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ANTI-DIABETIC ACTIVITY OF METHANOLIC AND ETHYL ACETATE EXTRACTS OF *WRIGHTIA TINCTORIA* R.BR. FRUIT

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ABSTRACT

The present work was undertaken to investigate various extracts of fruit of *Wrightia tinctoria* (F-Apocyanacea) for anti-diabetic activity in alloxan induced diabetic rats. A comparison was made between the action of extracts and known anti-diabetic drug glibenclamide (10mg/kg body weight). Oral administration of methanolic extract at a dose of 300 mg/kg/b. wt and ethyl acetate extract at a dose of 200 mg/kg/b. wt exhibited a significant ($p<0.001$, $p<0.001$) hypoglycemic activity in normal rats and significant ($p<0.001$, $p<0.001$) anti-hyperglycemic activity in alloxan induced diabetic rats respectively. The maximum reduction in blood glucose level was observed after 4 hours in case of methanolic and ethyl acetate extracts with a percentage protection of 37% and 42% respectively. In long term treatment of alloxan induced diabetic rats the degree of protection was determined by measuring blood glucose on 0, 1, 2, 4, 7, 14th day. Both the extracts showed a significant anti-diabetic activity comparable with that of glibenclamide. These results indicate that the *W. tinctoria* fruit extracts possess significant anti – diabetic activity.

INTRODUCTION: Diabetes mellitus is a potentially morbid condition with high prevalence worldwide, this has affected millions of people all over the world thus the disease constitutes a major health concern [1]. Currently global prevalence of the disease is around 200 million and would increase to 300 million by 2025.

In view of the increasing prevalence there is a growing need to develop integrated approaches towards the management and prevention of diabetes mellitus by exploring the potentials offered by the traditional phytotherapies. Despite considerable progress in the management of D. mellitus by conventional drugs, the search for natural antidiabetic plants as alternative is ongoing. Experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes leading to complications.

So, plants capable of neutralizing free radicals are effective in preventing diabetes and reducing the severity of diabetic complications.

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In the recent past many hypoglycemic agents are introduced still the diabetes and the related complications continue to be a major medical problem not only in developed countries but also in developing countries. Many Indian medicinal plants are reported to be useful in diabetes [2, 3]. Phenolics and Flavonoids can exert their antioxidant activity by various mechanisms like quenching the free radicals, by chelating of metal ions by inhibiting enzymatic systems responsible for free radical generation [4].

So poly-phenolics and flavonoids incorporation into diet could contribute to potential management of hyperglycemia. However as far as ascertained, no detailed scientific study seemed to have been carried out to assess the hypoglycemic activity of fruit of this abundantly wild grown plant. Therefore, the present study was conducted to determine the hypoglycemic activity of *Wrightia tinctoria* fruit extracts in normal and alloxan–induced diabetic rats.

W. tinctoria R.Br. Belongs to family Apocynaceae [5] (The wealth of India), is a small deciduous tree, generally up to 1.8 m tall and often under 60 cm girth, sometimes up to 7.5 m high, distributed all over India. It is commonly known as “indrajav”, has been important in the traditional healing and widely recognized medicinal plant [6]. The wrightial a new terpene and other phytoconstituents such as cycloartenone, cycloeucalenol were isolated identified by fractionation of methanol extract of the immature seed pods [7].

The ursolic acid and isoricinolic acid has been also isolated from the seed pods [8]. The characterization of lingo-cellulosic seed fiber from *W. tinctoria* has been carried out [9]. Almost every part of plant is useful - leaves pungent chewed for relief from tooth ache, bark and seeds are antidysenteric, antidiarrhoel and antihaemorrhagic [10]. Oil emulsion of leaves and pods is used to treat psoriasis [11, 12]. The plant is also reported for its antimicrobial, wound healing and hepatoprotective activity [13, 14, 15, 16].

The selection of inappropriate animal model has been identified as one of the common problem associated with ethno-botanical researches [17]. Among the currently available animal models like normoglycemic animal model, oral glucose loading animal model, chemical induction animal models(alloxan and streptozotocin), genetic model of diabetes and surgical model of diabetes, chemical induction with alloxan appears to be easiest and most popularly used and practicable method of inducing diabetes mellitus in rodents.

Alloxan is capable of inducing both Type I and Type II diabetes with proper dosage selection and makes easy to put to use the experimental animals within seven days after induction of diabetes mellitus and can be maintained to prevent death throughout the experimental period but surgical and genetic methods require high technical skills and may be associated with a high percentage of animal death and thus rarely used [18].

Moreover administration of appropriate antioxidants from plant source could prevent (or) retard the diabetic complications to some extent [19]. Thus, alloxan induced diabetes mellitus serves as a pathological biomodel for testing a substance with supposed antioxidant activities *in vivo* [20].

This study was carried to clarify the effect of *W. tinctoria* fruit extracts (methanol and ethyl acetate) as beneficial in the treatment of diabetes. Active solvent extracts of the plants are commonly used because they may contain more than one active ingredient and less expensive than a purified single compound. Therefore knowing the most effective solvent extract would be useful in development of new drugs from plants. Keeping these facts in view the present study has been undertaken to identify the active anti-diabetic extract of *W. tinctoria* pods. The most fascinating phyto nutrients flavanoids and phenolics in plant pods that give strong antioxidant activity made this plant to be more concern about the study.

MATERIALS AND METHODS:

Plant Material: Matured fruits of *W. tinctoria* (Family: Apocyanacea) were collected in the month of April 2011 from village Keesara gutta, Ranga Reddy district, Hyderabad, Andhra Pradesh, India. The plant was identified and authenticated by Professor Badraiah, Department of Botany, Osmania University, Hyderabad, India. A voucher specimen- 0573 was deposited at herbarium of the University, Hyderabad for future reference.

Preparation of Extracts: The collected fruits were cut in to small pieces and shade dried at room temperature and powdered to coarse powder. Extracted using Methanol, Ethyl acetate, and Chloroform by adopting simple maceration technique for seven days. The excess solvent was removed using Rotary flash evaporator. The obtained crude extract (Yield 9.2%w/w, 5.3%, 4.9% of methanolic, ethyl acetate, chloroform respectively) was stored in desiccator for further studies.

Preliminary Phyto-chemical investigations [21, 22]**:** The preliminary phyto-chemical investigations were carried out with all the three extracts of Fruits of *W. tinctoria* for qualitative identification of phyto-chemical constituents present in each extract and tests were carried out as per the standard methods. All the chemicals and reagents used were of analytical grade.

Experimental Animals [23]**:** Albino male rats (wistar strain) weighing between 150 - 200g were used in this investigation. Animals were Purchased from the National Institute of Nutrition c/o Teena biolabs Pvt . ltd. for the experimental purpose. Experimental procedure was approved by the Institutional Animal Ethical Committee (IAEC) of Gokaraju and Rangarju college of pharmacy, Osmania University, Hyderabad. Constituted for the purpose of CPCSEA Govt. of India (Registration number 177/99 /CPCSEA 6th Aug 2011) and all the procedures were followed as per rules and regulations.

All the animals were kept for acclimatization for 2 weeks under laboratory conditions and fed with pellet diet and tap water *ad libitum*. Dose selection was made on the basis of acute toxicity study as per the OECD guidelines. In toxicity studies no mortality observed up to 3000mg/kg, for methanol and 2000 mg/kg for ethyl acetate extract i.e. 1/10th and 1/20th dose of both the extracts was selected (300 and 150mg of methanol and 200 and 100 mg of ethyl acetate extract, so these doses were considered as the maximum tolerated dose.

Evaluation of extracts in normal healthy rats for Hypoglycemic Activity: Normal healthy rats were used for testing potential oral hypoglycemic agents. This is also a valid method used in addition to other diabetic animal models [24].

This method allows the effect of drug to be tested in animal with an intact pancreatic activity and hypoglycemic agent can also be detected. This also helps in selection of proper dose for a 30-40% of reduction of glucose level as excess hypoglycemia also leads to serious consequences.

Animals were divided in to 6 groups of 6 animals each.

- Group I- normal rats treated with vehicle (1%CMC) and served as normal control.
- Group II- normal rats treated with glibenclamide (10mg/kg body weight) served as standard reference.
- Group III and IV- normal rats were treated with methanol fruit extract of *W. tinctoria* at doses of 150 and 300 mg/kg body weight respectively.
- Group V and VI- normal rats treated with ethyl acetate fruit extract of *W. tinctoria* at a doses of 100 and 200 mg/kg body weight respectively.

Anti-hyperglycemic studies:

Induction of Diabetes: The diabetes was induced in rats as described by Trivedi *et al* [25]. Animals were allowed to fast overnight prior to injection. Hyperglycemia was induced in overnight fasted wistar strain albino rats weighing 180-240g by a single intra-peritoneal injection of freshly prepared alloxan monohydrate in normal saline (120 mg/kg body weight) within 50-75 seconds. The rats were kept on 5% glucose solution in the cages to prevent hypoglycemia.Hyperglycemia was confirmed by the elevated glucose level in serum, determined at 48 h after injection. The rats found hyperglycemic were screened for the anti-hyperglycemic study.

Experimental Design: Animals were divided in to four groups of six rats each. Test groups were administered methanol and ethyl acetate extracts at doses 300 and 200 mg/kg body weight respectively by oral route. Standard and control animals were treated with standard drug glibenclamide at an oral dose of 10 mg/kg body weight and 1% CMC respectively. All doses were started 48h after alloxan injection. Fasting blood glucose levels were estimated on Hour 0, 1, 2, 4, 6 (short-term study after a single administration of doses) and then on day 0, 1, 2, 4, 7, 14 (long-term

study). 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, data so obtained was used for analysis.

Statistical Analysis: The results are expressed as mean± S.E.M. the significance of various treatments were calculated utilizing ANOVA followed by students test Newman kuel multiple comparison using computerized Graph pad In stat version 3.05 and were considered statistically significant when P<0.05.

RESULTS: Methanolic extract revealed the presence of Steroids, Flavonoids and Phenolics. Ethyl acetate extract revealed the presence of Tannins, Steroids, Flavonoids and Phenolics, whereas chloroform extract revealed the presence of Alkaloids, Saponins and Steroids. Ethyl acetate and methanolic 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. Results were summarized in **Table 1**

Table 1: Preliminary Phytochemical Analysis of Extracts

Chemical Constituents	Methanol extract	Ethyl acetate extract	Chloroform extract
Alkaloids	-	-	+
Tannins	-	+	+
Saponins	-	-	+
Steroids	+	+	+
Flavanoids	+	+	-
Phenolics	+	+	-

(+)=Present; (-)=Absent

The hypoglycemic effects produced by the methanol and ethyl acetate extracts of fruit of *W. tinctoria* (WTM and WEA) in normal rats are summarized in the **Table 2**. Both the methanolic and ethyl acetate extracts showed a significant (p<0.001) decrease of blood glucose levels on fasting condition (41.34% and 36.06% reduction respectively after 4 h of administration) compared to the standard reference drug glibenclamide at a dose of 10mg/kg/body weight, showed a significant (p<0.001) decrease in blood glucose level 36.14% after 4 h of administration. From these Results $1/10^{th}$ of the dose was selected for further pharmacological studies.

TABLE 2: EFFECT OF METHANOLIC AND ETHYL ACETATE EXTRACTS OF MATURED FRUITS OF *W. TINCTORIA* IN NORMOGLYCEMIC RATS

Group	0 h	1 h	2 h	4 h	6 h
Normal control	85.33±3.04	78.26±2.30	85.93±3.03	85.13±1.55	85.46±2.90
WTM-150mg/kg	84.44±1.86	71.66±1.42	66.10±1.02*	62.77±1.59**	71.21±1.09
WTM-300mg/kg	89.33±2.66	66.26±3.15	58.86±2.62**	52.4±1.18**	75.06±0.99
WEA-100mg/kg	98±3.83	92±3.26	84.66±3.33	79.33±3.78	93.33±3.67
WEA-200mg/kg	101.66±3.92	76.66±4.55	69.44±3.69*	64.99±3.92**	70.55±6.52
STD-10mg/kg	92.21±1.64	70.55±2.34	63.88±2.90**	58.88±3.06**	52.77±3.15**

Values are given as mean± S.E.M (n=6);**p<0.001;*p<0.01 compared with control group

The effect of methanol extract (300mg/kg/body weight) and ethyl acetate extract (200 mg/kg/body weight) of fruits of *W. tinctoria on* alloxan – induced animals is indicated in **Table 3** (short-term study) and **Table 4** (long-term study).

The results in **Table 3** showed that after a single dose of both the extracts on alloxan induced diabetic rats, there was a significant reduction (p<0.001, at 4h) of fasting blood glucose level with a percentage reduction of 36.69% and 42.32% of methanol and ethyl acetate extracts respectively as compared with diabetic control group.

The anti diabetic effect was found comparable to that of standard drug glibenclamide (p<0.001, at 4 h) with a 40.48% reduction of blood glucose level. On chronic administration (**Table 4**), Significant difference was observed between experimental and diabetic control rats in lowering blood glucose level at a dose of 300 mg/kg body weight of methanol and 200 mg/kg/body weight of ethyl acetate extract.

Both the extracts significantly lowered blood glucose level and showed maximum reduction of 61.37% and 55.83% respectively (p<0.001) 0n Day 14, whereas inhibition of 43.18% (p<0.001) was found for glibenclamide on Day 14 as peak.

In this case the extracts were found to be more effective than that of standard drug glibenclamide.

TABLE 3: EFFECT OF METHANOLIC AND ETHYL ACETATE EXTRACTS OF MATURED FRUITS OF *WRIGHTIA TINCTORIA* IN DIABETIC RATS: (SHORT TERM STUDY)

Group	0 h	1 h	2 h	4 h	6 h
Diabetic control	292.48± 3.95	296.24±4.04	296.31±3.52	289.45±2.60	292.75±3.81
WTM-300mg/kg	316.53±6.41	260.47±5.16*	243.21±4.02**	200.39±4.59**	271.00±3.76
WEA-200mg/kg	300.39±8.35	227.26±9.72**	197.62±5.86**	173.25±4.25**	189.72±6.45**
STD-10mg/ kg	329.31±8.22	308.29±7.106	239.78±7.29**	195.98±7.67**	212.97±8.90

Values are given as mean± S.E.M (n=6);**p<0.001;*p<0.01 compared with diabetic control

TABLE 4: EFFECT OF METHANOLIC AND ETHYL ACETATE EXTRACTS OF MATURED FRUITS OF *WRIGHTIA TINCTORIA* (LONG TERM STUDY)

Group	0 day	1 day	2 day	4 day	7 day	14 day
Diabetic control	292.48±3.95	286.75±4.01	277.66±3.717	275.02±3.64	272.78±4.30	258.29±3.41
WTM-300mg/kg	316.53±6.41	298.08±5.83	206.18±4.64*	169.62±4.43*	142.81±3.39*	122.26±3.33*
WEA-200mg/kg	300.39±8.35	267.57±7.54	217.25±5.57*	194.55±5.40*	169.38±5.43*	132.67±2.99*
STD-10mg/kg	329.31±8.22	301.75±7.83	290.51±7.91	256.78±7.33*	223.51±8.11*	187.10±6.0*

Values are given as mean± S.E.M (n=6);*p<0.001 compared with diabetic control

DISCUSSION: The present study was conducted to evaluate the hypoglycemic and anti-hyperglycemic activity of fruit extracts of *W. tinctoria* a herbal drug, to get a berth in the group of anti-diabetic herbal drugs. In this study the methanol and ethyl acetate extracts of matured fruits of *W. tinctoria* showed significant maximum blood glucose level reduction in normal groups up to 6 h. Alloxan, a beta cytotoxin destroys B cells of islet of langerhans of pancreas resulting in a decrease in endogenous insulin secretion and paves the ways for the decreased utilization of glucose by the tissue. It results in elevation of blood glucose level. Expression of elevated fasting blood glucose level confirmed induction of diabetes in alloxan- induced experimental rats.

The experiment focused on exploring the competence of methanol extract of matured fruits of *W. tinctoria* for the correction of diabetes. The differences between the initial and final fasting blood glucose levels of different groups in both short-term and long-term studies exposed a significant elevation in blood glucose level in diabetic controls as compared with that of extract-treated and glibenclamide-treated animals. Maintenance of blood glucose level with extract-treated rats indicates the effectiveness of the extracts in experimental diabetic animals.

The methanolic extract at a dose of 300 mg/kg/body weight and ethyl acetate extract at a dose of 200 mg/kg/body weight only showed a better percentage blood glucose reduction.

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 since it decreased blood glucose in normoglycemic rats like glibenclamide. It is also known that alloxan selectively destroys insulin-secreting β-cells in the islets of langerhans [26].

In the present study, the dose of alloxan (120 mg/kg, intraperitoneally) 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 liberating oxygen-free radicals, which cause lipid peroxide-mediated pancreatic injury [27]. The extracts may scavenge free radicals and facilitate reconstruction of pancreatic cells to release more insulin and ultimately produces an anti-diabetic effect.

CONCLUSION: The results of this investigation revealed that methanolic and ethyl acetate extracts of matured fruits of *Wrightia tinctoria* possesses significant anti-diabetic activity. Preliminary phyto chemical screening indicated the presence of Natural anti oxidants like flavonoids and phenolics in both the extracts of fruits of *W. tinctoria*. Flavonoids and phenolics are known to be bioactive anti-diabetic principles, which strengthen the endogenous antioxidant defenses against the Reactive Oxygen Species and restore the optimal

balance, so they are gaining immense importance by virtue of their critical role in disease prevention. In this context *W. tinctoria* can be mentioned as a plant of considerable importance.

Further studies to fractionate the active principles and to elucidate the exact mechanism of action are, therefore, required to be undertaken.

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