Hypoglycemic and Hypolipidemic Activity of *Wrightia Tinctoria L.* in Alloxan Induced Diabetes in Albino Wistar Rats.

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Summary

Wrightia tinctoria L., a member of the Apocynaceae family, is used in folk medicine because of its treatment in piles, fever, diarrhea, roundworm and colic. The leaves are useful in treating Psoriasis, analgesic, anti-inflammatory, diuretic, antinociceptive and anti diabetic activity. Aim of this study to investigate the hypoglycemic and hypolipidemic activity of petroleum ether extract of *Wrightia tinctoria L.* (PWT) in alloxan induced diabetes in albino wistar rats. Alloxan was administered as a single dose (120mg/kg, b.wt) to induce diabetes. Administration of pet ether extracts from leaves of *Wrightia tinctoria L.* (200 &400mg/kg body weight/day) for 14 days, to alloxan-induced diabetic rats. The fasting blood sugar levels and serum biochemical analysis in alloxan-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 hypoglycemic activities of the pet ether extract of *Wrightia tinctoria L.* (PWT) were similar to those produced by glibenclamide at 2.5mg/kg (positive control, $p < 0.05$). The results of this work suggest that *Wrightia tinctoria L.* may have new clinical significant choice in diabetes mellitus illness, and could explain the basis for its traditional use to manage diabetes-related complications by rural community of southern of India.

Keywords: *Wrightia tinctoria L.*, Antidiabetic Activity, Alloxan Induced Diabetes, Total cholesterol and Triglycerides.

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Introduction

Diabetes mellitus is a global burden as its incidence is considered to be high (4–5%) all over the world[1]. However, quest for the development of more effective antidiabetic agents is being pursued relentlessly. Recently, herbal products have started gaining importance as complementary and alternative medicine to treat diabetic mellitus[2,3].

Hence a proper remedy for diabetes mellitus has to be found before the need reaches to its culmination. Though, many herbal products have been described for the treatment of diabetic mellitus, very few of them have been explored scientifically so far. The existing antidiabetic drugs encounter many adverse effects and need on prolonged treatment including questionable efficacy in the treatment. This forces the area of research to find improved treatments which will counteract the adverse effects and draw backs of the existing treatment.

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[4]. Psoriasis, analgesic, anti-inflammatory, diuretic, and antinociceptive activity was reported [5-8].

Materials and Methods

Plant collection

The Plant material of *Wrightia tinctoria* used for investigation was collected from S.V. university at Tirupathi, Chittor (Dist.), Andhra Pradesh, India. The plant was authenticated by Dr.K.Madhava Chetty, Assistant Professor, Department of botany, S.V.University, Tirupathi. The voucher specimen of the plant was deposited at the college for further reference.

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 [9].

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

The acute toxicity of Petroleum Ether extract of *Wrightia tinctoria leaves* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [10].

Antidiabetic Activity

The method of **Dash et al.**[11] was followed. The test samples were suspended in 2%v/v Tween 80 in distilled water. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered through p.o route.

Single Dose Study

In Alloxan induced diabetic rats

The acclimatized rats were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of Alloxan monohydrate in normal saline. After 1 h, the rats were provided feed *ad libitum*. The blood glucose level was checked before Alloxanisation and 24 h after Alloxanisation. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anesthesia. Plasma was separated following centrifugation the glucose was estimated by using Glucose estimation kit from **one touch ultra**, **Life Scan, Johnson and Johnson, Milpitas, C.A., U.S.A.**

Experimental Design

Rats were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This condition was observed at the end of 48 h after Alloxanisation. The rats were segregated into four groups of six rats in each. Group I - diabetic control and rats received only vehicle (2 ml/kg p.o) 25% Tween 80. Group II - rats received Petroleum Ether Extract of *Wrightia tinctoria* (200 mg/kg/day p.o) suspended in 2% v/v Tween 80. Group III - rats received Petroleum Ether Extract of *Wrightia tinctoria* (400 mg/kg/day p.o) suspended in 2% v/v Tween 80. Group IV – rats received Glibenclamide (2.5 mg/kg p.o) suspended in 2% v/v Tween 80. Blood glucose levels were examined after 1, 3, 5, 7 and 24 hr of administration of single dose of PWT (200 & 400 mg/kg/day p.o).

Multidose Study

In Alloxan induced diabetic rats.

The selected rats were treated with similar kind of test samples as above, but the blood glucose level was measured on 0, 3, 7, and 14 days of treatment.

Estimation of serum Lipid Profile

Estimation of Lipid profile such as Total Cholesterol, Triglycerides and serum glucose level by method of **Sood, 1999** [12].

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.

Effect of PWT on blood glucose level

There were observable changes in BGL and lipid profile of treated and untreated rats. Treatment of diabetic rats with pet–ether extract of *Wrightia tinctoria* and Glibenclamide significantly decreased the BGL compared to untreated diabetic rats. Dose dependent reduction in BGL, TC and TG was observed in Alloxan induced diabetic rats treated with petroleum ether extract of *Wrightia tinctoria*.

Single dose study

After single dose of the PWT (200 or 400 mg/kg, p.o) on the Alloxan induced diabetic rats, there was a significant reduction (P>0.01) in BGL of the diabetic rats with in the period of acute study which was seven hours compared to the control. The effect was significant like the standard drug, Glibenclamide. PWT at the dose of 400 mg/kg body weight exhibited better BGL reduction (70.74%) than 200 mg/kg body weight (68.7%) and that produced by the standard drug, Glibenclamide 2.5mg/kg (71.42%) at the same period (Table 1).

Multidose study

During prolonged study (14 days), the PWT (200 or 400 mg/kg) produced a significant (P>0.01) in BGL of the diabetic rats compared to control.PWT at the dose of 400 mg/kg body weight exhibited better BGL reduction (74.39%) than 200 mg/kg body weight (65.74%) and that produced by the standard drug, Glibenclamide 2.5mg/kg (75.77%) at the same period. (Table 2).

Serum lipid profile

Beneficial effects of PWT on serum lipids, one of the major cardiovascular risk factors in type 2 diabetes mellitus, can be observed from lipid-related data (Table 3). Compared with the control values, the PWT (200 or 400 mg/kg) groups showed significant reduction ($P > 0.01$) in the serum levels of total cholesterol and triglycerides.

Discussion and Conclusion

The petroleum ether extract of *Wrightia tinctoria* (PWT) was screened to explore the scientific basis of its utility for correction of biochemical changes in Alloxan-induced diabetic rats. Models of experimental diabetes that utilizes diabetogenic agent Alloxan induced blood glucose levels higher than 250 mg/dL which has been considered as severe diabetes [13]. Diabetes mellitus is one of the most common chronic disease and is associated with hyperlipidemia and co-morbidities such as obesity and hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes [14].

Several workers have shown that hyperglycemia and hyperlipidemia are the common characteristics of Alloxan-induced diabetes mellitus in experimental rats [15-17]. The maximum reduction (74.39%) in serum glucose levels was seen in PWT at the dose of 400 mg/kg (Table 2). Thus, we could say that PWT had a beneficial effect on carbohydrate metabolism in diabetic rats. The hypoglycemic effect of PWT may be its effect on potentiating the insulin activity either by increasing the pancreatic secretion of insulin from cells of islets of langerhans or its release from bound insulin [18].

Dyslipidemia is a frequent complication noted in chemical induced diabetes [16,17,19] and presents a serious risk of vascular disease. In this study, we have also observed an increase in the concentration of TC and TG in alloxan induced diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus [13,20]. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of the glucose [21]. Regarding the mechanism of action PWT may enhance activity of enzymes involved in bile acid synthesis and its excretion and this may have decreased in serum cholesterol and triglycerides[22].

Most of the hypolipidemic drugs do not decrease serum TG level, but PWT lowered it significantly since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis the triglycerides [23]. The total lipid profile in serum (total cholesterol, triglycerides) of the Alloxan induced diabetes rats treated with PWT (200 or 400 mg/kg, p.o) showed significant reduction, as compared to diabetic control rats (Table 3). This suggests that PWT can prevent or be helpful in reducing the complications of lipid profile observed in some diabetics in whom hyperglycemia and hypertriglyceremia coexist quite frequently. The strong anti-hyperglycemic effect of PWT could indirectly be related to beneficial action against the abnormal high concentration of serum lipids observed in diabetes rats.

Finally, although the present data clearly indicate the strong antihyperglycemic and antihyperlipidemic effect observed in Alloxan-induced diabetic rats justifies the use of *Wrightia tinctoria* for the treatment of diabetes and related complications. Additional studies are necessary to isolate and identify the active principle as well as identify possible links between Petroleum Ether Extract of *Wrightia tinctoria Linn* and plant's chemical composition.

Table 1: Effect of *Wrightia tinctoria* on blood glucose levels of Alloxan induced diabetic rats after a single dose

Groups	Drugs	Dose	Initial	1hr	3hr	5hr	7hr	24hr
Group I	Diabetic control	2% Tween 80 w/v soln p.o	277.50±1.26	274±1.47	275±1.77	276±1.39	281.5±2.03	294±1.37
Group II	Diabetic control + PWT	200 mg/kg p.o	289.67±2.26 ^{nsa}	226±1.69 ^{nsa}	200.33±1.49 ^{**a}	157.50±2.80 ^{**a}	109.67±2.14 ^{**a}	91.833±1.49 ^{**a}
Group III	Diabetic control + PWT	400 mg/kg p.o	277.83±1.83 ^{nsa}	218.83±3.88 ^{**a}	194±1 ^{**a}	149.17±2.10 ^{**a}	109.17±2.94 ^{**a}	86.167±2.17 ^{**a}
Group IV	Diabetic control + standard	Glibenclamide (2.5 mg/kg) p.o	281.67±1.94 ^{nsb}	205±1.37 ^{**b}	151.83±1.22 ^{**b}	139.17±1.70 ^{**b}	100.67±2.49 ^{**b}	84.667±1.89 ^{**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 with in 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 blood glucose levels of Alloxan induced diabetic rats after a prolonged treatment

Groups	Drugs	Dose	Initial	Third day	Fifth day	Seventh day	Fourteenth day
Group I	Diabetic control	2% Tween 80 w/v soln p.o	277.80±1.26	285±1.07	285±1.41	286.33±1.58	289.17±1.97
Group II	Diabetic control + PWT	200 mg/kg p.o	289.67±2.26^{nsa}	207±1.21 ^{nsa}	149.5±1.20^{**a}	117±2.67^{**a}	99±2.42^{**a}
Group III	Diabetic control + PWT	400 mg/kg p.o	277.83±1.83^{nsa}	132.16±2.19^{**a}	87.83±2.88^{**a}	85±2.25^{**a}	74±3.28^{**a}
Group IV	Diabetic control + standard	Glibenclamide (2.5 mg/kg) p.o	281.67±1.94^{nsb}	76.33±3.10^{**b}	78.83±1.51^{**b}	76.67±1.25^{**b}	70.17±2.45^{**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 3: Effect of *Wrightia tinctoria* on Total Cholesterol and Triglycerides levels of Alloxan induced diabetic rats after a prolonged treatment

Groups	Drugs	Dose	Total Cholesterol	Triglycerides
Group I	Normal Control	2% Tween 80 w/v soln p.o	72.80±2.40	74±2.07
Group II	Diabetic control	2% Tween 80 w/v soln p.o	289.5± 2.21**[a]	194 ± 1.88**[a]
Group III	Diabetic control + PWT	200 mg/kg p.o	138.66 ± 0.98*[b]	111.16 ± 0.87*[b]
Group IV	Diabetic control + PWT	400 mg/kg p.o	93 ± 1.21**[b]	71.66 ± 1.17**[b]
Group V	Diabetic control + standard	Glibenclamide (2.5 mg/kg) p.o	85.5 ± 0.99**[c]	68.33 ± 1.56**[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.

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