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Effects of Combined Formulation of Metformin, Pioglitazone and Aqueous Extract of *Delonix Regia* on Serum Levels of Gonadal Steroids (Testosterone and Progesterone) 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 (m6.25,d150mg) on progesterone and testosterone levels in streptozotocin-induced diabetic Albino wistar rats was verified. 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 samples collected and analyzed for various parameters. The result obtained showed a reduction in the levels of testosterone and progesterone hormones in diabetic-induced wistar albino rats compared with normal control rats. However, these were reversed 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, Diabetes, Progesterone, Testosterone*

1. Introduction

Nature has been a source of medicinal agents for thousands of years and impressive number of modern drugs has been isolated from natural source, many based on their use in traditional medicine or phytomedicine. Various medicinal plants have been used for years in daily life to treat diseases all over the world. According to world health organization (WHO), medicinal plants are the best source to obtain a variety of newer herbal drugs. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development program in the pharmaceutical industry. The use of plant extract with known antihyperglycemic properties may be of immense importance in therapeutic treatment. The exclusive use of herbal remedies to treat and manage ailments had served from the onset as the most important therapeutic approach available to man. However, the decline from its use due to the introduction of modern synthetic medicine started at about the beginning of the 20th century up to the 1970's (Wills *et al*., 2000). Traditional medicine accounts for about 80% of the health needs of the rural populace in most regions of Africa;

Delonix regia is one of the medicinal plants, the parts of which are of high medicinal value in many countries of Africa (Ateb and Edournal, 2003).

The prolonged use of these herbal products, without proper monitoring of the usage had brought about a number of health related problems like infertility, which is a common problem affecting most couples all over the world (Keke, 2008).

Diabetes mellitus is an endocrine and metabolic disorder that poses considerable threat worldwide in the 21st century (Adewole and Ojewole, 2009). The central identifying features of diabetes mellitus are hyperglycaemia, hypoinsulinemia, dyslipidemia. Currently diabetes is controlled by diet, exercise, insulin replacement therapy and by the use of herbal hypoglycemic agents (Mallick*et al*., 2007).However; available oral hypoglycaemic drugs have many side effects such as nausea and vomiting, cholestatic jaundice, agranulocytosis, aplastic and hemolytic anemias, generalized hypersensitivity reactions, dermatological reaction and lactic acidosis. Therefore, searching for effective, low cost and less side effected hypoglycaemic agents is important. World ethno botanical information about medicinal plants reports that almost 800 plants could be used to control diabetes mellitus (DM) (Udayakumar*et al*., 2009).

This study is designed to evaluate the effects of metformin, pioglitazone and aqueous extract of *Delonix regia* on serum levels of gonadal steroids (testosterone and progesterone) of streptozotocin-induced diabetic male and female wistar albino rats.

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2. Materials and Methods

2.1. Drugs and Equipment

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

2.2. 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).

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.

2.3.1. 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

Delonix regia 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 *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

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 (Guoxiaohua, *et al.,* 2006).

2.5.1. Collection of Blood Sample

Three animals were sacrificed by anaesthesing the animals with chloroform in desiccator's chamber after every two weeks of treatment with anti-diabetic agent from each group and blood samples were 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 hormone levels.

- 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 (Engvall, 1980).

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 ± SD (standard deviation).

3. Results

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

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

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

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

Testosterone (ng/ml)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR		3.3334	2.0664	3.4337	4.7337
grp1		±0.8819	±0.1201	±0.1763	±0.0881
DCR		1.6335	1.4336	1.6649	2.3647
grp2		±0.1945^{k}	±0.1201^{m}	±0.1154^{b}	±0.1453^{t}
		1.9765	2.005	3.1333	3.3658
Grp3	d200mg	±0.5018	±0.1154	±0.0333	±0.1732
		1.001	1.1338	1.5667	2.1667
Grp4	d300mg	0.5774^{k}	±0.0881^{m}	±0.1453^{b}	±0.1763^{t}
		1.2654	2.2385	2.3333	3.6656
Grp5	d400mg	±0.7321^{k}	±0.3785	±0.1763	±0.8368
		1.5667	1.3667	1.8376	1.6453
Grp6	m8.3mg	±0.1453^{k}	±0.2333^{m}	±0.2081	±0.1731^{t}
		1.5765	1.4333	1.6634	1.8725
Grp7	m12.5mg	±0.5275^{k}	±0.1201^{m}	±0.1527^{b}	±0.08217^{t}
		1.6333	1.6537	1.6455	2.5667
Grp8	m16.5mg	±0.6677^{k}	±0.2081	±0.1793^{b}	±0.2962^{t}
		1.7675	1.6335	1.5667	1.7667
Grp9	p0.5mg	±0.5774	±0.1633	±0.1201^{b}	±0.0881^{t}
		1.6333	1.1567	1.5667	1.9333
Grp10	p0.75mg	±0.2019^{k}	±0.2848^{m}	±0.0654^{b}	±0.0338^{t}
		1.6777	1.8667	1.8337	2.4667
Grp11	p1.0mg	±0.8559	±0.0338	±0.0666	±0.3480
		1.4356	1.6456	2.2333	3.5896
Grp12	m6.25d150mg	±0.1628^{k}	±0.1147	±0.2343	±0.0658

Table 2: The result of the effect of drugs/extract administration on testosterone in streptozotocin-induced diabetic male wistar albino rats, test results in various weeks.

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 $p<0.05$.

Progesterone (ng/ml)

Group	Treatment	Week 2	Week 4	Week 6	Week 8
NCR		3.6353	4.3736	3.7635	5.2673
grp1		±0.6524	±0.7265	±0.7161	±0.2827
DCR		1.1554	1.6333	1.7543	2.0667
grp2		±0.0577[a]	±0.0881[r]	±0.1154[w]	±0.1201[c]
		1.1333	0.7667	1.3087	1.1452
Grp3	d200mg	±0.1337[a]	±0.0881[f]	±0.1154[w]	±0.2516[c]
		1.9667	2.5667	2.6333	4.2667
Grp4	d300mg	±0.1763	±0.1453	±0.4977	±0.1333
		1.4667	1.6739	2.0333	3.9333
Grp5	d400mg	±0.1763	±0.0577[r]	±0.0881	±0.0819[c]
		2.1769	1.4667	1.8667	1.5667
Grp6	m8.3mg	±0.1527	±0.1666	±0.3527	±0.0881
		0.7333	1.0667	1.0667	0.9667
Grp7	m12.5mg	±0.1201	±0.1453[r]	±0.1201	±0.12019[c]
		0.6333	1.7046	1.1333	2.1333
Grp8	m16.5mg	±0.1201[a]	±0.0577[r]	±0.2962[w]	±0.0666
		1.2333	1.2057	1.1333	1.2543
Grp9	p0.5mg	±0.2333[a]	±0.0577[r]	±0.2905[w]	±0.5774[c]
		2.7333	2.6667	2.4667	3.5667
Grp10	p0.7mg	±0.6667	±0.1855	±0.2403	±0.2848
		1.6489	1.5333	2.3333	2.2048
Grp11	p1.0mg	±0.0881	±0.1666[r]	±0.4176	±0.3511
		1.4667	2.5046	2.6667	3.5653
Grp12	m6.25d150mg	±0.8559	±0.1547[r]	±0.8819	±0.1547

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

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 $p<0.05$.

4. Discussion and Conclusion

Diabetes is a chronic disease in which there are high levels of blood glucose resulting from defect 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) is selectively toxic to the beta cells of the pancreatic islets of Langerhans, and 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.

In investigating the effect of aqueous extract of *Delonix regia*, metformin, pioglitazone and the combined formulation of metformin and *Delonix regia* extract on testosterone and progesterone in stz-induced diabetic wistar albino rats, it was observed that there was a decrease in testosterone level in diabetic control rats compared with normal rats in table 2, this may be as a result of diabetes induced hypertension or elevated prolactin level since elevated prolactin condition can produce hypothalamic pituitary dysfunction and suppress gonadal function directly (Kirby *et al*., 1979), subsequently leading to low level of serum testosterone. Elevated prolactin level (mild hyperprolactinemia) is known to be associated with impotence (Franks *et al*., 1978; Nagulesparen *et al*., 1978).

There was a decreased level of progesterone in diabetic control rats compared with normal rats in table 3, this may also be as a result of elevated prolactin level leading to hypothalamic pituitary dysfunction and suppress gonadal function directly (Kirby *et al*., 1979), resulting in a low level of serum progesterone observed in diabetic control rats.

From the present findings, there was decreased level of testosterone and progesterone in the stz-induced diabetic albino wistar rats. 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 *Delonix regia* extract can be used in the treatment and management of infertility necessitated by decreased level of testosterone and progesterone elicited by diabetes.

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