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Effects of Metformin, Pioglitazone and Aqueous Extract of Delonix Regia on Blood Levels of Glycated Hemoglobin (Hba1c) of Streptozotocin-Induced Diabetic Male and Female Wistar Albino Rats

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Abstract: *The effects of Delonix regia extract (d200mg, d300mg, and d400mg), metformin (m8.3mg, m12.5mg and m16.5mg), pioglitazone (p0.5mg, p0.7mg and p0.9mg) and combined formulation of metformin and extract (m6.25d150mg) on* glycated hemoglobin *status in streptozotocin-induced diabetic Albino wistar rats. Diabetic status of these rats was assessed by estimating fasting blood glucose levels. A total of 150 albino rats were used for the investigation and were grouped into twelve groups of twelve rats each as follows; Group I: normal control rats (NCR). Group II: Diabetic control rats (DCR). Group III: Diabetic rats treated with d200mg. Group IV: Diabetic rats treated with d300mg. Group V: Diabetic rats treated with d400mg. Group VI: Diabetic rats treated with m8.3mg. Group VII: Diabetic rats treated with m12.5mg. Group VIII: Diabetic rats treated with m16.5mg. Group IX: Diabetic rats treated with p0.5mg. Group X: Diabetic rats treated with p0.75mg. Group XI: Diabetic rats treated with p1.0mg. Group XII: Diabetic rats treated with m125d300mg each for male and female respectively, for a total of 56 days. After every two weeks interval of treatment for eight weeks three rats from each group were sacrificed and blood sample were collected and analyzed for various parameters. The result obtained showed an elevated level of* glycated hemoglobin *in diabetic-induced wistar albino rats compared with normal control rats. However, there was reversal of the effects when treated with the drug/extract. Also there was reduction in the blood glucose level of the diabetic rats treated with metformin (from 6.37±0.69 to 5.20±0.62mmol/l), pioglitazone (from 7.30±0.21mmol/l to 4.70±0.46), aqueous extract of Delonixregia (from 8.20±0.81mmol/l to 6.10±0.60) and combined formulation of metformin and extract (from 7.81±0.34 to 4.80±0.17), at p<0.05 confidence level when compared with diabetic control rats in the various weeks of treatment respectively.*

Key Words: *Delonix regia, Diabetes, Glycated hemoglobin,*

I. Introduction

Glycated hemoglobin (hemoglobin A1c, HbA_{1c}, A1c, or Hb1c) is a form of hemoglobin that is measured primary to identify the average plasma glucose concentration over prolonged period of time. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. In diabetes mellitus, there is higher amount of glycated hemoglobin which indicates poor control of blood glucose levels (Larsen, 1990).

The 2010 American Diabetes Association standards of medical care in Diabetes added the A1c≥ 48mmol/l (≥6.5%) as another criterion for the diagnosis of diabetes (ADA, 2010)

Traditional medicines play an important role in health services around the globe. About three-quarters of the world population relies on plants and plant extracts for healthcare. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare.

Delonixregia (Family: Caesalpiniaceae) is a medium-sized tree found in greater parts of India. Delonixregiacommonly known as Krishnachura, Gulmohar, Malinche and Tabachine (Cowen, 1984) is grown commonly in different parts of Bangladesh. It is well adapted to the humid tropical climate (Mandalet al., 2008), usually grown as an ornamental tree, and given the name 'Royal Poinciana' or 'Flamboyant'. The decoction of the leaves is traditionally used in treating gastric problems, body pain, and rheumatic pains of joints (Parrota, 2000). Ethanolic extracts of flower and bark were investigated to have anti-inflammatory activity in rats (Srinivasanet al., 2001). The leaves are reported to have antibacterial (Parekh et al., 2005) and antimalarial effect(Ankrahet al., 2003). Plant extract has been reported to possess anti-bacterial, anti-malarial and anti-fungal properties (Aqil and Ahmad, 2007; Ankrahet al., 2003; Duttaet al., 1998). Delonixregia contains proteins, flavonoids, tannins, phenolic compounds, glycosides, sterols, and triterpenoids. The leaf of this tree is used by the folk medicinal practitioners of Bangladesh for controlling sugar level in diabetic patients. This information suggests that this plant may possess some anti-diabetic activities but no scientific work has been conducted to justify this hypothesis. The present study has been carried out to evaluate the activities of metformin,

pioglitazone and aqueous extract of Delonixregia on serum levels of glycated hemoglobin of streptozotocin-induced diabetic male and female wistar albino rats.

II. Materials And Methods

2.1 Drugs and Equipment

Metformin, pioglitazone were obtained from DrakooPharmarcy, Elekahia, Port Harcourt while Streptozotocin was obtained from NBUZOR Chemical No.96, Rumuola, Port-Harcourt Nigeria. All other reagents were of analytical grade.

2.2Collection of Plant Seeds/ Preparation of Delonixregia extract

Dried seed of Delonixregia (flamboyant tree) were collected from a biological garden in University of Port Harcourt, Rivers State and was identified and authenticated by the Plant Science and Biotechnology (PSB) Department of the University of Port-Harcourt, Rivers State, Nigeria. The dried pods of the Delonixregia were carefully plucked off from the plant and were opened to collect the seeds. The seeds were thoroughly washed and sundried for a period of two months to a constant weight. The dried seeds were then blended with high speed blender at Choba market until a fine smooth powder was obtained.

Exactly 44.5g of dried powdered sample were weighed using the weighing balance. Then the measured sample was transferred into a measuring conical flank and 600ml of distilled water was added to it. This was shaken vigorously for 10 minutes and allowed to stand for 24hours. At the end of the extraction, different concentrations of the extract were prepared (d200mg, d300mg and d400mg).

2.3 Animals

A total of one hundred and fifty (150) albino wistar rats weighing between 159-270g and between six to fourteen weeks old (of which seventy-five (75) were males and female each) were used for the study. The animals were purchased from the Department of Biochemistry, University of Port-Harcourt animal house. The animals were kept in cages of 12 rats per cage in the animal house laboratory to acclimatize for one week while they receive their normal feed and water ad libitum. The feed was purchased from the livestock feed shop, Rumuokoro, a division of livestock feeds Nigeria Limited, Port-Harcourt. The feed given to the animals were finisher mash.

Formulation of High Fat Diet

After one week of acclimatization, the animals were fed with high fat diet for one month. The high fat diet was formulated as follows; in every 1000g of the total feed, the following compositions were added.

Cholesterol	25g	2.5%
Sucrose	200g	20%
Lard	100g	10%
Finisher	675g	67.5%

These were thoroughly mixed together before given to the animals with water ad libitum for a period of the month.

2.4 Experimental Design

Delonixregia extract, metformin and pioglitazone were given orally once daily, presented in the table below.

Groups	Treatment received per day
1	Normal rat feed
2	High fat feed
3	High fat feed + stz + 200mg/kg of Delonixregia extract
4	High fat feed + stz + 300mg/kg of Delonixregia extract
5	High fat feed + stz + 400mg/kg of Delonixregia extract
6	High fat feed + stz + 8.3mg/kg of metformin
7	High fat feed + stz + 12.5mg/kg of metformin
8	High fat feed + stz + 16.5mg/kg of metformin
9	High fat feed + stz + 0.5mg/kg of pioglitazone
10	High fat feed + stz + 0.75mg/kg of pioglitazone
11	High fat feed + stz + 1.00mg/kg of pioglitazone
12	High fat feed + stz + m6.25d150mg/kg of met. &Delonixregia extract

2.5 Induction of Diabetes (streptozotocin)

The 150 albino wistar rats were housed in the plastic cages. Six rats were used for the pilot study to ascertain, the dose level at which the rats can be made diabetic. Animal were then weighed and divided into 12 groups of 12 animals each.

Group 1 received the normal rats feed (finisher).
Groups 2 to 12 received high fat feed composed of sucrose (20%), lard (10%) and cholesterol 25% for four weeks, aimed at inducing insulin resistance. After four weeks on high fat feed, the animals were re-weighed.
Groups 2 to 12 were also injected intraperitoneally with stz at dose of 60mg/kg. The stz was given as 4g in 160ml of distilled water (Guoxiaohua, et al., 2006).

Collection of blood sample

Three animals were sacrificed by anaesthesing the animals with chloroform in desiccator chamber after every two weeks of treatment with anti-diabetic agent from each group and blood samples was collected from retro-orbital venous plexus until the end of the 16^{th} weeks of study. All the animals were sacrificed and blood samples were collected into heparin for the estimation of prolactin.

Glucose Determination

The plasma glucose concentration was determined using the multiCarein™glucose strips and glucometer.

Glycated Hemoglobin Determination

The glycated hemoglobin was determined using high-performance liquid chromatography (HPLC). The HbA1c result is calculated as a ratio to total hemoglobin by using a chromatogram.

2.6 Statistical Analysis of Data

The Data were analyzed for statistical differences between treatment groups, by means of ANOVA and followed by multiple comparisons using least significant difference (post hoc LSD), on SPSS 19. In all, $p<0.05$ was considered significant. Data are presented as mean ± s.d (standard deviation).

III. Results

The results of the analyses carried out are presented in tables as shown below.

Table 1: The result of the effect drugs/extract administration on glucose level in streptozotocin-induced diabetic male wistar albino rats.

Drugs	GL STZ INDTN	GL B4 TRT	GLTRT WK4	GLTRT WK8
Metformin	6.00±0.05	6.37±0.69	6.40±1.39	5.20±0.62
Pioglitazone	4.17±0.15	7.30±0.21	6.27±0.18	4.70±0.46
Extract	5.63±0.09	8.20±0.81	5.30±0.49	6.10±0.60
Combined formulation	5.12±0.45	7.81±0.34	6.90±0.27	4.80±0.17

All values indicated in the table are mean±SD values. Superscripts with the same letter are not significant at $p<0.05$ while those with different letters were considered to be significant at the levels of $p<0.05$.
However, the Normal Control Rats (NCR) remained constant at average of 2.50±0.06mmol/l.

Key:

GL STZ INDTN: average glucose level 48hrs after stz induction
GL B4 TRT: average glucose level prior to drug/extract treatment
GL TRT WK4: average glucose level after week 4 of treatment
GL TRT WK 8: average glucose level after week 8 of treatment

Table 2: The result of the effectof drugs/extract administration on glycated hemoglobin in streptozotocin-induced diabetic male wistar albino rats.

Glycated hemoglobin (percent)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR		1.6398	1.7623	1.4887	2.3667
grp1		±0.8819	±0.1201	±0.1154	±0.1453
DCR		4.3389	4.1906	3.7843	4.1133
grp2		±0.8819^{d}	±0.1201^{n}	±0.1763^{p}	±0.0881^{g}
		3.9746	2.7966	2.8133	2.6736
Grp3	d200mg	±0.5018	±0.1154	±0.0333	±0.1732
		4.0547	4.7365	2.8566	2.1966
Grp4	d300mg	±0.0578	±0.0881^{n}	±0.1453^{p}	±0.1763
		3.2749	4.2366	3.8833	4.6758
Grp5	d400mg	±0.7321	±0.3785^{n}	±0.1763^{p}	±0.8368^{g}
		4.5667	3.3667	2.8973	2.6462
Grp6	m8.3mg	±0.1453^{d}	±0.2333	±0.2081	±0.1732
		4.5937	3.4337	2.6359	2.5798
Grp7	m12.5mg	±0.5275^{d}	±0.1219	±0.1527	±0.0817
		4.6337	2.7668	2.9764	2.5766
Grp8	m16.5mg	±0.6677^{d}	±0.2081	±0.1763	±0.2967
		3.7676	2.7663	1.8566	1.7667
Grp9	p0.5mg	±0.5774	±0.1633	±0.1219	±0.0881
		3.7633	2.5756	2.5667	1.9333
Grp10	p0.75mg	±0.1019	0.2848	±0.0654	±0.0333
		4.7677	4.8666	3.8333	4.3566
Grp11	p1.0mg	±0.8559^{d}	±0.0336^{n}	±0.0666^{p}	±0.3480^{g}
		3.4356	4.6864	3.8333	4.2389
Grp12	m6.25d150mg	±0.1628	±0.1147^{n}	±0.2343^{p}	±0.0658^{g}

All values indicated in the table are mean±SD values. Superscripts with the same letter are not significant at $p<0.05$ while those with different letters were considered to be significant at the levels of $p<0.05$.

Table 3: The result of the effect of drugs/extract administration on glycated hemoglobin in strepzotocin-induced diabetic wistar female albino rats.

Glycated hemoglobin (percent)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR		1.7336	1.6667	1.4667	1.4667
Grp1		±0.2027	±0.1768	±0.348	±0.1453
DCR		4.9873	4.8726	3.7363	4.1827
Grp2		±0.8373^{r}	±0.8373^{c}	±0.8363^{a}	±0.7282^{z}
		2.8333	3.6671	2.8067	2.2667
Grp3	d200mg	±0.1453	±0.1453	±0.7587	±0.4055
		6.3667	5.6998	4.7667	2.0667
Grp4	d300mg	±0.3179^{r}	±0.1527^{c}	±0.1855^{a}	±0.2905
		5.7056	3.2667	2.4333	1.1333
Grp5	d400mg	±0.6429^{r}	±0.1333	±0.3666	±0.4096
		4.8333	3.4335	3.6667	3.4072
Grp6	m8.3mg	±.0477	±0.9504	±0.3333	±0.3055
		6.5654	6.3667	4.7667	3.5333
Grp7	m12.5mg	±0.3214^{r}	±0.8819^{c}	±0.3333^{a}	±0.3711
		5.4333	6.2735	5.9333	6.2333
Grp8	m16.5mg	±0.3711^{r}	±0.2645	±0.5268^{a}	±0.6227^{z}
		2.5333	2.4667	1.9333	1.4054
Grp9	p0.5mg	±0.0881^{r}	±0.3333	±0.5811	±0.7371
		5.5333	5.3346	4.5376	4.8667
Grp10	p0.75mg	±0.2666^{r}	±0.0881^{c}	±0.2516^{a}	±0.3333^{z}
		6.2667	5.73656	4.3667	3.9675
Grp11	p1.0mg	±0.2666^{r}	±0.2081^{c}	±0.2666^{a}	±0.0577
		3.7667	2.5667	2.2899	3.2787
Grp12	m6.25d150mg	±0.1453	±0.1333	±0.3605	±0.3055

All values indicated in the table are mean±SD values. Superscripts with the same letter are not significant at $p<0.05$ while those with different letters were considered to be significant at the levels of $p<0.05$.

IV. Discussion And Conclusion

Diabetes is a chronic disease in which there are high levels of blood glucose resulting from defect in sugar in insulin secretion, insulin action or both. Diabetes is also associated with hyperlipidemia and co-morbidities such as obesity and hypertension. The present study was undertaken with the objective of exploring the effects of metformin, pioglitazone, Delonix regia extract and combined formulation of metformin and Delonix regia extract. A combination of a high fat extract and low dose of streptozotocin has been reported to induce type 2 diabetes in rats (Guoxiaohua, et al., 2006). This probably explains the significant elevation of blood glucose observed in this study in experimental rats given high fat diet and low dose streptozotocin when compared to normal rats. Streptozotocin (stz) which is selectively toxic to the beta cells of the pancreatic islets of Langerhans was used to induce diabetic type 2 in rats (Weiss, 1982). After the induction of diabetes with the stz, it was observed that the normal control rats retained their normal glucose level of 2.50±0.06mmol/l while the glucose level of the diabetic control rats significantly increased from 5.63±0.06mmol/l to 8.87±0.69mmol/l. After 8 weeks of administration of anti-diabetic agents, metformin significantly reduced blood glucose from 6.37±0.69 to 5.20±0.60mmol/l, pioglitazone reduced blood glucose level from 7.30±0.21 to 4.70±0.46mmol/l, Delonix regia extract reduced blood glucose from 8.20±0.81 to 6.10±0.60mmol/l while the combined formulation of Delonix regia extract and metformin also significantly reduced blood glucose level from 7.81±0.34 to 4.80mmol/l.

The investigation also analysed the effect of Delonix regia extract, metformin, pioglitazone and the combined formulation of metformin and Delonix regia extract on glycated hemoglobin in stz-induced diabetic wistar albino rats.

In table 2 and 3, there was an overall increase in the glycated hemoglobin level in stz-induced diabetic rats when compared with the normal control rats, since red blood cells are freely permeable to glucose and to each erythrocyte, glycated hemoglobin is formed continuously from hemoglobin (Keen et al., 1973).

From the present findings glycated hemoglobin increased drastically in stz-induced diabetic albino wistar rats resulting from increase in blood glucose level but was reduced on administration of the drugs and aqueous extract of Delonix regia. Therefore, the drugs and aqueous extract of Delonix regia can be used in the treatment and management of diabetes mellitus.

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