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Effects of ethyl acetate portion of *Indigofera pulchra* leaves extract on blood glucose levels of alloxan-induced diabetic and normoglycemic Wistar rats

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Abstract: The ethyl acetate portion of *Indigofera pulchra* leaves extract (papilionaceae) was assessed for blood glucose levels of alloxan induced diabetic and normoglycemic Wistar rats . Different doses of the extract were (50, 100 and 200 mg/kg) administered after 18 h fast and there blood glucose levels were measured at 0,2,4,8 and 24 h after treatment. Lowest dose (50 mg/kg) administered significant decrease ($p<0.05$) in the blood glucose levels of alloxan-induced diabetic and normoglycemic Wistar rats after 24 hours of treatment. The doses of 100 and 200 mg kg-1 did not show any significantly decrease the blood glucose levels of alloxan induced diabetic Wistar rats. The hypoglycemic and antihyperglycemic potentials of the ethyl acetate portion of the extract was comparable to that of biphasic isophane insulin (6.i.u/kg) .In relation to the normoglycemic groups there was a significant decrease in the blood glucose level ($p<0.05$) with the doses of 50 and 100 mg/kg after treatments, while there was no any significant change with the highest dose of 200 mg/kg after treatments. The preliminary phytochemical screening of the extract revealed the presences of alkaloids tannins and flavonoids. The lethal dose of the extract (LD_{50}) in rats was calculated to be 775 mg/kg bodyweight. Concluded that the ethyl acetate portion of hydromethanolic leaves extract of *Indigofera pulchra* possess anti-diabetic effects in alloxan- induced in diabetic and normoglycemic rats.

Key words: *Indigofera pulchra*, ethyl acetate hypoglycemic activity, normoglycemic, alloxan, Diabetes mellitus

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of impaired carbohydrates, fat and protein metabolism. It is characterized by hyperglycaemia expressed as abnormal glucose value, which is due to insulin deficiency and/or insulin resistance which results in decrease utilization of carbohydrate and excessive glycogenolysis and gluconeogenesis from amino acid by fatty acid. It has been defined by the World Health Organization (WHO) on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.3 mmol/L (140mg/dl) or greater than 11.1 mmol/L (200mg/dl) two hours after carbohydrate meal or two hours after oral ingestion of the equivalent of 7.5g of glucose.

This disease is on the increase all over the world, especially in Africa where it was formerly regarded as the disease of the affluent (Amos *et al.,* 1997).

Diabetes is one of the most common non-communicable diseases worldwide when compared with the general population, mortality and morbidity is increased in diabetes, due to the associated chronic complications – both specific microvascular retinopathy, nephropathy and non-specific macrovascular atherosclerosis). Acute metabolic complications (e.g. diabetic ketoacidosis) continue to be a cause of mortality in developing countries (Ayesha *et al.,* 2003).

Using current and projected estimates, it has been suspected that the global prevalence of type I diabetes mellitus will increase from 3.5 million in 1995 to 5.5 million in 2010 (Amos *et al.,* 1997). For Africa, the projected increase is from 85,000 (1995) to 219,000 (2010).

Because of the tendency of many professionals to cling to the belief that only pharmaceutical products are of value, therefore, possible source of new drugs to complement the action of oral hypoglycemic agents. Renewed attention to alternative medicines and natural therapies has stimulated a new wave of research interest into traditional practice. The World Health Organization (WHO) Expert Committee on Diabetes listed as one of its recommendations; further investigations into traditional methods of treating diabetes (WHO, 1980).

Indigofera pulchra(Wild) family: papilionaceae is an annual coma non climbing herb or shrub that can grow up to 1m tall. It is widely distributed throughout West-Africa (Herper,1976). In ethno medicine, the leaves are used to treat infected wound (Herper 1976; Burkill, 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) and also the

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crude hydromethanolic and n-butanol extracts have antidiabetic activities (Tanko *et al*., 2008a,b).

The present study was designed to test the hypoglycemic effect of ethyl acetate portion of *Indigofera pulchra* leaves extract on alloxan–induced diabetes and normoglycemic Wistar rats.

MATERIALS AND METHODS

Plant material: *Indigofera pulchra* sample 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. The leaves were air-dried and made into powder using pestle and mortar.

Extraction: The air- dried powdered plant material was extracted with 70% methanol using soxhlets apparatus, the solvent was removed *in-vacuo* to yield a residue (100gms) referred to as methanol extract. One hundred grams of the methanol extract was suspended in water and filtered, the water soluble part was extracted with ethyl acetate (4 x 500 ml) to give 2.5g ethyl acetate portion.

Chemicals used: All chemicals and drugs were obtained commercially and were of analytical grade. Alloxan monohydrate (Sigma).The Biphasic Isophane insulin AS Mixtard 30 HM Pen fill (Novo Nordisk AIS 2880 Bagsvaerd, Denmark. NAFDAC Reg.no 04-1601).

Acute toxicity study: The lethal dose (LD_{50}) of the plant extract was determined by method of Lork (1983) using 21 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 intraperitoneal (i.p). They were observed for 24 h for signs of toxicity. In the second phase 12 rats were divided into 4 groups of 3 rats each and were also treated with the extract at doses of 200, 400 and 800 and 1600 mg/kg bodyweight *(i.p).*The median lethal dose (LD_{50}) was calculated using the second phase.

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

Animals and Induction of Diabetes Mellitus: Wistar strain albino rats of both sexes weighing (150-200 g) bred in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences A.B.U Zaria, were used for the study of the effect of the ethyl acetate portion of *Indigofera pulchra* leaves extract on the blood glucose levels of the animals. The animals were fed *ad libitum* with pellet diet (Vital feeds, Jos, Nigeria) and water. They were kept and maintained under laboratory conditions of temperature, humidity and light (25±1 °C 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. The rats assigned to the diabetic groups were injected with alloxan monohydrate dissolved in sterile cold normal saline at a dose of 150 mg/kg body weight intraperitoneally as reported earlier by (Kamewara Rao *et al*.,1997).

Table 1: Phytochemcial analysis of n-butanol portion of the leaves extract of Indigofera pulchra

	Constituents/Test	Preference
1.	Alkaloids	+
	Dragendoff's	
	Mayers	
	Wagner's	
2.	Flavonoids	+
	Shinoda	
	Sodium hydroxide	
3.	Saponins	-
	Frothing	
4.	Tannins	+
	Feric chloride	
	Lead acetate	
5.	Steroidal nucleus	-
	Salkwoski	
	Liberman-Burchard	

Key: + = Present, -= Absent

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 (Stanley *et al*., 2001).They 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 a blood glucose levels greater than 170mg/dl were considered diabetic and used for this research work (Stanley *et al*., 2001).

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

- Group 1- Received normal saline i.p
- Group2- Received Biphasic Isophane Insulin 6 i.u/kg i.p (Stanley *et al*., 2001)
- Group3-Received 50mg/kg body weight of the ethyl acetate portion of the extract i.p
- Group4- Received 100mg/kg body weight of the ethyl acetate portion of the extract i.p
- Group5- Received 200mg/kg body weight of the ethyl acetate portion of the extract i.p

The normoglycemic Wistar rats were also randomly grouped into five, with five rats (n=5) in each group as follows:

- Group 6- Received normal saline i.p
- Group7- Received Biphasic Isophane Insulin 6 i.u/kg i.p (Stanley *et al*.,2001)

- Group8-Received 50mg/kg body weight of the ethyl acetate portion of the extract i.p
- Group9- Received 100mg/kg body weight of the ethyl acetate portion of the extract i.p
- Group10- Received 200mg/kg body weight of the ethyl acetate portion of the 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 by Dunnett's method. Values of $p<0.05$ or less were taken as significant (Duncan *et al.*, 1977). The alloxan-induced diabetic groups and normoglycemic groups were analyzed separately for statistical significance.

RESULTS

Acute toxicity study (LD_{50}): The sign of toxicity were first noticed after 4-6 hours of extract administration. There was decreased locomotor activity, decreased feed intake, and prostration after 10 hours of extract administration. The median lethal dose (LD_{50}) in rats was calculated to be 775 mg/kg body weight.

Blood Glucose levels of Alloxan-induced Diabetic Wistar rats: Table 2 showed the results of the effects of three doses (50, 100 and 200 mg/kg) of the extract insulin and control normal saline in alloxan- induced diabetic Wistar rats. There was no any significant decreased with two doses of the extract 100 and 200 mg/kg when compared with control normal saline group. However the gave lowest dose of the extract 50 mg/kg there was a significant decrease ($p<0.05$) in the blood glucose levels when compared to control after 24 hours of extract treatment. In relation to the reference drug biphasic isophane insulin there was a significant decrease in the blood glucose levels when compared to control normal saline group

Blood Glucose levels of Normoglycemic Wistar rats: Table 3 showed the results of the effects of three doses (50mg/kg, 100mg/kg and 200mg/kg bodyweight) of ethyl acetate portion of the leaves extract of *Indigofera pulchra*, Insulin and control groups in normal Wistar rats. There was no significant decrease in the blood glucose level with the highest dose of the extract (200 mg/kg) when compared to control normal saline group .Also in regards to the other two doses of 50 and 100 mg/kg there was a significant decrease ($p<0.05$) in the blood glucose levels when compared to the control normal saline group.

DISCUSSION

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 in 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).

In relation to the alloxan induced diabetes. There was no any significant decrease with the two doses of the extract 100 and 200 mg/kg when compared with control normal saline group. Also in regard to lowest dose of the extract 50 mg/kg there was a significant decrease ($p<0.05$) in the blood glucose levels when compared to control after 24 hours of extract treatment. In relation to the reference drug biphasic isophane insulin there was a significant decrease in the blood glucose levels when compared to control normal saline group

As regard to the normoglycemic group the effects of three doses (50mg/Kg, 100mg/Kg and 200mg/Kg bodyweight) of ethyl acetate portion of the leaves extract of *Indigofera pulchra*, Insulin and control groups in normal Wistar rats. There was no any significant decrease in the blood glucose level with the highest dose of the extract that is 200 mg/kg when compared to control normal saline group .Also in regards to the other two doses 50 and 100 mg/kg there was a significant decrease ($p<0.05$) in the blood glucose levels when compared to the control normal saline group.

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 ethyl acetate portion of the *extract* of revealed the presence of alkaloids and flavonoids. Flavonoid isolated from the other antidiabetic medicinal plants has been found to stimulate secretion or possess an insulin like-effect (Marles and Farnsworth 1995). Effect of the flavonoids quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini, *et al.*, (2004) as the mechanism by which they reduced hyperglycaemia caused by alloxan-diabetic rats. The flavonoids present in *Indigofera pulchra* may also be acting similarly thereby decreasing the high blood glucose levels of alloxan-diabetic and normoglycemic Wistar rats.

CONCLUSION

In conclusion, the results of the present study confirm the hypoglycemic effect of ethyl acetate portion of

Table 2: Effect of Different doses of ethyl acetate portion of *Indigofera pulchra* leaves extract on blood glucose levels (mg/dl) of alloxan-induced diabetic Wistar rats.

	BLOOD GLUCOSE LEVELS(Mg/dl)				
Treatment	0 hour	2 hours	4 hours	8 hours	24 hours
Group 1 Control $^{(N/Saline)}$	452.8 ± 62.3	473.4 ± 59.6	466.8 ± 52.8	455.4 ± 57.9	455.6 ± 38.3
Group 2(Insulin 6.i.u/kg)	439.8 ± 36.3	443.4 ± 40.6^{ns}	384.8± 30.4^{ns}	300.8± 34.3^{a}	191.2 ± 14.2^{a}
Group 3(50 mg/kg)	451.8± 59.5	464.6 ± 63.0^{ns}	390.0 ± 60.7^{ns}	348.8 ± 50.6^{ns}	318.8 ± 36.2^{a}
Group 4(100 mg/kg)	453.0 ± 41.7	467.2 ± 46.5^{ns}	432.6 ± 33.1^{ns}	392.6 ±28.4^{ns}	374.2 ± 19.5^{ns}
Group 5(200 mg/kg)	445.8 ± 73.0	447.4 ± 49.7^{ns}	414.0 ± 48.9^{ns}	376.2 ± 51.2^{ns}	348.8 ± 51.5^{ns}

Vales are given as mean ± SD 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

Table 3: Effect of Different doses of ethyl acetate portion of *Indigofera pulchra* leaves extract on blood glucose levels (mg/dl) of normoglycemic Wistar rats.

	BLOOD GLUCOSE LEVELS(Mg/dl)				
Treatment	0 hour	2 hours	4 hours	8 hours	24 hours
Group 6 Control $^{(N/Saline)}$	81.2±5.17	90.4±3.90	91.8±4.30	94.6±5.03	90.0±3.72
Group 7 (Insulin 6.i.u/kg)	83.8 ± 4.68^{ns}	77.6 ±5.46^{ns}	65.8±4.93^{a}	52.2±2.59^{a}	41.8±1.98^{a}
Group 8 (50 mg/kg)	83.2±2.74^{ns}	88.0 ±2.38^{ns}	72.6±5.19^{a}	54.6± 7.95^{a}	46.2±6.47^{a}
Group 9 (100 mg/kg)	81.2 ±5.03^{ns}	88.2 ±7.76^{ns}	74.0±5.35^{ns}	63.8±7.31^{a}	59.0±6.97^{a}
Group 10 (200 mg/kg)	83.8±6.76^{ns}	88.0±7.92^{ns}	84.4±8.17^{ns}	80.0±8.48^{ns}	73.4±7.91^{ns}

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

Indigofera pulchra leaves extract when administered to alloxan -induced diabetic and normoglycemic animal model which suggest the presence of biologically active components which may be worth further investigation and elucidation. Further studies are in fact currently under way to isolate and characterize the active principle (s) of the crude extract.

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