid metabolism (Sperling and Saunders, 2000).

Hyperlipidemia was reported as common in adults with diabetes and it is characterized most often by increased triglyceride and reduced HDL cholesterol levels. This is generally observed in both type 1 and type 2 diabetes, representing the defect of insulin action in each, either due to inadequate secretion or resistance (Garg and Grundy, 1990). Increased fatty acid concentrations also increased the β -oxidation of fatty acids, producing more acetyl-CoA and cholesterol in diabetics (Yokogoshi and Oda, 2000, Mard *et al.*, 2010). Alterations in plasma lipoprotein metabolism are common in diabetes, which tend to exaggerate any pre-existing tendencies towards elevated lipid levels (Merzouk *et al.*, 2004). The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis (Bopanna *et al.*, 1997). Previous studies in *Smilax chinensis* has reported similar results in the fasting blood glucose levels and lipid profile in Streptozotocin induced diabetic rats (Venkidesh *et al.*, 2010). On the basis of the present study, it could be concluded that the methanolic extract of *S.wightii* exerted a significant antidiabetic and antihyperlipidemic effect. This could be due to different types of active principles present in the extract which serve as a good adjuvant in the present armamentarium of antidiabetic drug and has therapeutic value.

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