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Ameliorative effects of metformin, pioglitazone and aqueous extract of *Delonixregia* on serum levels of luteinizing and follicle stimulating hormones of streptozotocin-induced diabetic male and female wistar albino rats

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ABSTRACT

The effects of Delonixregia 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 (m6.25,d150mg) on follicle stimulating hormone and luteinizing hormone levels in streptozotocin-induced diabetic Albino wistar 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 a reduction in the level offollicle stimulating hormone and luteinizing hormonein 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) an d combined therapy 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: *Delonxregia*, Diabetes, luteinizing hormone, Follicle stimulating hormone

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INTRODUCTION

*Delonixregia*also known as Flamboyant tree are species of flowering plant in the family Fabaceae, subfamily Caesalpiniaiceae. It originated from Madagascar (Zambia) where it is now almost extinct. It is now widespread in most tropical areas of the world such as Brazil, Burkina Faso, Cyprus, Egypt, Eritrea, Ethiopia, India, Jamaica, Kenya, Mexico, Niger, Nigeria, PuetoRico, Singapore. South Africa, Sri Lanka, Sudan, Tanzania, Uganda, United States of America.[1]In many tropical parts of the world it is grown as an ornamental tree and often used to prepare extracts with antimicrobial, antioxidant and antifungal activities. Phytochemical analysis of different parts of *Delonixregia* indicated the presence of alkaloids, sterols and saponins. These phytochemical constituents have antimicrobial, anti diarrheal, anti-malarial, anti-convulsive and anti-inflammatory effects. Thus, these parts of plants have been used in traditional or forklore medicine for the treatment of various ailments such as malaria, diarrhoea, diabetes and arthritis etc.[2]. *Delonixregia*, with medicinal and biological properties, have been used in folk

medicine system of ancient and even modern civilizations for treatment of constipation, inflammation, arthritis, hemiplagia, leucorrhoea and rheumatism [3].

Diabetes Mellitus is a clinical syndrome, characterized byhyperglycemia caused by relative or absolute deficiencyof insulin at the cellular level. It is the most commonendocrine disorder, affecting mankind all over the world, prevalence of which is increasing, daily[4]. Experimental diabetes in animals hasprovided considerable insight into the physiologic andbiochemical derangement of the diabetic state. Many of thederangement have been characterized in hyperglycaemic animals. Significant changes in lipid metabolism andstructure also occur in diabetes[5].Inthese cases the structural changes are clearly oxidative innature and are associated with development of vasculardisease in diabetes[6].

In humans, both male and female, LH is essential for reproduction. In *females*, LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production. At the time of menstruation, FSH initiates follicular growth, specifically affecting granulosacells[7].

The aim of the present study, therefore, is to determine the effects of metformin, pioglitazone and aqueous extract of *Delonixregia* on Reproductive hormones (luteinizing hormone and follicle stimulating hormone) in streptozotocin-induced diabetic male and female wistar albino rats.

MATERIALS AND METHODS

2.1 Drugs and Equipment

Metformin, pioglitazone were obtained fromDrakooPharmarcy, Elekahia, PortHarcourt whileStreptozotocin 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 sun-dried 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) wistar albino 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 one month.

2.4 Experimental Design

Delonixregia 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 *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. 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 [8].

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 16th weeks of study. All the animals were sacrificed and blood samples were collected into heparin for laboratory investigations.

Glucose Determination

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

Biochemical Analyses: The hormonal analyses were performed using the ELISA (a solid base enzyme-linked immunosorbent assay) method, which is based on the sandwich principle [9].

2.6 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 as Mean ± S.D (standard deviation).

RESULTS

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

Table 1: The result of the effect of 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 follicle stimulating hormone in streptozotocin-induced diabetic malewistar albino rats

Follicle stimulating hormone (u/l)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR grp1		7.4393 ±0.6958	8.2967 ±0.3760	9.6727 ±0.2097	10.6865 ±0.6735
DCR grp2		3.2553 ±0.5242^{p}	4.5242 ±0.6171^{f}	4.8171 ±0.6151^{y}	5.6133 ±0.7161
Grp3	d200mg	2.6667 ±0.1763^{p}	4.7335 ±0.5783	7.1333 ±3.4801	6.5667 ±0.4977
Grp4	d300mg	3.2596 ±0.4041	5.7133 ±0.3095	4.4667 ±0.5206^{y}	6.9667 ±0.3756
Grp5	d400mg	2.4333 ±0.7218^{p}	5.5667 ±0.4977	7.2333 ±0.4096	6.0333 ±1.2991^{k}
Grp6	m8.3mg	2.2667 ±0.5456^{p}	6.9636 ±0.5859	7.2333 ±0.4096	8.3477 ±1.8583
Grp7	m12.5mg	3.6566 ±0.3511	4.3566 ±0.0577^{f}	7.3667 ±0.0333	3.0333 ±0.3333^{k}
Grp8	m16.5mg	2.0333 ±0.5783^{p}	2.8795 ±0.5291^{f}	4.03333 ±0.5787^{y}	6.2333 0.3333
Grp9	p0.5mg	4.4546 ±0.0534	4.0667 ±0.6064^{f}	4.6331 ±0.8413^{y}	5.5797 ±0.3383^{k}
Grp10	p0.75mg	1.9576 ±0.2516^{p}	4.4667 ±0.1201	7.3686 ±0.3055	7.5045 ±0.3605
Grp11	p1.0mg	3.9667 ±0.8333	3.1667 ±0.3666^{f}	6.0333 ±0.3333	3.4333 ±0.9562^{k}
Grp12	m6.25d150mg	3.7667 ±0.4409	6.6786 ±0.3055	6.9333 ±0.4667^{y}	8.1333 ±0.6691

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 drugs/extract administration on follicle stimulating hormone in streptozotocin-induced diabetic femalewistar albino rats

Follicle stimulating hormone (u/l)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR Grp1		12.1667 ±0.2603	13.5667 ±0.4667	16.5667 ±0.6359	14.8667 ±0.0881
DCR Grp2		6.2202 ±0.8292^{a}	8.1355 ±0.0292^{s}	8.9282 ±0.8225^{e}	8.6252 ±0.2622^{d}
Grp3	d200mg	6.7333 ±0.1666	11.7657 ±0.0333	13.3333 ±0.0881	12.8232 ±0.7578
Grp4	d300mg	6.1667 ±0.0333^{a}	7.6077 ±0.3511^{s}	9.4766 ±0.0527	8.1667 ±0.2027^{d}
Grp5	d400mg	6.1333 ±2.0366^{a}	7.6045 ±0.2309^{s}	11.1045 ±0.2081	10.1333 ±0.4233
Grp6	m8.3mg	5.6467 ±0.1587^{a}	8.4333 ±0.4255	9.6066 ±0.30551	11.7333 ±0.3711
Grp7	m12.5mg	7.2333 ±0.2848	8.2795 ±0.1527	12.8034 ±0.1154	11.6667 ±0.2848
Grp8	m16.5mg	5.6865 ±0.0577^{a}	6.1333 ±0.1855^{s}	8.8769 ±0.1527^{e}	7.4496 ±0.1154^{d}
Grp9	p0.5mg	6.4000 ±0.2081	7.3330 ±0.2027^{s}	10.5667 ±0.2848	10.3487 ±0.2134
Grr10	p0.75mg	5.5467 ±0.1627^{a}	8.5667 ±0.2333	8.8667 ±0.0333^{e}	7.9142 ±0.1527^{d}
Grp11	p1.0mg	6.7333 ±0.0333	7.5333 ±0.2666^{s}	8.5666 ±0.2333^{e}	7.5333 ±0.2728^{d}
Grp12	m6.25d1500mg	7.35783 ±0.53099	11.1667 ±0.3666	14.0333 ±0.3333	13.1153 ±0.8962

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 4:The result of the effect of drugs/extract administration on luteinizing hormone in streptozotocin-induced diabetic malewistar albino rats

Luitenizing hormone (u/l)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR grp1		8.4667 ±0.2848	7.1667 ±0.1667	10.3667 ±0.2185	8.0367 ±0.9805
DCR grp2		4.7645 ±0.5423^{e}	3.9262 ±0.7265^{m}	5.6252 ±0.6298^{k}	4.0928 ±0.9805^{p}
Grp3	d200mg	4.3466 ±0.2309^{e}	3.0333 ±0.4176^{m}	2.8333 ±0.0888^{k}	3.5333 ±0.2603^{p}
Grp4	d300mg	5.6667 ±0.1666	5.2468 ±0.7937	3.5333 ±0.5364^{k}	3.7333 ±0.5607^{p}
Grp5	d400mg	3.9333 ±0.3844^{e}	3.4333 ±0.2666^{m}	5.7046 ±0.6245	4.8667 ±0.4333
Grp6	d8.3mg	5.0577 ±0.1154	6.6678 ±0.5915	8.3336 ±0.0333^{k}	7.4656 ±0.2309
Grp7	m12.5mg	5.7567 ±0.1527	5.2667 ±0.3666	5.3333 ±0.3666^{k}	3.7898 ±0.7549^{p}
Grp8	m16.5mg	5.4000 ±0.7654	4.7667 ±0.0666	7.5667 ±0.3844	7.8684 ±0.5773
Grp9	p0.5mg	4.8333 ±0.5811	5.5609 ±0.2514	4.0333 ±0.1333^{k}	5.9333 ±0.5487
Grp10	p0.75mg	4.4667 ±0.1763^{e}	3.4333 ±0.7423^{m}	5.8666 ±0.0333	7.9667 ±0.4055
Grp11	p1.0mg	4.3667 ±0.1666^{e}	5.6342 ±0.37800	6.3333 ±0.3333	7.6333 ±0.0881
Grp12	m6.25d150mg	3.5333 ±0.2905^{e}	3.7667 ±0.0881^{m}	6.4487 ±0.6475	6.3486 ±0.3856

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 5:The result of the effect of drugs/extract administration on luitenizing hormone in streptozotocin-induced diabetic female wistar albino rats

Luitenizing hormone (u/l)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR grp1		12.1667 ±0.2603	16.5667 ±0.4667	18.5667 ±0.6359	14.8866 ±0.7881
DCR grp2		6.8202 ±0.8292^{t}	9.6355 ±0.2978^{d}	8.6822 ±0.8225^{l}	8.6252 ±0.2622^{v}
grp3	d200mg	5.0333 ±0.1453^{t}	7.0667 ±0.4055^{d}	3.6667 ±0.4096^{l}	8.2333 ±0.6691^{v}
grp4	d300mg	9.2686 ±0.3055	11.8333 ±0.3929	17.8667 ±0.3179	11.2687 ±0.5131
grp5	d400mg	8.2333 ±0.1703	17.6956 ±0.5859	15.7667 ±0.2333^{l}	15.9667 ±0.1201
grp6	m8.3mg	7.5567 ±0.5437	10.9333 ±0.0666	14.575 ±0.7549^{l}	16.3333 ±0.3283
grp7	m12.5mg	7.6333 ±0.9683	10.7667 ±0.9243	11.7578 ±0.8888	10.9333 ±0.5487
grp8	m16.5mg	11.1453 ±0.5507	13.4053 ±0.6245	12.2562 ±0.3511	13.3667 ±0.7535
grp9	p0.5mg	10.3333 ±0.2728^{t}	8.8054 ±0.5507^{d}	10.5667 ±0.3333	10.9333 ±0.4977
grp10	p0.75mg	6.4667 ±0.2848^{t}	7.7549 ±0.2586^{d}	5.5333 ±0.1453^{l}	8.7333 ±0.4333
grp11	p1.0mg	5.5064 ±0.3511^{t}	3.4667 ±0.2333^{d}	3.6333 ±0.8819^{l}	6.2333 ±0.24037^{v}
grp12	m6.25d150mg	4.8333 ±0.0333^{t}	5.4687 ±0.8819^{d}	7.3333 ±0.2403^{l}	8.2667 ±0.8576^{v}

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

DISCUSSION AND CONCLUSION

Since there was a decreased level of both follicle stimulating hormone and luteinizing hormone observed in the stz-induced diabetic albino rats when compared with the normal control rats as shown in tables2 and 3for follicle stimulating hormone and tables 4 and 5 for luteinizing hormone, an elevated prolactin secretion can suppress the secretion of luteinizing hormone and gonadotropin releasing hormone (GnRHs), leading to hypogonadism[10][11].

Synergically, luteinizing and follicle stimulating hormones binds to the receptors in the testis and ovary to regulate gonadal function by promoting sex steroid production and gametogenesis.

From the present findings, there was decreased level of follicle stimulating hormone and luteinizing hormone in the stz-induced diabetic albino wistar rats resulting from increase in blood glucose level. However, on administration of different concentrations of *Delonixregia* extract, Metformin, Pioglitazone and combined formulation of Metformin and *Delonixregia* extract, the effect was reversed.

Therefore, the drugs and *Delonixregia*aqueous extract can be used in the treatment and management of decreased levels of follicle stimulating and luteinizing hormones in diabetic condition.

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