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Research Article

Hypoprolactinemic Properties of combined therapy of metformin, pioglitazone and aqueous extract of *Delonix regia* in streptozotocin-induced diabetic male and female Albino wistar 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 Prolactin levels in streptozotocin-induced diabetic wistar albino rats was investigated. 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 prolactin in diabetic-induced wistaralbino 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 Delonix regia (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.
Keywords: *Delonix regia*, Prolactin, Diabetes.

INTRODUCTION

Diabetes mellitus, simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood glucose because the cell does not produce enough insulin, does not respond to the insulin that is produced or both. This high blood glucose produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Diabetes mellitus, is initially characterized by loss of glucose homeostasis resulting from defect in insulin secretion, insulin action or both resulting in impaired metabolism of glucose and other energy yielding fuels such as lipids and proteins [2]. It is also worthy to note that insulin action may be altered by an abnormally high amount of glucagon and other insulin antagonist like growth hormones and corticosteroids by causing diabetes [1].

When blood glucose is poorly controlled over long periods in diabetes mellitus, blood vessels throughout the body begin to function abnormally and undergo structural changes that result to inadequate blood supply to the tissue. This in turn leads to increased risk of heart attack, stroke, kidney disease, retinopathy and blindness and gangrene of the limbs. In diabetic conditions there are significant changes in lipid metabolism and structure[3]. These structural changes are clearly oxidative in nature and are associated with the development of vascular disease [4], and it gives rise to the complication associated with diabetes mellitus. Oxidative stress resulting from oxidation reaction is produced under diabetic conditions and possibly causes various forms of tissue damage in patients with diabetes.

In diabetes mellitus, two main classifications exist as idiopathic and secondary diabetes mellitus. The idiopathic diabetes mellitus is divided into two main types; the insulin dependent diabetes mellitus (IDDM) also called type 1 diabetes and the non-insulin dependent diabetes mellitus (NIDDM) also known as the type 2 diabetes.

Type 1 diabetes may be caused by injury to the beta cells of the pancreas resulting in impaired insulin production, viral infection, autoimmune disorders which may be involved in the destruction of beta cells even hereditary also plays an important role in determining the susceptibility of the beta cell.

Prolactin (PRL) also known as luteotropic hormone is a protein that in humans is encoded by the PRL gene. It also acts in a cytokine-like manner [5] and as an important regulator of the immune system. Prolactin has many effects including regulating lactation and stimulating proliferation of oligodendrocyte precursor cells. It stimulates the mammary glands to produce milk (lactation) [5].

The aim of the present study, therefore, is to determine the Effects of combined therapy of metformin, pioglitazone and aqueous extract of *Delonix regia* on serum levels of prolactin on streptozotocin-induced diabetic male and female Albino wistar rats.

MATERIALS AND METHODS

Drugs and Equipment

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

Collection of Plant Seeds/ Preparation of *Delonix regia* extract

Dried seed of *Delonix regia* (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 *Delonix regia* 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).

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 *adlibitum* for a period of the month.

Experimental Design

Delonix regia extract, metformin and pioglitazone were given orally once daily, as 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 *Delonix regia* extract
4	High fat feed + stz + 300mg/kg of *Delonix regia* extract
5	High fat feed + stz + 400mg/kg of *Delonix regia* 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. &*Delonix regia* extract

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. Animals 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 [6].

Collection of blood sample

Three animals were sacrificed by anaesthesing the animals with chloroform in desiccators 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 16th weeks of study. All the animals were sacrificed and blood samples were collected into heparin for the estimation of Prolactin.

Glucose Determination

Five days after streptozotocin injection, 3ml of blood specimen was collected from the tail vein and blood glucose measured using Accu-chek Glucometer. Stz injected rats were observed to possess fasting plasma glucose concentration greater than 7.0mmol/L confirmed diabetes.

Prolactin Determination

The concentrations of prolactin were determined using prolactin quantitative test kit (ELISA) on authomated microplate reader machine.

Statistical Analysis of Data

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

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 malewistar 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 effect of drugs/extract administration on Prolactin levels in streptozotocin-induced diabetic malewistar albino ra ts. Prolactin (ng/ml)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR Gp1		1.5467 ±0.8762	1.7653 ±0.7262	1.8735 ±0.7652	1.9876 ±0.9876
DCR Grp2		2.1333 ±0.8819[a]	3.5667 ±0.1666	2.3330 ±0.0881[c]	2.7667 ±0.1201[d]
Grp3	d200mg	2.3331 ±0.0819[a]	2.4589 ±0.5774	2.6667 ±0.0333[c]	2.4333 ±0.0881
Grp4	d300mg	1.5333 ±0.2403	1.8058 ±0.5774	2.0333 ±0.3333	2.4333 ±0.0881
Grp5	d400mg	1.5586 ±0.6119	1.5667 ±0.1453	2.4333 ±0.2185[c]	1.7667 ±0.1855
Grp6	m8.3mg	1.3333 ±0.1333	1.3587 ±0.7321	1.7054 ±0.1527	1.5333 ±0.1201
Grp7	m12.5mg	1.3666 ±0.2019	1.6333 ±0.1453	2.4956 ±0.5774[c]	2.8576 ±0.5774
Grp8	m16.5mg	2.9365 ±0.5774[a]	2.2333 ±0.3333	2.1661 ±0.1219	2.8054 ±0.1547[d]
Grp9	p0.5mg	2.4666 ±0.4666[a]	2.3339 ±0.2927	1.8333 ±0.1201	2.2667 ±0.3333
Grp10	p0.75mg	1.5667 ±0.2276	1.9709 ±0.05774	1.4333 ±0.2019	2.7333 ±0.3596
Grp11	m1.0mg	1.8333 ±0.3333	1.6597 ±0.5774	1.5333 ±0.1763	2.3354 ±0.5166
Grp12	M6.25d150mg	3.3666 ±0.2027[a]	1.4766 ±0.1154	1.8333 ±0.0333	2.3667 ±0.2634

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 effectof drugs/extract administration on prolactin levels in streptozotocin-induced diabetic female wistar albino rats. Prolactin (ng/ml)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR grp1		2.6746 ±0.5242	3.7262 ±0.6252	3.788 ±0.8673	3.6837 ±0.8272
DCR grp2		5.2767 ±0.4415	5.2667 ±0.4819	6.8333 ±0.1836	6.5667 ±0.2333[w]
Grp3	d200mg	4.5498 ±0.2887	3.5347 ±0.5166	3.7333 ±0.1559	3.8667 ±0.2158
Grp4	d300mg	2.5987 ±0.5774	3.8333 ±0.4333	3.7333 ±0.3179	3.3333 ±0.2848
Grp5	d400mg	3.1667 ±0.1453	3.3333 ±0.3551	3.3670 ±0.5275	3.5872 ±0.2658
Grp6	m8.3mg	3.6333 ±0.2633	3.3765 ±0.3055	3.5667 ±0.2634	4.1667 ±0.0333
Grp7	m12.5mg	2.2333 ±0.2848	5.5667 ±0.2848[m]	5.9667 ±0.3198	6.5687 ±0.2648[w]
Grp8	m16.5mg	2.2795 ±0.3519	4.8566 ±0.2566	4.6667 ±0.2437	6.1667 ±0.1209
Grp9	p0.5mg	2.5333 ±0.3283	4.5333 ±0.6888	4.4576 ±0.7636	3.8333 ±0.5238
Grp10	p0.75mg	3.9759 ±0.2645	5.2667 ±0.1768	5.8667 ±0.2962	5.7689 ±0.2648
Grp11	p1.0mg	2.6333 ±0.2276	4.1667 ±0.1201	3.7876 ±0.1527	2.8333 ±0.6667
Grp12	m6.25d150mg	2.6333 ±0.3929[k]	4.9956 ±0.1527	4.7667 ±0.1201	4.7667 ±0.5487

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 $p<0.05$.

DISCUSSION AND CONCLUSION

It was observed from tables 2 and 3; that there was elevated mean fasting serum prolactin level in diabetic albino wistar rats compared with the normal control rats. Differences in dietary habit would be possible explanation since high fat diet is known to influence prolactin level [7,8,9]. Alternative possibilities would be abnormalities developing from micro-vascular infarcts of pituitary stalk which interfere with the inhibitory stimuli of prolactin secretion. It is noteworthy in this regard that pituitary infarction has been associated with diabetes mellitus [10]. Saller and Chiodo [11] reported, glucose administration in the rats suppresses the firing of central dopamine neurons; hence it is not tempting to speculate that chronically elevated blood glucose in diabetes could raise serum prolactin level suppressing dopaminergic neuronal activity, a known inhibitor of prolactin release [12]. The correlation between serum prolactin and fasting plasma glucose found from this study is supportive of this speculation.

In conclusion, from the present findings, there was elevated level of prolactin in the streptozotocin-induced diabetic albino wistar rats resulting from increase in blood glucose level. However, on administration of different concentrations of *Delonix regia* extract, Metformin, Pioglitazone and combined formulation of Metformin and *Delonix regia* extract, the effect was reversed. Therefore, the drugs and *Delonixregi a*aqueous extract can be used in the treatment and management of elevated level of prolactin in diabetic condition.

REFERENCES

1. White JP, Campbell RK; Diabetes in Clinical Pharmacy and therapeutic, 5thEdn.London: Williams Publisher, 1992: 307-320.
2. Sheen JA; Drug treatment of non-insulin dependent diabetes mellitus in the 1990's.Achievements and future development. Drug, 1997; 54(3): 355-368.
3. Sochar M, Baquer NZ, Mclean P; Glucose under utilization in diabetes. Comparative on the changes in the activity of enzymes of glucose metabolism in rat's kidney. Molecular Physiology,1995; 7:51-68.
4. Morel WF, Clusolin NT; Association of subclinical hypercortisolism with type 2 diabetes mellitus: a case-control study in hospitalized patients. European Journal of Endocrinology, 1989; 153(6): 837-844.
5. Zinger M, McFarland M, Ben-Jonathan N; Prolactin expression and secretion by human breast glandular and adipose tissue explants. The Journal of Clinical Endocrinology and Metabolism, 2003; 88(2): 689-696.
6. Guoxiaohua ZL, Heng L; Type 2 diabetes mellitus induced by diets and its feature of renal involvement in rat.Chinese Journal of diabetes, 2006.
7. Hill P, Wynder EL; Diet and Prolactin release .Lancet, 1976; 308(7989): 806-807.
8. Hill P; Diet and plasma Prolactin.America Journal of Clinical Nutrition, 1981; 34: 1162.
9. Quingley ME, Ropert JF, Yen SSC; Acute Prolactin release triggered by feeding. Journal of Clinical Endocrinology and Metabolism, 1981; 52: 1043.
10. Kovacs K; Necrosis of anterior pituitary in humans. Neuroendocrinology, 1969; 4: 201.
11. Saller CF, Chiodo LA; Glucose suppresses basal firing and haloperidol-induced increases in the firing rate of central doperminergic neurons. Science, 1980; 210: 1269.
12. Macleod RM; Regulation of Prolactin secretion. In frontier in neuroendocrinology, Martini, L. and Ganong, W.F., (eds), New York: Raven Press, 1976; 169.