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Effect of Residual Aqueous Portion of Hydro-methanolic Leaves Extract of *Indigofera Pulchra* on Blood Glucose Levels of Alloxan-induced Diabetic Wistar Rats

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Abstract: This aim of this study was to evaluate the hypoglycemic effect of residual aqueous portion of hydro-methanolic leaves extract of *indigofera pulchra on* blood glucose levels of alloxan-induced diabetes rats. Three doses of the extract (250,500 and 1000 mg/kg) were administered intraperitoneally. After 2, 4 and 6 h of extract administration there was no significant change in the blood glucose levels in all the three doses of the extract administered when compared to the control. Also after 8 and 24 hours of extract administration there was a significant ($p<0.05$) decrease in the blood glucose levels with the dose of 1000mg/kg the extract administered. In regard to the dose of 500mg/kg there was a significant decrease ($p<0.05$) after 24 h of extract administration. In relation to the reference drug there was a significant decrease ($p<0.05$) after 4, 8 and 24 h of administration when compared to control. The Preliminary phytochemical screening revealed the presence of alkaloids, tannins, flavonoids and saponins. The median lethal dose (LD_{50}) in rats was calculated to be 2,154 mg/kg body weight. In conclusion the residual aqueous portion of hydromethanolic leaves extract of *Indigofera pulchra* possess anti-diabetic effect in alloxan- induced in diabetic rats.

Key words: *Indigofera pulchra,* Diabetes mellitus, Residual aqueous portion, Hypoglycemia.

INTRODUCTION

Diabetes mellitus, often simply diabetes, is a syndrome characterized by disordered metabolism and inappropriate hyperglycemia (high blood sugar) resulting either from low levels of the hormone insulin secretion or to a combination of resistance to insulin's effects and inadequate insulin secretion to compensate. An international expert committee recommended the use of the terms "type 1 and type 2 diabetes" (Tierney *et al.,* 2002). Type 1 diabetes mellitus-formerly known as insulin-dependent diabetes mellitus (IDDM), childhood diabetes or juvenile diabetes-is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to a deficiency of insulin. The main cause of this beta cell loss is a T-cell mediated autoimmune attack (Rother, 2007). There is no known preventive measure that can be taken against type 1 diabetes, which comprises up to 10% of diabetes mellitus cases in North America and Europe (though this varies by geographical location). Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of cases of diabetes affecting children (Rother, 2007). In ethnomedicine, the leaves are used to treat infected wound (Hepper's, 1976 and Burkhill's, 1995) while the decoction of the aerial part is used as prophylactic against snake-bite (Sule *et al.,* 2003) and as anti-inflammatory (Abubakar *et al.,* 2007). Previous pharmacological studies on the methanol extract of the aerial part of this plant showed that it exhibited venom detoxifying activities (Abubakar *et al., 2006*). Also it has been reported that the hydro-methanolic and n-butanol portion of the leaves extract has anti-diabetic property (Tanko *et al.,* 2008a, b)

The present study was designed to test the hypoglycemic effect of residual aqueous portion of hydro-methanolic leaves extract of *Indigofera pulchra* on alloxan induced diabetic on Wistar rats.

MATERIALS AND METHODS

Plant material: *Indigofera pulchra* leaves was collected from Samaru-Zaria in the month of October 2007 and was authenticated by A.U.Gallah of the Biological Sciences Department, Ahmadu Bello University Zaria-Nigeria where a voucher specimen (No.6558) was deposited.

Extract preparation: The leaves of *Indigofera pulchra* were air-dried under the shade and ground into powder. The powder 300g was in macerated 70% methanol and 30% aqueous at room temperature for 72 hours. It was then filtered using a filter paper (Whatman No. 1). The filtrate was partitioned with n-butanol and ethyl acetate and the residue was evaporated to dryness in an oven at

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40ºC to get the residual aqueous portion of the plant. The residual aqueous portion was then stored in an air-tight container at 20ºC until use.

Chemicals used: All chemicals and drugs were obtained commercially and were of analytical grade.

Experimental animals: Twenty five Wistar rats of both sexes were used for the study of the effects of *Indigofera pulchra* extract on the blood glucose levels of the animals. The animals obtained from the animal house facilities of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria. They were kept in standard cages at 25^0C and 12 hour light/dark condition in the animal room of the Department of Human Physiology, ABU, Zaria. The animals were fed on commercial feeds and were given water *ad libitum* .The animals were fasted from feeds for 12 hours before the commencement of each experiment, but were allowed water.

Acute toxicity study: The lethal doses (LD_{50}) of the plant extract was determined by method of Lorke (1983) using 12 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the extract at doses of 1600, 2900 and 5000 mg/kg bodyweight (i.p). The median lethal dose (LD_{50}) was calculated using the second phase.

Phytochemical screening: The preliminary phytochemical screening of the crude extract of *Indigofera pulchra* was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols (Trease and Evans, 1983).

Animals and Induction of Diabetes Mellitus: Wistar strain albino rats of both sexes weighing (180-200 g) bred in the Department of Human physiology, faculty of Medicine A.B.U Zaria, were used for this study. The animals were kept and maintained on standard laboratory animal feed and water *ad libitum,* were housed in polypropylene cages at room temperature throughout the study, under laboratory conditions of temperature, humidity and light (25±1ºC and 12 h light/dark cycle) respectively. We followed the Guide for the care and Use of Laboratory Animals, 1985, issued by the US Department of Health and Human Services, Public Health Service, National Institute of Health, NIH Publication No.86-23. Diabetes was induced by single intraperitoneal injection of alloxan monohydrate dissolved in sterile cold normal saline at a dose of 150 mg/kg body weight as reported by (Kameswara Rao *et al.,* 1999). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic release of Insulin, the rats were treated with 20% glucose solution intraperitoneally after 6 hours The animals were kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia .After a period of three days the rats with blood glucose levels greater than 200 mg/dL were considered diabetic and used for this research work.

Experimental Design: The Alloxan-induced diabetic Wistar rats were randomly assigned into five groups (1-5) of five rats (n = 5) each as follows, namely:

Group 1 Received normal saline i.p
Group 2 Received Biphasic Isophane Insulin 6 i.u/kg i.p (Stanley *et al.*, 2001)
Group 3 Received 250 mg/kg body weight of the *Indigofera pulchra extract i.p*
Group 4 Received 500 mg/kg body weight of the *Indigofera pulchra extract i.p*
Group 5 Received 1000 mg/kg body weight of the *Indigofera pulchra extract i.p*

Determination of blood glucose levels: All blood samples were collected by cutting the tail-tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 2, 4, 8 and 24 h. Determination of the blood glucose level was done by the glucose-oxidase principle (Beach and Turner, 1958) using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl (Rheney and Kirk, 2000).

Statistical analysis: Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of $p<0.05$ were considered as significant (Duncan *et al.*, 1977).

RESULTS

Phytochemical analysis: Table 1:showed the result of the preliminary phytochemical screening test for various constituents which revealed the presence(+) of tannins, saponins, flavonoids and its glycosides and alkaloids and absences (-) of steroids,

Acute toxicity study (LD_{50}): The sign of toxicity were first noticed after 8-10 hours of extract administration. There was decreased locomotive activity, decreased feed intake, and prostration after 14 hours of extract administration. The median lethal dose (LD_{50}) in rats was calculated to be 2,154 mg/kg body weight.

Table 2 below showed the results of the effects of three doses (250, 500 and 1000 mg/kg) of *Indigofera pulchra* extract, Insulin and control groups in alloxan induced diabetic Wistar rats. The dose of Insulin and the three doses of the extract did not show any significant change in the blood glucose levels when compared to untreated control after 2 h of extract administration while after 4 hours of extract administration there was a

Table 1: Phytochemcial analysis of Residual aqueous portion of the leaves extract of *Indigofera pulchra*

Constituents	Preference
Alkaloids	
Dragendoff's	+
Picric acid	+
Wagner's	+
Flavonoids	
Shinoda	+
Sodium hydroxide	+
Saponins	
Frothing	+
Tannins	
Ferric chloride	+
Lead acetate	+
Steroidal nucleus	
Salkwoski	-
Liberman-Burchard	-

Key: + = Present - = Absent

the extract showed a significant ($p<00.5$) decrease in the blood glucose levels when compared to control. With regard to the dose of 250 mg/kg there was no significant decrease ($p<0.05$) in the blood glucose level at all the time intervals when compared to control

In regard to the biphasic Isophane Insulin (6.i.u/kg), treated group, there was a significant decrease ($p<0.05$) in the blood glucose level when compared to control after 4, 8 and 24 hours of treatment.

The extract might possess Insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of residual aqueous portion of the extract as showed in Table 1 revealed the presence of tannins, saponins and flavonoids. Flavonoid and tannins

Table2: Effect of Residual aqueous portion of hydro-methanolic extract of *Indigofera pulchra* on alloxan- induced diabetes

	Blood Glucose Level (mg/dl)				
	0 hour	2 hours	4 hours	8 hours	24 hours
Group 1 Control (Normal/Saline)	510.0±51.4	570.4±21.3	534.4±30.4	515.6±31.8	533.8±15.2
Group 2 (Insulin 6.i.u/kg)	537.4±35.7 ^{ns}	539.4±24.9 ^{ns}	391.2±31.7 ^{a}	354.4±38.39 ^{a}	335.6±26.9 ^{a}
Group 3 (250 mg/kg)	496.0±4.70 ^{ns}	534.4±43.6 ^{ns}	467.0±23.1 ^{ns}	445.4±20.0 ^{ns}	466.0±24.6 ^{ns}
Group 4 (500 mg/kg)	527.8±27.0 ^{ns}	527.8±27.0 ^{ns}	454.8±27.3 ^{ns}	424.4±38.3 ^{ns}	401.2±27.6 ^{a}
Group 5 (1000 mg/kg)	540.4 ±35.3 ^{ns}	540.4±35.3 ^{ns}	467.8±18.2 ^{ns}	305.8±18.8 ^{a}	375.0±35.9 ^{a}

Values are given as mean ± SEM for 5 rats in each group; experimental groups are compared with diabetic control.
Values are statistically significant at $^{a=}P<0.05$ $^{ns=}$not significant

significant decrease in the blood glucose level in the insulin group. After 8 and 24 h however, there was a significant decrease in blood glucose level in 500 mg/kg as well as 1000 mg/kg when compared to control untreated.

DISCUSSIONS

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial destruction of the β-cells of Islets of Langerhan's (Abdel-Barry *et al.,* 1997).This results leads to decreased Insulin levels and hyperglycemia leading to type 1 diabetes mellitus. However, animal models of diabetes differ significantly from each other and none can be taken, without reservation, to reproduce the essentials of human diabetes (Bell and Hyde, 1983).

Many secondary metabolites participate in a variety of anti-diabetic functions in *vivo* (Kako *et al.*, 1997). The glycemic change in blood glucose levels of diabetic rats at different time intervals after intraperitoneal administration of *Indigofera pulchra* extract at the doses of 250, 500 and 1000 mg/kg as shown in table 2 serves as evidence to support this fact.

In the alloxan-induced diabetic groups, the effect of three doses (250, 500 and 1000 mg/kg) of the aqueous portion of *Indigofera pulchra*, Insulin and control groups were evaluated. The dose of Insulin and the three doses of the extract did not show any significant change in the blood glucose levels when compared to untreated control after 2 hours of treatment. However, after 4, 8 and 24 hours of treatments the doses of 500 and 1000 mg/kg of isolated from the other anti-diabetic medicinal plants has been found to stimulate secretion or possess an Hypoglycemic activity and residual aqueous portion of *Indigofera pulchra* insulin like-effect (Marles and Farnsworth, 1995). The flavonoids present in the residual aqueous portion of *Indigofera pulchra* may also be acting similarly thereby decreasing the high blood glucose levels of alloxan-diabetic on Wistar rats.

CONCLUSION

In conclusion, the evidence obtained in the present laboratory animal study indicate that residual aqueous portion of hydro-methanolic leaves extract of *Indigofera pulchra* possess anti-diabetic properties which suggests the presence of biologically active components which may be worth further investigation and elucidation.

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