



Polyherbal Extract of *Ocimum Gratissimum* and *Gongronema Latifolium* on Reproductive Functions in Alloxan Induced Diabetic Male Rats

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Abstract

Background: Reproductive functions are impaired in diabetes mellitus. Polyherbal extracts from ocimum gratissimum and gongronema latifolium extracts on reproductive hormones and sperm parameters were studied in alloxan induced diabetic rats.

Materials and Methods: Twenty five adult male albino rats were used in the study. Twenty rats were made diabetic using 100 mg/kg body weight of alloxan monohydrate (intraperitoneal injection). The rats were divided into five groups I (control), II (diabetic control), III, IV and V. The rats were treated with 200mg/kg of ocimum gratissimum (group III), 200mg/kg of gongronema latifolium (group IV), and 200mg/kg per extract of ocimum gratissimum and gongronema latifolium (group V) for 21 days.

Results: The rats treated with polyherbal extracts showed significantly reduced levels of FSH, LH, testosterone, sperm count, sperm motility, sperm morphology and viability. The same results was obtained in rats treated with gongronema latifolium (200mg/kg) except in rats treated ocimum gratissimum extract (200mg/kg) that showed elevated testosterone levels.

Conclusion: These findings showed that polyherbal treatment from ocimum gratissimum and gongronema latifolium do not restore reproductive functions in alloxan induced diabetic rats.

Keywords: gongronema latifolium. ocimum gratissimum. testosterone. follicle stimulating hormone. luteinizing hormone. sperm parameters

INTRODUCTION

Ocimum gratissimum and *gongronema latifolium* leaves are among the commonest spices used in the preparation of delicacies such as pepper soup,

“nkwobi” and making of soup and stew in local restaurants and homes in Nigeria. *Ocimum gratissimum* (lamiaceae) commonly known as “effinrin-nia” by the Yoruba’s, “Nchumou” in

Igbo and “diadoya” in Hausa in Nigeria¹; it is believed to originate from Asia and Africa². It is a perennial plant that is woody at the base with an average height of 1-3 m high and extensively used throughout West Africa as an antimalarial and anti-convulsant³, treatment of respiratory tract infection⁴, diarrhea⁵ and possess hypoglycemic⁶⁻⁸ and antioxidant activity.⁹⁻¹¹ Our recent report also showed that *ocimum gratissimum* extract increased hematological parameters in diabetic rats.¹²

Gongronema latifolium (Asclepiadaceae) commonly locally called “Utazi” and “Arokeke” in the Southeastern and Southwestern States in Nigeria.¹³ It is an edible rainforest plant native to the South Eastern part of Nigeria, has been widely used in folk medicine as a spice and vegetable.¹⁴ Pharmacological actions of both aqueous and ethanol extract include hypolipidemic¹⁵ and anti-lipid peroxidative,¹⁶ antidiabetic,¹⁷ hepatoprotective,¹⁸⁻¹⁹ and increased hematological parameters.¹²

Diabetes mellitus caused reproductive impairment in both males and females.²⁰⁻²¹ Testicular function is primarily controlled by pituitary hormones FSH and LH. The FSH regulates spermatogenesis, whereas the LH controls Leydig cell function.²² Therefore, alloxan was used to induce experimental diabetes mellitus because it is well known to induce type I diabetes in experimental animals by varying the dose of alloxan used.²³⁻²⁴ Alloxan induces diabetes due to a specific necrosis of the pancreatic beta cells in experimental animals.²⁵

The present study was designed to investigate the effects of polyherbal extracts from *ocimum gratissimum* and *gongronema latifolium* on some reproductive hormones and sperm parameters in alloxan induced diabetic rats.

MATERIALS AND METHODS

Experimental Animal

Twenty five male albino rats of Wistar strain weighing 200 -250 g were used. The rats were divided into five groups I, II, III, IV and V. Twenty overnight fasted rats from groups II, III, IV and V rats were made diabetic using single intraperitoneal injection (i.p.) of freshly prepared solution of alloxan monohydrate (100 mg/kg body

weight) dissolved in physiological solution. The alloxanized rats were kept for two days with free access to food and water. The rats were fasted on the 3rd day for 12 hours and their blood glucose levels were determined using Finetest glucometer and its corresponding strips. The twenty rats exhibited glucose level above 200 mg/dl.

Extraction of Plant Material

The leaves of *ocimum gratissimum* and *gongronema latifolium* were purchased from a local market in Elele, Rivers State. The plants were taxonomically identified and authenticated in a herbarium of Pharmacognosy Department, Madonna University, Elele. The fresh leaves were washed and sundried for seven days. The dried leaves were grounded into fine powder and packed separately. About 200 g of the fine powder of the two leaves each was separately extracted with 1000 ml of ethanol by cold maceration for 48 hours and filtered. The preparation was filtered using Whatman filter paper (No 1). The filtrate was then concentrated to dryness at 35°C in an electric oven (gallenkamp) for 24 hours to obtain about 100 g of the ethanol extract. The extracts were separately kept in the refrigerator (-4°C) until ready for use.

Treatment Protocol

As previously described,¹² the administration of extracts lasted for 21 days. Group I (Control): consists of 5 rats received rat chow plus tap water. Group II consists of 5 rats received rat chow plus tap water. Group III consists of 5 rats received 200 mg/kg of *ocimum gratissimum* extract twice daily plus tap water and rat chow. Group IV consists of 5 rats received 200 mg/kg of *gongronema latifolium* plus tap water and rat chow. Group V consists of 5 rats received 200 mg/kg of *ocimum gratissimum* and 200 mg/kg of *gongronema latifolium* plus tap water and rat chow.

Sample Collection, Hormonal and Sperm Analysis

The rats were sacrificed under chloroform anesthesia; 5 ml of blood of blood was collected via cardiac puncture and put in a well labeled EDTA bottles for hormonal assay for testosterone, LH and FSH whereas right testes with caudal

epididymis were excised and subjected to sperm analysis.

Assay for FSH, LH and Testosterone

Blood serum was introduced into microplate well for each sample to be measured. An enzyme-linked conjugate for FSH with blue cover was added to all and rocked for 10 seconds and thereafter incubated for 1 hour at room temperature. The plate was then washed to remove all unbound materials. After washing, excess fluids were taped off using dry towel paper. Then colour was developed by adding colour reagent to determine the bound hormone. Quantitative test result was obtained by measuring the absorbance. The colour intensity was checked by taking the ELISA reader which is attached to spectrophotometer which the read the absorbance. LH assay follows similar with that of FSH but the slight difference is that the microplate well for LH is specifically coated with the antibody against LH and the enzyme-linked conjugate for LH is different with yellow cap.

Testosterone assay: blood serum was introduced into microplate well following the same procedure for FSH for each sample to be measured except an enzyme-linked conjugate for testosterone was added.

Sperm Count Procedure

The caudal epididymis was separated from the testes and lacerated to collect the semen. Nineteen drops of semen diluent (1:20 dilution of semen diluent) was introduced into a test tube and a drop of semen was then added. The test tube was not shook vigorously in order to avoid cutting-off the head, mid-piece or tail. One drop of the semen suspension was charged into Neubauer counting chamber and the sperms were counted in ten random fields. Counts for the sperm were averaged and expressed as the number of sperm per cauda epididymis.

Sperm Motility Procedure

A drop of well mixed liquefied semen was placed on a glass slide and covered with a cover slide. The sperm was focused using x10 objective. The condenser was closed sufficiently to give good contrast ensuring spread of spermatozoa. Using x40 objectives, several fields were examined to

access motility. The number of motile and non-motile sperms was counted. A thin film of the semen was prepared and a drop of normal saline was added into a well cleansed grease free slide. A spreader was placed few distances from the drop. Then a contact was made with the spreader in a drop. The spreader was pushed forward for even distribution of the film to obtain head, mid-piece and tail. Alcohol was then used to fix the semen sample on the slide to avoid washing away of the semen during staining. The number of motile sperm was then expressed as the percentage of the total sperm.

Sperm Viability Procedure

A viability study (percentage of live spermatozoa) was done using eosin stain. A drop of semen was mixed with two drops of 0.5 % eosin solution on a glass slide focusing the specimen with x10 objective, it was counted using x40 objective and an average value for each was recorded in percentage. The motile (viable) sperm cells were unstained while the non-motile (non-viable) sperm cells absorbed the stain.

Sperm Morphology Procedure

A thin film of semen was prepared using a microscope glass slide to obtain head, mid-piece and tail. The glass slide was then flooded with 75 % alcohol and then left to air-dry for ten minutes. It was afterwards rinsed under a low running tap water and allowed to air-dry as well. The glass slide was taken to the staining rack and flooded with 1:20 dilution of carbon fuchsin for about three minutes. The slide was however rinsed under a low running tap water. Notwithstanding, the glass slide was also counter-stained using methylene blue and left to stand for five minutes and then rinsed under low running tap water and allowed to air-dry. The slide was taken to x100 objective oil immersion to observe the head defect and mid-piece defect (such as microcephalus, detached head, flattened head, doubled head and bent neck), and tail defect in percentage (such as coiled tail, bent tail and doubled tail) was determined.

Statistics

The data obtained was analyzed using the Statistical Package for Social Sciences (SPSS

version 18.0 for windows). Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $P < 0.05$.

RESULTS

Table 1 showed the blood glucose level before and after administration of alloxan induced diabetes mellitus. Table 2 showed that blood glucose level of groups III, IV and V was significantly reduced ($P < 0.05$) at the 3rd week of experiment compared to group II. There was significant difference ($P < 0.05$) in the blood glucose at the 3rd week between group I compared to group III, IV and V respectively. The testosterone levels (table 3) showed statistically significant decrease ($P < 0.05$) in groups IV, V compared to group I. There was no statistically significant difference ($P > 0.05$) between II compared to groups IV and V. There was also no statistically significant difference ($P >$

0.05) between group III compared to group I. FSH and LH levels showed statistically significant decrease ($P < 0.05$) in groups III, IV and V compared to group I. There was also statistically significant difference ($P < 0.05$) between group II compared to groups III, IV and V. Results did not show (table 4) statistically significant difference ($P > 0.05$) in abnormal cells between group I compared with groups III, IV and V. There was statistically significant difference ($P < 0.05$) in normal cells between group I compared to groups III, IV and V. Sperm count and motility were significantly decreased ($P < 0.05$) in groups III, IV and V compared to group I. Sperm viability was significantly reduced ($P < 0.05$) in groups IV and V compared to group I. However, there was no statistically significant difference ($P > 0.05$) between group III and group I.

Table 1:

Blood glucose level before and after administration of diabetes mellitus

Groups	Blood glucose before induction of diabetes (mg/dl)	Blood glucose after induction of diabetes (mg/dl)
I Control	77.25 ± 8.70	77.25 ± 8.70
II Diabetic Control	63.50 ± 4.09	245.50 ± 20.99
III 200mg/kg O.G.	78.75 ± 6.66	308.75 ± 53.07
IV 200mg/kg G.L.	86.25 ± 2.56	290.75 ± 43.67
V 400mg/kg of O.G + G. L	78.25 ± 3.61	382.00 ± 27.36

Table 2:

Blood glucose level at different weeks and at the end of experiment after treatment with *ocimum gratissimum* (O. G) and *gongronema latifolium* (G. L)

Treatment groups	Blood glucose level after first week of treatment (mg/dl)	Blood glucose level after second week of treatment (mg/dl)	Blood glucose level at the third week of experiment (mg/dl)
I Control	69.50 ± 4.21	79.25 ± 2.29	72.50 ± 2.02
II Diabetic control	262.25 ± 20.30 ^a	266.75 ± 20.37 ^a	284.00 ± 32.88 ^a
III 200mg/kg O.G.	237.50 ± 34.96 ^a	192.75 ± 54.29 ^{ab}	128.75 ± 28.81 ^{ab}
IV 200mg/kg G.L.	250.25 ± 41.96 ^a	142.75 ± 23.48 ^{ab}	96.75 ± 7.44 ^{ab}
V 400mg/kg of O.G+ G.L	283.50 ± 40.47 ^a	141.88 ± 45.23 ^{ab}	107.25 ± 4.96 ^{ab}

Data represented as mean + SEM; (^a) $P < 0.05$ significant difference between control (^b) $P < 0.05$ significant difference between diabetic control

Table 3:

Synergistic effects of *gongronema latifolium* and *ocimum gratissimum* on testosterone, FSH and LH

Treatment groups	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)
I Control	2.60 ± 0.20	12.77 ± 1.58	0.60 ± 0.12
II Diabetic control	0.52 ± 0.15 ^a	3.60 ± 0.15 ^a	0.11 ± 0.01 ^a
III 200mg/kg O.G.	2.47 ± 0.18 ^b	9.77 ± 0.03 ^{ab}	0.37 ± 0.03 ^{ab}
IV 200mg/kg G.L.	1.60 ± 0.12 ^{ab}	9.17 ± 0.09 ^{ab}	0.37 ± 0.09 ^{ab}
V 400mg/kg of O.G+ G.L	1.80 ± 0.12 ^{ab}	9.60 ± 0.12 ^{ab}	0.33 ± 0.33 ^{ab}

Data represented as mean + SEM; (^a) P < 0.05 significant difference between control (^b) P < 0.05 significant difference between diabetic control

Table 4:

Synergistic effects of *gongronema latifolium* and *ocimum gratissimum* on sperm morphology, sperm count, sperm motility and viability.

Treatment groups	Sperm morphology (%)		Sperm count (x10 ⁶ /ml)	Sperm motility (%)	Sperm viability (%)
	Abnormal cells	Normal Cells			
I Control	30.67 ± 0.67	69.33 ± 0.67	180.00 ± 6.36	72.67 ± 0.88	81.67 ± 0.88
II Diabetic Control	65.00 ± 0.50 ^a	35.00 ± 0.50 ^a	120.50 ± 1.20 ^a	65.71 ± 0.52 ^a	65.20 ± 0.20 ^a
III 200mg/kg of O. G	35.00 ± 1.61 ^b	65.00 ± 1.61 ^{ab}	144.33 ± 4.84 ^{ab}	69.00 ± 0.58 ^a	81.33 ± 0.68 ^b
IV 200mg/kg of G. L	42.25 ± 1.64 ^b	57.75 ± 1.64 ^{ab}	131.00 ± 2.65 ^a	68.33 ± 1.20 ^a	75.00 ± 0.58 ^{ab}
V 400mg/kg of O. G. and G. L.	49.25 ± 0.66 ^b	50.75 ± 0.66 ^{ab}	122.00 ± 0.58 ^a	67.33 ± 1.20 ^a	72.00 ± 1.16 ^a

Data represented as mean + SEM; (^a) P < 0.05 significant difference between control (^b) P < 0.05 significant difference between diabetic control

DISCUSSION

Results (Table 2) showed that polyherbal extracts significantly reduced blood glucose levels in a similar pattern as previously described.¹² The significant reduction (table 3) in testosterone, FSH and LH following treatment with polyherbal extracts from *ocimum gratissimum* and *gongronema latifolium* observed in this study could depend on the actions of individual extracts. The extract from *ocimum gratissimum* extract (200 mg/kg) caused statistically significant increase in testosterone level with a decrease in both LH and FSH, whereas extract from *gongronema latifolium* extract (200 mg/kg)

caused statistically significant decrease in testosterone, LH and FSH levels. LH is tropic to the Leydig cells and stimulates the secretion of testosterone, which in turn feeds back to inhibit LH secretion.²⁶ In diabetic animals, there are both pituitary and testicular abnormalities²⁷ in the manner that resulted in the impairment of pituitary responsiveness to gonadotropin releasing hormone (GnRH). Consequently, this impairment of pituitary responsiveness to GnRH resulted in the reduction of LH pulse amplitude in streptozotocin-induced diabetic rats.²⁸ In the present study, both extracts decreased LH possibly by potentiating

pituitary unresponsiveness to GnRH thereby causing a decrease in testosterone.

Our previous work had shown that synergy of *vernonia amygdalina* and *ocimum gratissimum* increased LH, FSH and testosterone in streptozotocin induced diabetic rats,²⁹ and the mechanism of action may be dependent on pancreatic regeneration and availability of insulin. Bucholtz et al.³⁰ have reported that insulin and/or insulin-dependent changes in glucose availability modulate LH (GnRH) pulse frequency, an effect that is potentiated by, but not dependent upon gonadal steroids. Because these extracts have been implicated in the reduction of blood glucose in diabetic animals^{7-8,32,36}; therefore, we suggest that the polyherbal treatment may not have potentiated insulin release thereby causing a decrease in LH level. This suggestion seemed credible because in the result (table 2), polyherbal effect did not significantly reduced blood glucose level. This could imply that polyherbal extract may not have stimulated insulin release. Conversely, *gongronema latifolium* extract (200 mg/kg) caused a significant decrease in blood glucose without a concomitant increase in LH suggesting that the mechanism of action may be insulin independent. Polyherbal effect may otherwise act on the hypothalamic-pituitary axis to cause in a decrease in LH. As stated ab ovo, testicular function is controlled by the LH, therefore, the decrease in testosterone may be dependent on the availability of LH that triggers the Leydig cells to produce testosterone.

FSH is tropic to the Sertoli cells alongside with androgens maintain the gametogenic function of the testes.²⁶ Results (table 3) showed a decrease in FSH level. Several studies have reported that lack of insulin in streptozotocin induced diabetic rats did not impair spermatogenesis via a direct effect on the epithelium of seminiferous tubules but alteration in the serum FSH levels.³¹⁻³² These reductions of FSH and testosterone in the present study were expected to distort the gametogenic function of the testes which could present as dysfunction of sperm parameters.

Thus, we studied sperm parameters in order to determine gametogenic function of the testes. Indeed, results (table 4) showed increased abnormal sperm cells, decreased normal sperm cells, decreased sperm count, motility, and

viability after treatment with polyherbal extracts. These decreases in the sperm parameters may have resulted from the reduction in FSH and testosterone levels suggesting that gametogenic function could have been disrupted. The significant reduction in sperm parameters (table 4) following treatment with 200 mg/kg of *ocimum gratissimum* agreed with previous reports in male rats.³³⁻³⁴ Although controversial view exist between the effect of bi-herbal action of *gongronema latifolium* and *ocimum gratissimum*³⁵ and the results obtained in the present study. It was pointed out that bi-herbal extracts effects on testosterone and sperm parameters were dose dependent as increased dose concentration of bi-herbal extracts increased testosterone and decreased sperm parameters whereas decreased dose concentration decreased testosterone and increased sperm parameters. Even though establishing the mechanism could not be explained whether the polyherbal extracts effects were insulin dependent or/and direct effect on the hypothalamic-pituitary axis.

CONCLUSION

The present study conclude that polyherbal extracts of *ocimum gratissimum* (200 mg/kg) and *gongronema latifolium* (200 mg/kg) caused a decrease in sperm parameters due to their reducing effects on pituitary gonadotropins and testosterone levels.

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