Original Article

Lack of hypoglycemic activity in total flavonoid fraction of *Wrightia tinctoria* on alloxan induced hyperglycemia

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Abstract

Objective: The objective of this study was to investigate the hypoglycemic activity of *Wrightia tinctoria* (Roxb) (R. Br. Linn., Family- Apocynaceae) total flavonoid (WTTF) fraction on alloxan induced hyperglycemia. **Materials and Methods:** Total flavonoid fraction from WTTF seed was separated and LD_{50} calculated as 1006.93 mg/kg, i.p., following Organization for Economic Co-operation and Development guideline 423. The effect of total flavonoid fraction was observed in three different dose levels 50, 75, and 100 mg/kg. Hypoglycemic activity was carried out using alloxan induced hyperglycemia model. The parameters observed were body weight, blood glucose, total cholesterol, triglycerides (TG), low density lipoprotein, high density lipoprotein, urea and creatinine along with relative organ weight of liver, kidney, and pancreas. **Results:** Total flavonoid fraction of *W. tinctoria* did not have any significant effect on reducing glucose level. WTTF had significantly reduced creatinine ($P < 0.001$), TG ($P < 0.05$) level and relative weight of liver in diabetic rat at the dose of 100 mg/kg. **Conclusion:** All the findings indicate absence of antidiabetic activity in *W. tinctoria* seed but possible presence of liver and kidney protective property.

Key words: Alloxan monohydrate, flavonoid, seed, *Wrightia tinctoria*

INTRODUCTION

Worldwide diabetes mellitus (DM) has reached epidemic proportions as per the World Health Organization, which is strongly related to lifestyle and economic changes. Over the next decade the number of diabetic patient worldwide will exceed the figure of 200 million. By the year 2025, India is predicted to have the most number of people with DM in the world.[1] Diabetes requires continuing medical care to prevent acute complications and the risk of long-term complications. Many diabetic people turn to complementary therapies to control the chronic nature and threat to quality of life eventually reducing the complications.[2] For a long time plants based herbal medicines have been the major source of drugs for treatment of DM in Ayurveda and other ancient systems of medicine, as plant products are generally considered to be less toxic and free from side-effects compared with modern synthetic drugs.[3,4] Ethnobotanical information refers to suggestive antidiabetic potential in about 800 medicinal plants and many herbs have been reported to possess hypoglycemic activity in the literature.[5,6]

Wrightia tinctoria (Roxb.) R.Br. is a small deciduous tree of the family Apocynaceae distributed in Central India, Burma and Timor. This plant is extensively used in the Indian system of medicine. Fresh leaves are pungent and are chewed for relief from toothache. Bark and seeds are antidysenteric, carminative, astringent, aphrodisiac and diuretic, used in flatulence, stomach pain and bilious affections.[7] *W. tinctoria* has reported antipsorasis,[8] antinociceptive,[9] wound healing effect,[10] hepatoprotective activity,[11] antiulcer and

Access this article online	
Quick Response Code:	Website: www.pnrjournal.com
	DOI: 10.4103/0976-9234.136793

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immunomodulatory effect.[12] The methanolic, ethanolic and ethyl acetate extract of *W. tinctoria* showed rich presence of alkaloid, glycoside, triterpene, tannins and phenolic compounds.[13,14] Until date, no pharmacological evidence for effectiveness of *W. tinctoria* phenolic and flavonoid content has been reported. Flavonoids contain wide range of biological activity and lot of research has been carried out on their potential role in treating diabetes and other diseases. Flavonoids are capable of improving, stabilizing and long sustaining the insulin secretion, human islets and pancreatic cell respectively.[15] This study was performed to assess the antidiabetic efficacy of *W. tinctoria* seed to explore the hypoglycemic effect of total flavonoid fraction.

MATERIALS AND METHODS

Collection and identification of plant material

Wrightia tinctoria seeds were purchased from local market of Bhopal, Madhya Pradesh, India. The plant was identified and authenticated by Dr. Tariq Hussain, Head, Herbarium Department, National Botanical Research Institute, Lucknow, Uttar Pradesh. A voucher specimen number was allotted (No. 97314/2009) and kept for future reference.

Isolation of total flavonoid from *Wrightia tinctoria* seed

Ground seed was extracted in a soxhlet extractor for 18 h with methanol and filtered through Whatman fiter paper. The mixture was concentrated under reduced pressure using Rotatory Evaporator (Jyoti Scientific, India) at temperature not >50°C and dried. The obtained methanolic extract was dissolved in aqueous acetone (75%), filtered, evaporated under reduced pressure and dried which gave positive result for flavonoid. The percentage yield of total flavonoid was 4.40% w/w, designated as WTTF.

Experimental animals

Laboratory bred Swiss Albino mice (20–25 g) and Wistar albino rats (120–150 g) of both sex were maintained under standard laboratory conditions at 22°C ± 2°C, relative humidity 50% ±15% and photoperiod (12 h dark and light), were used for the experiment. Commercial pellet diet (Hindustan Lever, India) and water were provided *ad libitum*. Ethical Committee approval was obtained from Institutional Animal Ethical Committee (approved body of "committee for the purpose of control and supervision of experiments on animals" Chennai, India) of Radharaman College of Pharmacy, Bhopal, before carrying out the experiments (IAEC/RCP/July 2011/05).

Acute toxicity studies (LD_{50})

LD_{50} was determined according to the guidelines of Organization for Economic Co-operation and Development (OECD) following the up and down method (OECD Guideline No. 423) and fixed dose method (OECD Guideline No. 420). Based on these guidelines, a limit test was performed at 1000 mg/kg (i.p.) for WTTF, which showed 40.00% mortality. A main test was performed to determine the exact LD_{50} value following OECD up and down method.[16]

Induction of diabetes in rats

Diabetes was induced on overnight fasted rats by a single dose administration of freshly prepared alloxan monohydrate (150 mg/kg, ip.) in normal saline.[17] Blood glucose (BG) level was measured by using one-touch glucometer and diabetes was confirmed after 72 h of alloxanization. Rats with fasting BG level >150 mg/dL were considered to be diabetic and were selected for studies.

Study protocol

Animals were randomly divided into six groups with six rats in each.

- Group I: Vehicle control group (0.2 ml/100 g body weight, i.p): 1% tween 80 solution in water for injection
- Group II: Diabetic control
- Group III: Metformin (20 mg/kg body weight, p.o.)
- Group IV: WTTF (50 mg/kg body weight, i.p)
- Group V: WTTF (75 mg/kg body weight, i.p)
- Group VI: WTTF (100 mg/kg body weight, i.p).

During the study period, body weight and BG level were recorded at 1st, 7th 14th and 21st day. On the 21st day animals were sacrificed and organs (liver, pancreas, and kidney) were isolated and kept in ice cold saline solution, blotted and weighed. Blood samples collected by heart puncture, serum was separated after coagulating at 37°C for 30 min and centrifuged at 3000 rpm for 10 min.[18] Serum was analyzed for various biochemical parameters total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), urea, and creatinine.

Biochemical estimations

Serum content of glucose, TC, TG, HDL, urea and creatinine were estimated in Span Auto analyzer (India) using commercial kit (Span Diagnostics Pvt. Ltd., India). Serum LDL cholesterol content was calculated with formula using serum concentration values of TC, TG, and HDL cholesterol.

Statistical analysis

Experimental data were analyzed using one-way ANOVA followed by Turkey-Kramer multiple comparison test. $P < 0.05$ were considered statistically significant. GraphPad Prism version 3.02 (GraphPad Software, Inc., USA) was used for statistical calculations.

RESULTS

Acute toxicity

LD_{50} was calculated as 1006.93 mg/kg from graphical representation. A dose range of 50, 75 and 100 mg/kg was selected for WTTF to evaluate the pharmacological activities.

Effect on body weight

Body weight gain in vehicle control group after 21 days was 6.14%. Diabetes prevents the weight gain on 14th day and 21th day by 7.38% and 8.94% respectively. Standard drug metformin treated group and WTTF (50, 75 and 150 mg/kg) treated groups showed decrease in body weight gain by 2.41%, 7.05%, 8.82% and 6.37% on 21th respectively [Table 1].

Effect on liver, kidney and pancreas weight

Liver kidney and pancreas weight were determined on 21st day after sacrifice of animals. The increase in weight of liver and pancreas was highly significant ($P < 0.001$) in diabetic animals compared with vehicle control group. Metformin significantly reduced the liver ($P < 0.001$) and pancreas ($P < 0.01$) weight compared with diabetic control group. WTTF at dose of 100 mg/kg dose treatment significantly ($P < 0.001$) decreased liver weight in diabetic animals [Table 1].

Effect on serum blood glucose

Fasting BG levels were determined on day 1st and every 7th day for 3 weeks [Table 2]. BG in the diabetic group increased gradually from the 1st day to the termination of experimental period ($P < 0.001$) in comparison to vehicle control group. WTTF in dose of 50, 75 and 150 mg/kg has nonsignificant effect on BG level. Metformin treated groups significantly suppressed ($P < 0.001$) serum BG level after 14th day to the termination of experimental protocol.

Effect on biochemical parameters

Alloxan induced diabetes has significantly ($P < 0.001$) increased the serum level of TC, TG, LDL and urea level in animals compared to vehicle control group. Effect of WTTF at 50, 75 and 150 mg/kg dose on serum TC, LDL, HDL and Urea was nonsignificant. WTTF had significantly reduced creatinine ($P < 0.001$) and TG ($P < 0.05$) level in diabetic rat at the dose of 100 mg/kg [Table 3].

DISCUSSION

In this study, hypoglycemic effect of total flavonoid isolated from *W. tinctoria* seed was evaluated by assessing body weight change, relative organ weight, BG level, and serum lipid parameters. Obesity is mostly associated with DM, indicating weight control as an important aspect of diabetes management. Our results showed that all the animals treated with WTTF have lost weight during the study period. The weight loss was highest in the diabetic control group while metformin treated group showed minimum weight loss. Metformin has effective reduced the elevated liver and pancreas weight in diabetic animals. WTTF had no effect on

Table 1: Effects of WTTF treatment on body weight of alloxan induced diabetic rats

Treatment (mg/kg)	Body weight Average weight in g (percentage change)				Weight/100 g body weight in g		
	0 day	7th day (%)	14th day (%)	21st day (%)	Liver	Kidney	Pancreas
Vehicle control	140.06±18.61	142.92±19.76 (+2.07)	145.92±21.34 (+4.21)	148.63±18.46 (+6.14)	2.485±0.02	0.812±0.03	0.284±0.02
Diabetic control	147.52±16.45	150.81±19.56 (+1.55)	136.60±16.29 (−7.38)	134.38±15.47 (−8.94)	4.342±0.04[a]	0.680±0.06	0.549±0.04[a]
Metformin (20, p.o.)	145.02±18.65	154.32±20.41 (+6.41)	148.65±18.85 (+2.48)	141.57±14.41 (−2.41)	2.847±0.09***	0.77±0.04[ns]	0.409±0.01**
WTTF (50, i.p.)	125.61±17.89	129.41±18.14 (+3.02)	124.18±16.88 (−1.13)	116.75±15.14 (−7.05)	4.61±0.01	0.59±0.01	0.49±0.02
WTTF (75, i.p.)	132.51±17.57	133.64±17.65 (+0.85)	126.14±17.12 (+4.80)	120.81±16.49 (−8.82)	4.11±0.06	0.61±0.03	0.51±0.03
WTTF (100, i.p.)	143.42±18.42	145.35±19.21 (+1.34)	142.51±18.50 (−0.63)	134.27±16.94 (−6.37)	3.88±0.07***	0.64±0.04	0.47±0.05

The values are expressed as mean±SEM, $n=6$ in each group. [a]$P<0.001$ compared to vehicle control group, ***$P<0.001$, **$P<0.01$ compared with diabetic control group. Values in parenthesis signify percentage change in body weight compared to initial body weight (0 day). SEM: Standard error of mean; WTTF: *Wrightia tinctoria* total flavonoid

Table 2: Effects of WTTF treatment on blood glucose level of alloxan induced diabetic rats

Treatment (mg/kg)	Day 0	Day 7	Day 14	Day 21
Vehicle control	82.52±3.84	80.33±6.41	84.62±5.41	83.70±3.11
Diabetic control	277.25±18.41[a]	282.50±16.24[a]	284.51±19.41[a]	278.45±18.74[a]
Metformin (20, p.o.)	288.60±17.24	232.61±15.87	185.48±14.57***	98.47±13.46***
WTTF (50, i.p.)	258.24±15.17	262.48±18.14	254.14±14.78	260.21±16.57
WTTF (75, i.p.)	235.17±16.24	238.18±15.57	232.79±16.14	254.71±18.64
WTTF (100, i.p.)	247.44±15.58	252.34±17.32	240.21±16.81	251.78±18.45

The values are expressed as mean±SEM, $n=6$ in each group. [a]$P<0.001$ compared to vehicle control group, ***$P<0.001$ compared with diabetic control group. SEM: Standard error of mean; WTTF: *Wrightia tinctoria* total flavonoid

Table 3: Effects of WTTF treatment on serum biochemical parameter of alloxan induced diabetic rats

Treatment (mg/kg)	Serum biochemical parameters					
	TC	TG	LDL	HDL	Urea	Creatinine
Vehicle control	98.22±2.94	98.50±1.30	55.46±1.62	22.05±0.55	32.09±1.10	0.62±0.04
Diabetic control	149.16±4.40[a]	149.16±4.22[a]	141.81±10.46[a]	16.85±1.20[ns]	64.63±1.11[a]	1.43±0.03[a]
Metformin (20, p.o.)	86.53±4.20***	80.39±9.23***	50.42±2.72***	26.24±1.14*	35.97±1.54*	0.64±0.01***
WTTF (50, i.p.)	138.12±5.14	157.18±6.87	134.71±8.74	18.24±2.27	44.47±2.18	1.31±0.09
WTTF (75, i.p.)	140.21±4.12	144.72±8.24	156.14±11.27	14.57±3.14	56.51±3.84	1.24±0.06
WTTF (100, i.p.)	132.14±5.22	118.24±5.22*	141.23±10.49	13.66±2.16	59.31±4.17	0.87±0.02***

The values are expressed as mean±SEM, n=6 in each group. [a]P<0.001 compare to vehicle control group, ***P<0.001, *P<0.05 compared with diabetic control group. TC: Total cholesterol; TG: Triglyceride; LDL: Low density lipoprotein; HDL: High density lipoprotein; WTTF: *Wrightia tinctoria* total flavonoid; SEM: Standard error of mean

pancreas though the liver weight was significantly normalized. Bigoniya and Rana (20 10) reported significant reduction of serum glutamate oxaloacetate transaminase level without effecting serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase level by the alcoholic extract of *W. tinctoria* bark signifying its hepatoprotective effect. The triterpenoidal fraction of bark is reported to have hepatoprotective against carbon tetrachloride induced acute rat liver toxicity.[19] This indicates protective effect of WTTF on liver either by reducing oxidative damage or by normalizing liver lipid metabolizing and turnover mechanism.

The diabetic control group animals showed fasting BG level above 250 mg/dl, which has remained higher throughout the study period of 21 days. Metformin (20 mg/kg) was used as a standard drug, showed decrease in BG level on 7th day onwards. The WTTF in all treated doses showed nonsignificant change in BG level throughout the study period. On long-term prolonged use *W. tinctoria* bark showed increase in lipid profile along with increased body weight, hemopoiesis and glucose suggesting its anabolic effect.[20] Methanolic extract of *W. tinctoria* bark with rich flavonoid content has potential antianemic and hematopoietic effect.[14]

Kedar and Chakrabarti have reported that the cholesterol, TG and SGPT levels are increased in case hyperglycemia. Deficiency of insulin caused the increase in the level of these enzymes in liver and serum of diabetic animals. It was also reported that the elevated level of enzymes in the liver and serum decreases significantly with the treatment of antidiabetic drugs like phenformin, metformin with improved body weight gain.[21]

In this study TC, TG, LDL, urea, and creatinine was increased in diabetic group and metformin effectively control all these disturbed parameters. WTTF treated group showed nonsignificant change in serum lipid parameter except decrease in TG and creatinine level. Extract induced decrease in TG level may be due to its effectiveness against diabetes induced fatty liver. Phytoconstituents like flavonoids are known to possess hepatoprotective activity.[22,23]

Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine, and ions. During renal diseases, the concentration of these metabolites increases in blood.[24] In this study, the diabetic animals showed significantly higher level of serum creatinine, which has been reduced to normal by WTTF at 100 mg/kg dose treatment. This effect may be due to the diuretic action of *W. tintoria* ethanolic extract as reported by Bigoniya *et al*., 2008. Normalization of creatinine level by WTTF indicates that it has protective effect on the kidney and further studies are required to explore effect of WTTF on kidney. The WTTF in all treated doses showed absence of antidiabetic effect indicating no effect on β-cells of the pancreas or not related to increased utilization of glucose by cells. On many cases beneficial flavonoids are less effective due to poor solubility, decreased bioavailability, first pass metabolism and intestinal degradation.[15]

CONCLUSION

The effect of *W. tinctoria* seed flavonoid fraction was not significant on hyperglycemia and other disturbed biochemical parameter induced by alloxan, but it has significant effect on normalization of serum creatinine level and lowering of TG and relative weight of liver indicating possible presence of kidney and liver protective property.

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How to cite this article: Shukla AK, Bigoniya P. Lack of hypoglycemic activity in total flavonoid fraction of *Wrightia tinctoria* on alloxan induced hyperglycemia. J Pharm Negative Results 2014;5:34-8.

Source of Support: AICTE, New Delhi under Research Promotion Scheme (RPS). **Conflict of Interest:** None declared.