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Pharmacological evaluation of traditional claims of Jharkhand region: Using seeds of *Wrightia tinctoria*, a adulterant of kurchi in hyperglycemia and hyperlipidemia

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Abstract

The seeds of *Wrightia tinctoria* (Roxb) R.Br., is known as adulterant of kurchi seed. *W. tinctoria* has been widely used for treatment of various medical complications in traditional system of medicine in north and central India. Commonly *Wrightia tinctoria* R. Br. Known as sweet indra-jao, dudhi, Pala Indigo Plant, Paalai plant. The present investigation deals with Hyperglycemic and Hyperlipidemic effect of ethanolic extract of seed of *W. Tinctoria* in alloxan induced diabetic albino rats. This claim was based on tradition practice followed by some traditional healers of Jharkhand region for treatment of diabetes and management of high cholesterol level. In this study diabetic rats were treated with extracts of *Wrightia tinctoria* (WT) 200 & 400 mg/kg body weight in (1% CMC) for 21 days. The results were compared with that of the standard drug glibenclamide. Blood glucose levels were estimated and serum total cholesterol (TCH), Triglycerides (TG) and High density lipoprotein (HDL) levels were also evaluated in normal and alloxan induced diabetic rats. Both the extracts (WT₁ and WT₂) significantly lowered blood glucose level. The WT₁ and WT₂ extracts also produced a significant decrease in the serum levels of total cholesterol (TCH) and (TG) triglycerides and significant increase in (HDL) high density lipoprotein levels.

Keywords: *Wrightia tinctoria*, hyperglycemic, hyperlipidemia, alloxan, total cholesterol, triglycerides high density lipoprotein

1. Introduction

Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments which also forms a rich source of knowledge [1]. Over 80,000 species of plants are in use throughout the world. In India around 20,000 medicinal plant species have been recorded but more than 500 traditional communities use about 800 plant species for curing different diseases [2]. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels [3].

Roughly 80% of people with diabetes are in developing countries, of which India and China share the larger contribution [4]. A multitude of herbs spices and other plant materials have been described for the treatment of diabetes throughout the world. The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads [5].

The plant kingdom is a wide field to search for natural effective oral hypoglycaemic or hypolipidemic agents that have slight or no side effects. More than 400 plants with glucose-lowering potential are known [6]. Also a number of plants are known to have hypolipidemic activity [7-9]. However, there is little information about plants with both hypoglycaemic and hypolipidemic effects.

The present investigation deals with Hyperglycemic and Hyperlipidemic effect of ethanolic extract of seed of *Wrightia tinctoria* in alloxan induced diabetic albino rats. This claim was based on tradition practice followed by some traditional healers of Jharkhand region for treatment of diabetes and management of high cholesterol level. *Wrightia tinctoria* R.Br. belongs to family Apocynaceae, commonly known as Indrajau and dudhi, the common vernacular names known are as: sweet indrajao, ivory wood, pala indigo plant in English, duhi, mitaindarjau kapar in Hindi, dandappala, ayyappala, vettupala in Malayalam, kala kuda in Marathi, Paalai in Tamil. It is distributed throughout the world and found in north and central part of India [10]. The plant is reported to contain the presence of flavanoid, glycoflavones-isorientin, and phenolic acids and so many other pharmaceutically significant natural compounds [11].

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The GC-MS analysis revealed 22 components from pet ether fraction obtained from elution of ethanolic extract of *W. tinctoria* packed in silica column. The prevailing significant compounds from fraction F₆ to F₉ were 6, 9, 12, 15-Docosatetraenoic acid, methyl ester (3.39%, Anticholesterol compound) and Trilinolein (7.74%, anti-ischemic, antiarrhythmic, and antioxidant) [12].

2. Methodology

2.1 Plant Material

The *Wrightia tinctoria* seed were collected from its natural habitat in Jharkhand, identified by botanist at (NBRI) National Botanical Research Institute, Lucknow, India, voucher specimens (NBRI-SOP-202) were preserved at the Herbarium of the same institute for future reference.

2.2 Preparation of *Wrightia tinctoria* Extracts

The seeds of *Wrightia tinctoria* washed dried and powered, extracted with per ether, chloroform, ethanol and water in successive session in soxhlet percolator. Extractable value calculated by evaporating the solvent in vacuum evaporator. Pet. Ether, Chloroform, Alcohol, Water soluble extractives were reported 20%, 6%, 10%, 4% w/w. In pet ether no crystalline component obtained, while chloroform and ethanolic extracts were semisolid in nature. Ethanolic extract of the plant was selected to carry out the pharmacological activity.

2.3 Animals

Adult albino rats of 16-19 weeks age of either sex, weighing 200-250 g, were procured from the Disease-Free Small-Animal House, United Institute Pharmacy Allahabad, Naini (India). The animals were kept in clean and dry plastic cages, with 12 h: 12 h light-dark cycle at 25 ± 2 °C temperature and 45-55% relative humidity. The animals were fed with standard pellet diet and water was given *ad libitum*. For experimental purpose the animals were kept fasting overnight but allowed free access to water. The Institutional Animal Ethics Committee (IAEC) of the United Institute Pharmacy, UCER Registration. Number-1451/PO/a/11/CPCSEA (Allahabad, Naini (India)) approved the study.

2.4 Chemicals

All chemicals and solvents used for various study were analytical grade and procured from approved chemical supplier.

2.5 Selection of doses

The selection of dose for ethanolic extract of *Wrightia tinctoria* 200 and 400mg/ kg body weight based on previous literature available on toxicity study [13].

2.6 Drug administration

The quantities of the individual drugs to be administered were calculated and suspended in vehicle (1% w/v suspension of carboxy methylcellulose (CMC) in water (10 ml/kg b.w.)). The drug was administered continuously for 21 days orally using an infant feeding tube. The results were compared with that of the standard drug glibenclamide which was also given continuously for 21 days

2.7 Induction of experimental diabetes through alloxan induced model

The numbers of animal models for induction of diabetes were available in various literatures like chemical induction model,

genetic model, surgical model, normoglycemic model, oral glucose loading model. Among the entire model the chemical induction by alloxan is easy method of inducing diabetes mellitus in rats. The present study was performed to justify the effect of *W. tinctoria* ethanolic seed extracts in the treatment of diabetes and its related complications like hyperlipidemia and hypercholesterolemia.

2.8 Experimental Design

All animals were allowed to adapt to cages for 3 days, after which they were fasted overnight. A single dose (120 mg/kg b.w, i.p.) of alloxan monohydrate (1%) dissolved in distilled water was used for induction of diabetes mellitus in the rats. Diabetes was confirmed 6 hr after alloxan injection by determining the blood glucose concentration; only animals with blood glucose of 200-300 mg/dl (mild diabetes) were used for the experiment. The diabetic animals were allowed free access to tap water and standard pellet diet and were maintained at room temperature in plastic cages. Since the injection of alloxan can provoke fatal hypoglycaemia due to a reactive massive release of pancreatic insulin, the rats were also orally given 5–10 ml of a 20% glucose solution after 6 h. The animals were then kept with free access to 5% glucose solution for the next 24 h to prevent severe hypoglycaemia. The rats with moderate diabetes having glycosuria and hyperglycaemia (i.e. with blood glucose levels of 200–300 mg/dl) were chosen for the experiments. Five groups of rats were used to study the effect of ethanolic extract of plants. Each groups consisting of six rats.

Group I – Normal Control rats received vehicle solution (1% CMC)

Group II - Diabetic control rats received vehicle solution (1%CMC)

Group V and VI - Diabetic rats treated with extracts of *Wrightia tinctoria* (WT₁) 200 & (WT₂)400 mg/kg body weight in (1% CMC), respectively.

Group VII- Diabetic rats treated with standard drug glibenclamide 10 mg/kg body weight in (1% CMC).

All doses were started 48h after alloxan injection. Fasting blood glucose levels were estimated by glucometer on day 0, 1, 2, 4, 7, 14, 21. After blood glucose estimation on 21 day the blood samples were collected by puncture of retro orbital plexus with capillary tube. Blood glucose levels were estimated by glucose-oxidase method using semi auto analyzer. Serum total cholesterol (TCH), Triglycerides (TG) and High density lipoprotein (HDL) levels were also evaluated in normal and alloxan induced diabetic rats. Data so obtained was used for statistical analysis.

2.9 Biochemical estimation

Analytical methods were used for assessment of the lipid profile.

2.10 Estimation of Total Cholesterol (T.C.)

Total plasma cholesterol (T.C.) was assayed according to cholesterol oxidase method, Modified Roeschlau's Method [14]. In brief the estimation of cholesterol involves the following enzyme catalysed reaction.

Cholesterol ester^{CE} → Cholesterol fatty acid

Cholesterol + O₂^{Chod} → Cholest-4-en-3-one + H₂O₂

2H₂O₂ + 4AAP + Phenol^{Pod} → 4H₂O + Quinoneimine

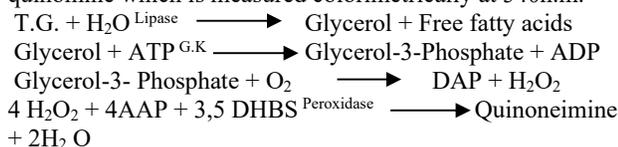
Where CE: Cholesterol esterase, CHOD: Cholesterol Oxidase, 4AAP: 4-Aminoantipyrine

Absorbance of Quinoneimine so formed is directly proportional to cholesterol concentration in the specimen. The

absorbance was measured spectrophotometrically at 550 nm. Using a Jenway 6105UV spectrophotometer. Calibration curve was constructed using cholesterol acid standard.

2.11 Estimation of (T.G) Total triglyceride

Total triglyceride (T.G.) was determined following the method of glycerol phosphate oxidase method by Wako and the modification by McGowan *et al* and Fossati *et al* [15]. This method resides simply on the enzymatic hydrolysis of triglycerides to glycerol, which reacts with ATP to form hydrogen peroxide, which in turn reacts with 4-aminoantipyrine in the presence of p-chlorophenol to form a quinomine which is measured colorimetrically at 540nm.



Where G.K: Glycerol Kinase, GPO: Glycerol Phosphate Oxidase, DAP: Dihydroxyacetone phosphate, ATP: Adenosine triphosphate, 4-AAP: 4 Aminoantipyrine, DHBS: 3,5 Dichloro-2-hydroxy benzene sulfonate. Intensity of chromogen (Quinoneimine) formed is proportional to the Triglycerides concentration in the sample when measured at 510 nm (500-540nm)

2.12 Estimation of High density lipoprotein (HDL)

HDL cholesterol was determined according to the method of Warnick *et al* [16].

2.13 Statistical analysis

All values of results were presented as mean \pm standard error

of mean (S.E.M.) The statistical analysis involving two groups which were evaluated by means of Student's t-test, whereas one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test was used for statistical comparison between control and various treated groups by using graph pad inStat software. Statistical significance was accepted at the $P < 0.01$ values.

3. Results and Discussion

The effect of Wrightia tinctoria (WT) alcoholic extracts on blood glucose level in alloxan induced diabetic rats

Ethanollic extract revealed the presence of Steroids, Flavonoids and Phenolics. Ethanollic extracts were selected for the study based on the preliminary phyto-chemical evaluation as they are rich in flavonoids and phenolics which possess antioxidant properties. The results of hypoglycaemic effects produced by the ethanollic extract of seed of *W. tinctoria* at two dose levels (WT₁ and WT₂) in normal and alloxan induced diabetic rats were summarized in the Table-1. During prolonged study (21 days), the WT₁ and WT₂ (200 or 400 mg/kg) produced a significant ($p < 0.01$) reduction in Blood Glucose Level (BGL) of the diabetic rats compared to control. WT₂ at the dose of 400 mg/kg body weight exhibited better BGL reduction (64%) than 200 mg/kg body weight (60%) and that produced by the standard drug, Glibenclamide 10 mg/kg (48.28%) at the same period (Table-1). Both the extracts (WT₁ and WT₂) significantly lowered blood glucose level and showed maximum reduction of 64% and 60% respectively ($p < 0.001$) on Day 21, whereas inhibition of 48.28% ($p < 0.001$) was found for standard drug glibenclamide on Day 21 as peak. In this case the extracts were found to be more effective than that of standard drug glibenclamide

Table 1: The effect of oral administration of *Wrightia tinctoria* (WT) ethanollic extracts at doses of 200mg (WT₁) and 400 mg (WT₂) on blood glucose level in alloxan induced diabetic rats.

	0 day	1 day	2 day	4 day	7 day	14 day	21 day
Gp I	84.83 \pm 0.41	85.00 \pm 0.0	84.82 \pm 0.40	84.83 \pm 0.75	85.00 \pm 0.00	84.83 \pm 0.41	84.00 \pm 0.00
Gp II	291.66 \pm 3.14	288.16 \pm 0.70	285.33 \pm 0.50	280.33 \pm 0.50	278.33 \pm 0.50	270.50 \pm 0.50	260.17 \pm 0.70
Gp V	300.66 \pm 0.85	265.33 \pm 0.52	214.50 \pm 0.55	190.50 \pm 0.84	164.33 \pm 0.51	140.33 \pm 0.52	120.66 \pm 0.82
Gp VI	300.66 \pm 1.03	299.33 \pm 0.52	250.50 \pm 0.55	160.50 \pm 0.84	140.33 \pm 0.52	120.83 \pm 0.41	110.50 \pm 0.55
Gp VII	290.16 \pm 0.75	288.33 \pm 0.52	284.33 \pm 0.81	281.00 \pm 0.63	250.83 \pm 0.75	200.33 \pm 0.52	150.83 \pm 0.98

Value represents the mean \pm S.D. for 6 rats per groups. $p < 0.01$ compared to normal control and diabetic control.

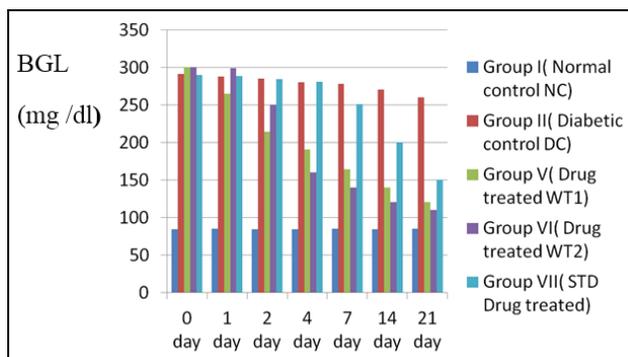


Fig 1: The effect of oral administration of *Wrightia tinctoria* (WT) ethanollic extracts at doses of 200 mg (WT₁) and 400 mg (WT₂) on blood glucose level in alloxan induced diabetic rats.

3.1 Serum lipid profile of rats treated with *Wrightia tinctoria* (WT₁ and WT₂) alcoholic extracts

Beneficial effects of *Wrightia tinctoria* on serum lipids, which is considered as one of the major cardiovascular risk factors in type 2 diabetes mellitus, could be observed from lipid-related data (Table 2). On Comparison with the control values, the

WT₁ and WT₂ (200 or 400 mg/kg) groups showed significant reduction ($P < 0.01$) in the serum levels of total cholesterol and triglycerides and increase in HDL levels. During prolonged study (21 days), the WT₁ and WT₂ (200 or 400 mg/kg) produced a significant ($p < 0.01$) decrease in the serum levels of total (TC) cholesterol and (TG) triglycerides and increase in (HDL) high density lipoprotein levels of the diabetic rats compared to diabetic control. WT₂ at the dose of 400 mg/kg body weight exhibited better reduction in TC (59.98%) than 200 mg/kg body weight (52.14%) and that produced by the standard drug, Glibenclamide 10 mg/kg (62.3%), at the same period WT₂ at the dose of 400 mg/kg body weight exhibited better reduction in TG (40.44%) than 200 mg/kg body weight (27.69%) and that produced by the standard drug, Glibenclamide 10 mg/kg (45.49%), at the same period while an better increase in HDL level (30.71%) was observed in WT₂ at the dose of 400 mg/kg body weight as compared to (13.24%) increase with WT₁ and that produced by the standard drug, Glibenclamide (35.64%). Both the extracts the WT₁ and WT₂ produced a significant ($p < 0.01$) decrease in the serum levels of total (TC) cholesterol and (TG) triglycerides and significant increase in (HDL) high

density lipoprotein levels of the diabetic rats compared to diabetic control. In this case standard drug glibenclamide showed better response as compared to *Wrightia tinctoria* extracts.

Table 2: The effect of oral administration of *Wrightia tinctoria* ethanolic extracts at doses of 200 mg (WT₁) and 400 mg (WT₂) on total cholesterol, triglyceride, HDL level in alloxan induced diabetic rats after 21 days.

	TCH	TG	HDL
Group I	88.05±0.054	81.01±0.075	36.03±0.05
Group II	251.50±0.547	151.66±0.520	29.50±0.55
Group V	120.37±0.570	109.68±0.530	34.00±0.63
Group VI	100.67±0.520	90.33±0.520	41.85±0.42
Group VII	94.82±0.400	82.67±0.520	45.83±0.41

Value represents the mean ± S.D. for 6 rats per groups. $p < 0.01$ compared to normal control and diabetic control.

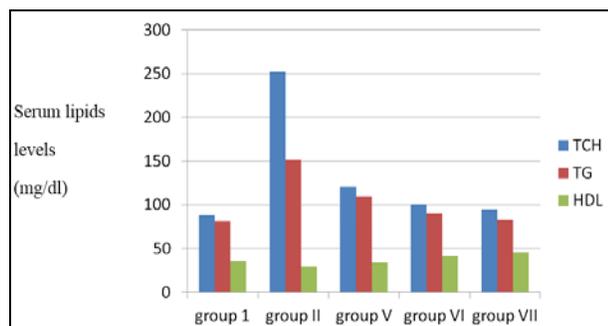


Fig 2: The effect of oral administration of *Wrightia tinctoria* (WT) ethanolic extracts at doses of 200 mg (WT₁) and 400 mg (WT₂) on total cholesterol, triglyceride, HDL level in alloxan induced diabetic rats after 21 days.

4. Discussion

Wrightia tinctoria claimed to be useful in diabetes. As per reports the local practitioners in Chittor District of India, claimed that the leaves are useful for treatment of diabetes [17]. The five significant compounds mainly flavanoid and indole compounds were reported in leaves of *Wrightia tinctoria* as indirubin, indigotin, isatin, anthranillate, rutin, tryptanthrin [18]. A new sterol, 14 α -methylzymo sterol is isolated from seed lipid [19] and a terpene Wrightial, cycloartenone, cycloeucaenol, were extracted from methanolic extract of seed pods of *Wrightia tinctoria* [18]. Lupeol, campestosterol, stigmasterol were isolated from the stem of *Wrightia tinctoria* [20]. 3,4-Seco-lup-20(29)-en-3-oic acid (1), a triterpene, was isolated from *Wrightia tinctoria* [21]. Ethanolic extract revealed the presence of Steroids, Flavonoids and Phenolics. Ethanolic extracts were selected for the study based on the preliminary phyto-chemical evaluation as they are rich in flavonoids and phenolics which possess antioxidant properties. Taking into account, in the present study the anti-diabetic evaluation of *Wrightia tinctoria* seed extracts was performed.

Results of anti-diabetic activity of the ethanolic extracts established the scientific basis for the utility of this plant in the treatment of diabetes. The ethanolic extract of *Wrightia tinctoria* (WT) was screened to explore the scientific basis of its utility for correction of biochemical changes in Alloxan induced diabetic rats. Both the extracts of *Wrightia tinctoria* seed (WT₁ and WT₂) significantly lowered blood glucose level and showed maximum reduction of 64% and 60% respectively ($p < 0.001$) On Day 21, whereas inhibition of 48.28% ($p < 0.001$) was found for standard drug glibenclamide on day 21 as peak. In this case the extracts were found to be

more effective than that of standard drug glibenclamide. 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. Alloxan acts as diabetogenic by the destruction of the islets of langerhans and causes massive reduction in insulin release thereby increasing hyperglycaemia [22]. Insulin deficiency leads to various metabolic alterations in the animals viz increase blood glucose, increase cholesterol, increase level of alkaline phosphate and transaminases etc. Single dose of intra-peritoneal (i.p.) treatment of rats with alloxan monohydrate (120 mg/kg) significantly increase blood glucose as shown in table 1. Two dose levels of ethanolic extract of *Wrightia tinctoria* (WT₁, WT₂) test drug and Standard drug glibenclamide 10 mg/kg body weight were found to reduce the elevated blood glucose level significantly in alloxan induced diabetic rats during 21 day treatment. The maximum reduction (64%) in serum glucose levels was seen in WT₂ at the dose of 400 mg/kg (Table 1). Thus, we could say that *Wrightia tinctoria* had a beneficial effect on carbohydrate metabolism in diabetic rats. The differences between the initial and final fasting blood glucose levels of different groups in three weeks studies exposed a significant elevation in blood glucose level in diabetic controls as compared with that of extract-treated and glibenclamide-treated animals. The percentage blood glucose reduction produced by the extracts in diabetic groups is greater than the percentage reduction observed in normal groups, so it can be hypothesized that *W. tinctoria* could have a sulphonyl urea like mechanism. It is also known that alloxan selectively destroys insulin-secreting β -cells in the islets of langerhans. The hypoglycaemic effect of WT 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 [23]. In the present study, the dose of alloxan (120 mg/kg, intraperitoneal) was selected in order to partially destroy the pancreatic β -cells. In this condition, insulin was secreted but not sufficiently to regulate the blood glucose. Sulphonyl urea compounds lower the blood glucose in normal and diabetic animals by stimulating insulin release from β -cells. Alloxan produces diabetes by producing oxygen-free radicals, which results lipid peroxide-mediated pancreatic cell injury [24]. The extracts may scavenge free radicals and facilitate reconstruction of pancreatic cells to release more insulin and ultimately produces an anti-diabetic effect.

Diabetes mellitus is one of the most common chronic diseases and is associated with hyperlipidemia and related complications such as obesity, atherosclerosis and hypertension. Hyperlipidemia is a metabolic disorder related to both clinical and experimental diabetes [25]. Several workers have shown that hyperglycemia and hyperlipidemia are the common characteristics of alloxan-induced diabetes mellitus in experimental rats [26, 27]. Both the extracts the WT₁ and WT₂ produced a significant ($p < 0.01$) decrease in the serum levels of total (TC) cholesterol and (TG) triglycerides and significant increase in (HDL) high density lipoprotein levels of the diabetic rats compared to diabetic control. In this case standard drug glibenclamide showed better response as compared to WT extracts.

Hyperlipidemia is a frequent complication noted in chemical induced diabetes and produces a serious risk of cardiovascular 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. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the underutilization of the glucose. Regarding the mechanism of action WT may enhance activity of enzymes involved in bile acid synthesis and its excretion and this may have decreased in serum cholesterol and triglycerides [28]. The experimental results obtained with the use of ethanolic extracts of *Wrightia tinctoria* with two dose levels revealed strong hypoglycaemic and hypolipidemic action thus proves the claims based on tradition practice followed by some traditional healers of Jharkhand region for treatment of diabetes and management of high cholesterol level.

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