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ANTI-DIABETIC EFFECT OF *WRIGHTIA TINCTORIA* EXTRACTS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Wrightia tinctoria leaves are used for treating diabetes by native practitioners of Chitoor District, India. In the present study, the antidiabetic effect of Petroleum Ether extract of *Wrightia tinctoria* leaves (PWT) was investigated in streptozotocin (STZ)-induced diabetic rats. Albino wistar rats were rendered diabetic by STZ (55 mg/kg, intraperitoneally). PWT was orally administered to STZ-induced diabetic rats at 200 and 400 mg/kg, p.o doses for 14 days to determine antihyperglycemic activity. The fasting blood sugar levels and serum biochemical analysis in STZ-induced diabetic rats were investigated. Oral administration of PWT (200 & 400 mg/kg) for 14 days exhibited a significant reduction in serum glucose, total cholesterol, and triglycerides in alloxan diabetic rats. The anti-diabetic and hypolipidemic activities of the pet ether extract of *Wrightia tinctoria L.* (PWT) were similar to those produced by glibenclamide at 600µg/kg (positive control, $p < 0.01$). The results demonstrate that PWT possesses potent antihyperglycemic and hypolipidemic activity in STZ-induced diabetic rats. These very encouraging results for diabetes control by Petroleum Ether extract of *Wrightia tinctoria* make the need for clinical studies in humans evident.

Keywords: *Wrightia tinctoria,* STZ-induced diabetic rats, anti-diabetic, hypolipidemic activity.

INTRODUCTION

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). The number of people suffering from the diabetes mellitus worldwide is increasing at an alarming rate with a projected 366 million peoples likely to de diabetic by the year 2030 as against 191 million estimated in 2000 (Wild et al., 2004).

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The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. In modern medicine (insulin, sulphonylureas, biguanides and thiazolidinediones), no satisfactory effective therapy is till available to cure the diabetes mellitus. In recent years, there has been renewed interest in plant medicine (Prince et al., 1998; Ladeji et al., 2003) for the treatment against different diseases as herbal drugs are generally out of toxic effect (Geetha et al., 1994; Rao et al., 2003) reported from research work conducted on experimental model animal.

Wrightia tinctoria is a deciduous tree with a light grey, smooth bark, amenable for carving. *Wrightia tinctoria* is called *dhudi* (Hindi) because of its preservative nature. Supposedly a few drops of its sap in milk prevent curdling

and enhance its shelf life, without the need to refrigerate. The native practitioners in and around Chitoor District, India, have claimed that The leaves are used for treating diabetes (Madhava Chetty, 2008). Psoriasis, analgesic, anti-inflammatory, diuretic, and antinociceptive activities were reported (Mitra et al., 1998; Krishnamoorthy and Ranganathan, 2000; Chopra et al., 1956; Bigoniya et al., 2006). In previous study hypoglycemic and hypolipidemic activity of *Wrightia tinctoria L*. in alloxan induced diabetes in albino wistar rats was reported. Therefore the present studies to conform the anti-diabetic activity of *Wrightia tinctoria L*. in STZ induced diabetic rats model.

MATERIALS AND METHODS

Preparation of extracts

The leaves of *Wrightia tinctoria* were collected, washed, cleaned, dried and pulverized in a grinder- mixer to obtain a coarse powder and then passed through 40 mesh sieves. About 180 g of powdered drug was extracted successively with petroleum ether using Soxhlet apparatus. The extraction was carried out until the extract becomes colorless. The solvent was completely removed from marc in case before the next extraction was carried out. The solvents are removed from either extract by distillation under reduced pressure. The dried extract thus obtained was kept in desicator and was used for further experiment. Percentage yield of Petroleum Ether Extract of *Wrightia Tinctoria* Leaves was found to be 5.8% w/w.

Phytochemical Screening

The phytochemical examination of Petroleum Ether extract of *Wrightia tinctoria* was performed by the standard methods (Harbone, 1973).

Animals Used

Albino wistar rats, weighing 150–200 g were used. The selected animals were housed in acrylic cages in standard environmental conditions (20–25 ◦C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee. (Reg. No. IAEC/ 930/a/06/ CPCSEA).

Acute toxicity study

Acute oral toxicity of Petroleum Ether extract of *Wrightia tinctoria leaves* was performed on albino wistar rats, according to OECD Guideline 423. Two groups of six rats each were used for the study. Group I served as control and received distilled water. Group II received single oral dose of PWT (2000mg/kg). The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 14 days. Food consumption was monitored daily and body weights were recorded weekly. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study (OECD, 2002).

Experimental induction of diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoenal injection of a freshly prepared solution of streptozotocin (STZ) (55 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5) (Rakieten et al., 1963). The animals were allowed to drink 1% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The treatment was started on the fourth day after STZ injection and this was considered as first day of treatment. The treatment was continued for 14 days.

Experimental design

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

Group I. Control rats receiving 0.1 M citrate buffer (pH 4.5).

Group II. Diabetic control and rats received only vehicle (2 ml/kg p.o) 25% Tween 80.

Group III. Diabetic rats received Petroleum Ether Extract of *Wrightia tinctoria* (200 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group IV. Diabetic rats received Petroleum Ether Extract of *Wrightia tinctoria* (400 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group V. Diabetic rats treated with glibenclamide (600 µg/kg b.w/day) suspended in 2% v/v Tween 80 orally for 14 days (Pari and Uma Maheswari, 2000).

Testing of fasting blood glucose level

Fasting blood glucose levels were measured on 0, 3, 7, and 14 days of treatment of Petroleum Ether extract of *Wrightia tinctoria leaves* supplement from the animals of all these groups. Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer (Atkin et al., 1991). The results were expressed in terms of milligram per deciliter of blood.

At the end of the experimental period, all the animals were sacrificed under light ether anesthesia. The guide line of our institutional ethical committee for this purpose was followed strictly. The rats were sacrificed by decapitation and blood was collected with anti-coagulant and the serum was used for the estimation of total cholesterol and triglycerides. Total cholesterol was estimated by the method of Parekh and Jung (1970). Triglycerides were estimated by the method of Rice (1970).

Histopathological study of pancreas

Pancreas were isolated and preserved in 10% formalin. Histopathological observation of the tissue was carried out at the Sri Venkateswara University, Pathology Laboratory, Tirupati, Andhra Pradesh -517 502.

Statistical Analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

Results

Phytochemical Screening

The results of preliminary phytochemical screening of Petroleum Ether Extract of *Wrightia tinctoria* (L.) revealed that presence of carbohydrates, gums and mucilage, fixed oils, alkaloid, glycosides, steroids and triterpenoids.

Acute oral toxicity study

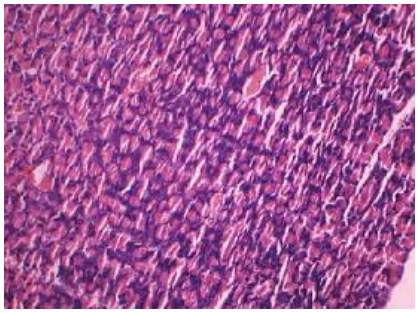
In acute toxicity study, PWT treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weights and food consumption when compared to the vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded that PWT was safe at 2000 mg/kg.

Effect of PWT on fasting blood glucose in STZ-induced diabetic rats

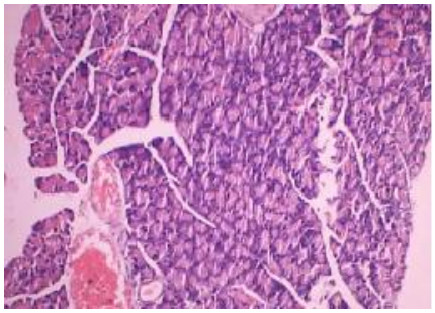
The effect of repeated oral administration of FRAE on blood glucose levels in STZ-diabetic rats is presented in Table 1. PWT, administered at three different doses of 200 & 400 mg/kg to STZ-
treated diabetic rats caused significant ($P < 0.001$) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 14. PWT 400 mg/kg exhibited maximum glucose lowering effect in diabetic rats compared to the other two doses. Glibenclamide exhibited significant reduction in blood glucose levels at the end of the study when compared to diabetic control.

Effect of PWT on serum lipids in STZ-induced diabetic rats

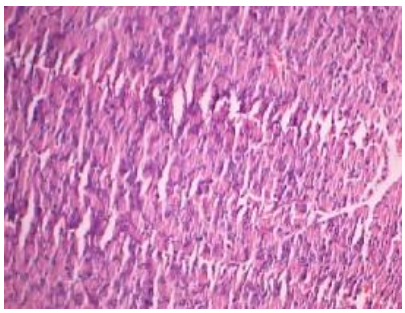
PWT showed a dose related significant ($P < 0.01$) reduction in triglycerides compared to pretreatment levels (Table 2). PWT at the doses of 200 and 400 mg/kg was more effectively reduced the cholesterol levels than diabetic control rats.



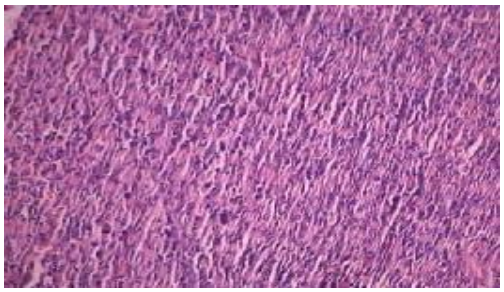
Group-I (Normal Control)



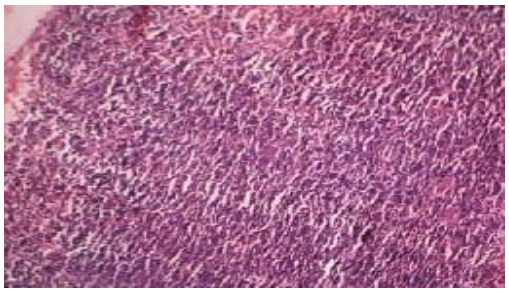
Group-II (Diabetic Control)



Group-III (PWT-200mg/kg)



Group-IV (PWT-400mg/kg)



Group-V (Glibenclamide - 600 µg/kg)

Table 1: Effect of Wrightia tinctoria on fasting blood glucose levels of STZ- induced diabetic rats

Groups (n=6)	Fasting Blood Glucose Levels			
	0th Day	5th Day	10th Day	15th Day
Group-I (Normal Control)	95 ± 1.15	95.66 ± 1.14**	95.16 ± 0.79**	95.5 ± 0.76**
Group-II (Diabetic Control)	279.5 ± 2.47	272.33 ± 2.19	282.33 ± .04	293.66 ± 2.96
Group-III (PWT-200mg/kg)	276.5 ± 2.75[a]	231.5 ± 1.97**[a]	167.66 ± 2.46**[a]	123.16 ± .41**[a]
Group-IV (PWT-200mg/kg)	282.16 ± 3.89[a]	229 ± 3.44**[a]	181.83±1.04**[a]	120.5 ± 1.28**[a]
Group-V (Glibenclamide - 600 µg/kg)	281.5 ± 3.05[b]	206.33 ± 2.24**[b]	138.16 ± 3.56**[b]	100 ± 0.66**[b]

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: **a**. diabetic + PWT - 200 & 400 treated rats vs. diabetic control rats. **b**. diabetic + Glibenclamide treated rats vs. diabetic control rats.

Table 2: Effect of *Wrightia tinctoria* on Total lipid profile of STZ- induced diabetic rats

Groups (n=6)	Biochemical Parameters				
	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group-I (Normal Control)	80.72±0.43**[a]	90.86±0.17**[a]	34.39±0.50**[a]	33.14±0.63**[a]	15.01±0.90**[a]
Group-II (Diabetic Control)	137.57±1.10	152.72±5.89	15.87±4.19	75.52±4.19	48.2±5.12
Group-III (PWT-200mg/kg)	112.45±0.59*[b]	97.58±0.44**[b]	25.39±0.25**[b]	38.65±0.59**[b]	28.52±0.44**[b]
Group-VI (PWT-400 mg/kg)	95.00±0.47**[b]	89.04±0.49**[b]	27.93±0.39**[b]	35.07±0.35**[b]	24.07±0.47**[b]
Group-V (Glibenclamide - 600 µg/kg)	84.14±0.61**[c]	82.00±0.49**[c]	28.71±0.34**[c]	32.17±0.25**[c]	20.52±0.58**[c]

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: **a**. Normal control rats vs. diabetic control rats. **b**. diabetic + PWT - 200 & 400 treated rats compared with diabetic control rats. **c**. diabetic + Glibenclamide treated rats vs. diabetic control rats.

Discussion and conclusion

This study was undertaken to evaluate the antidiabetic activity of PWT in streptozotocin-induced diabetic rats. Oral administration of PWT for 14 days caused a significant decrease in blood glucose levels.

STZ, a highly cytotoxic agent of pancreatic β-cells (Elsner et al., 2000), induces diabetes by damaging the cells that causes reduction in insulin release. So STZ significantly induced hyperglycemia accompanied by hypoinsulinemia. It is reported that treatment of diabetic animals with medicinal plant extracts resulted in activation of β-cells and granulation returned to normal, showing an insulinogenic effect (Kedar and Chakrabarti, 1982). The possible mechanism by which PWT mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals. In this context, a number of other plants have been reported to have antihyperglycemic activity with a stimulatory effect on insulin release (Esmaeili and Yazdanparas, 2004; Sharma et al., 2006). Since the extract produced highly significant antihyperglycemic effect even in streptozotocin-induced diabetic rats in which most of the β- cells are damaged, it is likely that PWT might have extrapancreatic mechanism of action. From the results of the present study, it may be suggested that the mechanism of action of PWT may be similar to glibenclamide action.

In diabetes, hyperglycemia is accompanied with dyslipidemia (Bierman et al., 1966; Garber, 2002), i.e. characterized by increase in TC, LDL, VLDL, TG and fall in HDL. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart diseases which are the secondary complications of diabetes (Ananthan *et al.,* 2003). This altered serum lipid profile was reversed towards normal after treatment with the PWT. PWT exhibited hypocholesterolemic and hypotriglyceridemic effects, while increased the levels of HDL in streptozotocin-induced diabetic rats. However, PWT was found to be more effective in reducing the levels of TG and LDL as compared to its effect on TC. The elevated atherogenic index, i.e. TC/HDL ratio, which is a useful determinant of cardiovascular risk (Grover et al., 1999), was also shifted towards normal after PWT treatment. Thus, it is reasonable to conclude that PWT could modulate blood lipid abnormalities. Since PWT up to 2000 mg/kg dose did not reveal any physical signs of toxicity or mortality even after 14 days of treatment, it can be considered relatively safe.

Thus, the significant antidiabetic effect of PWT could be due to the presence of various phytoconstituents detected in the phytochemical screening which alone or in synergism can impart therapeutic effect. It is concluded from the data that PWT possesses potent antihyperglycemic and hypolipidemic activity and it may prove to be effective for the treatment of both types of diabetes, i.e. IDDM and NIDDM. Further studies to isolate, identify and characterize the active principle(s) are in the progress.

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