

Case Study

EFFECTS OF *OCIMUM GRATISSIMUM* AND *GONGRONEMA LATIFOLIUM* ON FERTILITY PARAMETERS: A CASE FOR BI-HERBAL FORMULATIONS

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Abstract

The effect of aqueous extracts of *O.gratissimum*, *G.latifolium* and a bi-herbal formulation of both herbs on epididymal sperm characteristics and testosterone levels in male albino rats were investigated. After 6 weeks of treatment at various doses, *Ocimum gratissimum* and *Gongronema latifolium* showed not only an appreciable dose dependent morphological changes but also decreases in sperm motility, sperm count, and sperm viability at $p<0.05$ when compared against the control. There was a moderate change in the groups treated with the bi-herbal formulation at similar doses when compared with the control as against the mono-extracts which had a more pronounced negative effects. The testosterone levels were appreciably similar across all groups. These findings suggest that an extended treatment of *Ocimum gratissimum* or *Gongronema latifolium* on rats can negatively affect the sperm characteristics of the animals. The findings showed the possibility of protection on seminal parameters by an herbal formulation of both herbs.

Keywords: *Ocimum gratissimum*, *Gongronema latifolium*, Bi-herbal formulation, sperm characteristics, Testosterone

INTRODUCTION

Ocimum gratissimum is of the family lamiaceae, commonly known as 'scent leaf' is a perennial plant mostly used as spice. The Scent leaf has been reported to be rich in plant chemicals. Phytochemical evaluation of the plant has shown that it is rich in alkaloids, tannins, phytates, flavonoids and oligosaccharides (Ijeh *et al*, 2004). In the trado-medical practice, scent leaf is extensively used throughout West Africa as anti-malarial, mosquito repellent and anti-convulsant. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves has been shown to possess antiseptic, antibacterial, and antifungal activities (Ezekwesili *et al*, 2004). *O. gratissimum* has proved to be an effective anti-microbial (Orafidiya *et al*, 2001) and hypoglycemic and hepatoprotective agent (Egesie *et al.*, 2006). Njoku *et al* (2011) gave the LD₅₀ of the herb as 2450mg/kg body weight.

Gongronema latifolium is of the family Asclepiadaceae, locally known as 'utazi' in the Igbo dialect of eastern Nigeria, it is a tropical rainforest plant primarily used as spice and vegetable in the traditional folk practice (Ugochukwu and Babady, 2002; Ugochukwu *et al*, 2003). Phytochemical evaluation of the plant has shown that it is rich in essential oils, saponins and pregnanes amongst others (Schneider *et al*, 1993; Morebise and Fafunso, 1998). Alkaloids, tannins, phytates, flavonoids and oligosaccharides were identified in phytochemical analysis of the plant (Morebise *et al*, 2002). Traditionally, the southern part of Nigeria uses this herb in the treatment of malaria, diabetes and hypertension as well as a laxative. (Nwanjo *et al*, 2006) reported the lipid per oxidative activity of the plant. The study suggested that the plant possess antioxidant properties and is able to mop up

reactive oxygen species in the system. Effiong et al (2012) gave the LD₅₀ of the herb as 5000mg/kg by oral route and 1500mg/kg intraperitoneally.

The use of herbs in the treatment of ailments in Africa has been an age long practice. Man's continuous reliance on herbs for therapeutic and nutritional benefits cannot be overemphasized (Ezeonwu, 2011) and the use of plants for variety of purposes predates human history and forms the origin of what is known as modern medicine. Plant materials have remained central to trado-medical practices and have become useful source of new drugs. It is no longer news that a lot of plants have played effective roles in the production of drugs. Although, orthodox medical practice is generally acceptable, alternative healthcare is still relied on all over the world (Ozulua and Alonge, 2008). Medicinal plants are important sources of drugs for the treatment of several ailments. The plants can be used alone or combined with other plants (Avwioro, 2010). Several plants have been associated with nutritional and therapeutic benefits. *Ocimum gratissimum* and *Gongronema latifolium* are among such plants proven to have both nutritional and medicinal properties. Both plants traditionally are normal constituents of food taken predominantly in the eastern part of Nigeria and on several occasions used for the treatment of diseases. Combination of herbs to achieve a desired aim has been practiced along different cultures and in trado-medical practice. This crude form of combinatorial chemistry is not new and the practioneers have several motives to combine herbs. Amongst the reasons for such combination is the possibility of a synergic activity of both herbs, the moderation of effects/activity of one herb using another by reducing the toxicity and undesired effects, enhancing the activity or as a supplement.

In the absence of preliminary information on the effect of these herbs, individually and combined on the fertility of male rats, this research is undertaken to determine if there are such negative or positive effects on certain parameters such as sperm count, sperm viability, sperm motility and serum testosterone levels.

MATERIALS AND METHODS

Animals

Thirty-five male albino rats (200-250g) privately reared and nurtured were used in the study. They were weighed before commencement of treatment and housed in wooden cages with metal nettings. Throughout the period of the experiment the animals were fed normal feed and water.

Preparation of the extract

Fresh *Ocimum gratissimum* leaves and *Gongronema latifolium* leaves were collected from the field. Both plant materials were shade dried for 12days before powdering using manual grinder and then sieved with cheese cloth. Equal amount of both plants (250g each plant) was soaked in 1000ml of distilled water in a beaker, the mixture was shaken and allowed to stand for 24hours, boiled for 5minutes before filtering with a cheese cloth. The filtrate was decanted and then evaporated. Appropriate weights of the residue were prepared in distilled water to obtain the various concentrations used for the experiment. Each herb was also prepared separately following similar pattern described except the fact there was no combination of both herbs.

Experimental design

Seven experimental groups of five albino rats were used in the experiment. Each group was treated and fed as follows for two weeks

Group A: served as the control and received nothing but normal feed

Group B: 300mg/kg body weight of *O.gratissimum* extract

Group C: 500mg/kg body weight of *O.gratissimum* extract

Group D: 300mg/kg body weight of *G.latifolium* extract

Group E: 500mg/kg body weight of *G.latifolium* extract

Group F: 300mg/kg body weight of bi-herbal formulation

Group G: 500mg/kg body weight of bi-herbal formulation

Body and organ weights

Initial and final body weights of the animals were recorded. At the end of the treatment period, the animals were sacrificed 18hours after the last dosage. The testis were removed and weighed, the mean value was recorded.

Hormonal assay

Twelve to eighteen (12-18) hours after the last doses were administered, the animals were sacrificed and whole blood was collected by cardiac puncture. The blood sample was spun at 2500rpm for 10min using a centrifuge. Serum sample were assayed for testosterone using enzyme linked immunoassay technique.

Sperm characteristics

The seminal fluid was collected by macerating the reweighed and dissected testis in normal saline; after centrifuging at 12000rpm for 5minutes, the supernatant were assayed for sperm qualities and characteristics were assayed as described by Cheesbrough,(1984).

Statistical analysis

All values are expressed as Mean \pm SEM (Standard Error of Mean). The data was analyzed using Student T-test at $P < 0.05$ level of significance.

RESULTS

Body and organ weight changes

Table 1 show the body and organ weight of the rats. There was appreciable body weight gain across the entire groups. There was significant increase in the weight of the testis of animals treated with the extracts when compared with the control. The increased body weight can be possibly due to extended study time.

Table1. The Body and organ weight of the animals

	Mean initial body weight (g)	Mean final body weight (g)	Mean body weight change (g)	Mean testis weight (g)
GROUP A(control)	228.16 \pm 7.58	235.22 \pm 5.22	7.06	1.03 \pm 0.02
GROUP B	212.16 \pm 8.46	221.04 \pm 8.63	8.88	1.63 \pm 0.01*
GROUP C	227.72 \pm 6.73	234.28 \pm 5.82	6.56	1.91 \pm 0.14*
GROUP D	231.43 \pm 3.52	238.41 \pm 3.29	6.98	1.51 \pm 0.09*
GROUP E	214.18 \pm 6.41	221.19 \pm 4.30	7.01	1.88 \pm 0.11*
GROUP F	233.85 \pm 8.80	238.90 \pm 6.44	5.05	0.99 \pm 0.07
GROUP G	216.41 \pm 4.34	222.77 \pm 7.09	6.06	1.19 \pm 0.03*

All values are expressed in Mean + SEM (Standard Error of Mean), $P < 0.05$ * = significantly different from group A (control group)

Sperm motility

The sperm mobility was significantly reduced ($p < 0.05$) in rats treated with the 300mg/kg b.w and 500mg/kg bow of *O. gratissimum* and *Gongronema latifolium* extract when compared to that of the control (Table 2).

Table2. sperm motility, sperm count, sperm viability and testosterone *($p < 0.05$) significantly different from the control

GROUP	TREATMENT	SPERM MOTILITY (%)	SPERM COUNT (%)	SPERM VIABILITY (%)	TESTOSTERONE LEVEL (mg/ml)
A	Normal Control	73 \pm 9.11	77 \pm 4.56	91 \pm 7.53	1.62 \pm 0.14
B	300mg/kg O.g	50 \pm 5.72*	43 \pm 7.54*	55 \pm 9.82*	1.58 \pm 0.07
C	500mg/kg O.g	46 \pm 12.52*	38 \pm 9.28*	49 \pm 6.63*	1.70 \pm 0.61
D	300mg/kg G.l	51 \pm 4.04*	47 \pm 6.31*	60 \pm 11.75*	1.59 \pm 0.35
E	500mg/kg G.l	43 \pm 9.35*	41 \pm 7.60*	56 \pm 9.19*	1.61 \pm 0.19
F	300mg/kg Bi	68 \pm 14.23	66 \pm 3.71*	79 \pm 8.16	1.63 \pm 0.52
G	500mg/kg Bi	59 \pm 6.77*	63 \pm 6.67*	62 \pm 13.44*	1.73 \pm 0.18

Sperm counts

There was a visible reduction ($p < 0.05$) in the sperm counts of groups treated with *O. gratissimum* and *G. latifolium* when compared with the control (Table 2).

Sperm viability

The sperm viability of all treated groups shows a significant deviation ($p < 0.05$) from what was obtained in the control (Table 2).

Serum testosterone level

The serum testosterone level of both the treated rats and that of the control showed no significant differences.

DISCUSSION

The use of herbs in the management of ailments has been a regular practice in Africa. These herbs usually lead to the expected therapeutic results; however, the aftermath of such treatments are usually not noticed or ignored. *Ocimum gratissimum* and *Gongronema latifolium* are plants widely praised for their nutritional and therapeutic benefits. They somehow in one instance or another form part of weekly menu of most south eastern Nigerians. Some plants have been said to possess antifertility properties due to the nature of their phytochemical constituents. Such plants with antifertility properties usually result in reducing the sperm counts, motility, viability and visible alteration alters the morphology of the sperms; such impairment of male fertility has been reported in herbs that have anti-malarial activity, Oze et al (2007) and Raji et al (2003) reported antifertility effects of *Alstoniaboonei* and *Azardidichta indica* respectively while Ezeonwu (2011) gave such reports about *phyllanthus niruri*. *O. gratissimum* and *G. latifolium* have been associated with a high anti-malarial property, rich in alkaloids, flavonoids and glycosides: plant chemicals associated with anti-malarial and anti-fertility potentials. The findings of the present study showed that a prolonged treatment of albino rats with aqueous extract of *O. gratissimum* or *Gongronema latifolium* could significantly alter the fertility potential of male rats. From the tables above, *O. gratissimum* aqueous extract negatively impacted on the entire parameters studied just as *G. latifolium*.

An increase in the weight of an organ can be a pointer to toxicity. Simons et al (1995) noted that increase or decrease in weight of an organ after the administration of a chemical agent is an indicator of a toxic effect of such agent. The increases recorded in the body weight of the animals can be due to the prolonged time of study. The significant decreases in sperm viability, sperm motility and sperm counts in groups treated with *O. gratissimum* and *G. latifolium* show the antifertility properties of both plants in male rats. Such extreme effects were not recorded in animals treated with a bi-herbal extract of both plants. It would be noted that though there were effects, but the possibility of reduced toxicity cannot be ruled out. A bi-herbal aqueous extract of both plants showed a possible moderation of the effects of *O. gratissimum* by *G. latifolium* in fertility parameters.

Any compound capable of affecting fertility status of animals probably has the potential to penetrate the blood-testis barriers. Baddessarini (1980) reported that effect of chemical agents on sperm composition is attributed to their ability to penetrate this barrier. The decrease in sperm qualities points to reduction in the circulating androgen level. Any chemical agent that can affect reproductive activity will as well affect the quality and quantity of the sperm, such decrease can be attributed anti-androgenic property of the extract. This study points to the dangers of extended administration of both plants. The active plant chemical and mode action involved in these effects was not identified at the time of the study, however it's suggestive that they possibly follows same format as most anti-malarial plants implicated in such cases.

CONCLUSION

Summarily, these observations show that the aqueous extract of *O. gratissimum* and *G. latifolium* may have antifertility effects in albino rats under extended treatment.

References

- Avwioro OG(2010). Effectiveness of some medicinal plant decoction in the treatment of malaria in Nigeria. *Annals of Biological Research*, 1 (2):230-237
- Baddessarini RJ(1980). In drugs and treatment of psychiatric disorders. The pharmacological basis of therapeutics Ed. By Goodman and Gilman. Macmillan Pub.Co. Inc. Pp. 301-417.
- Cheesbrough M(1984). Microbiology collection, transport and laboratory examination of Semen. *Medical Laboratory Manual for tropical countries*. 2: 184-188
- Effiong GS, Udoh IE, Mbagwu HO, Ekpe IP, Asuquo EI, Atangwho IJ, Ebong IP(2012). *Acute and chronic toxicity studies of Gongronema latifolium leaf extract*. *International Res. J. Biochem. Bioinformatics*. 2 (7):155-161
- Egesie UG, Adelaiye AB, Ibu JO, Egesie OJ(2006). Safety and hypoglycemic properties of aqueous leaf extract of *Ocimum gratissimum* in STZ-induced diabetic animals. *Nig. J. Physiological Sci*. 21(1-2): 31-35

- Ezekwesili, CN, Obiorah KA, Ugwu OP(2004). Evaluation of anti-diarrheal effect of *Ocimum gratissimum* crude extract on albino rats. *Biokemistri* 16 (2):122-131
- Ezeonwu VU(2011). *Antifertility* Activity of Aqueous Extract of *Phyllanthus niruri* in Male Albino Rats. *The Internet J. Lab. Med.* (4): 2
- Ijeh II, Njoku OU, Ekenze EC(2004). Medicinal evaluation of *Xylopias aethiopia* and *Ocimum gratissimum*. *J. medicinal aromatic sci.* 26(1): 19-21
- Morebise O, Fafunso MA, Makinde JM, Olajide OA, Awe EO(2002). Anti-inflammatory property of the leaves of *Gongronema latifolium*. *Phytother. Res.* 16:75-77
- Morebise O, Fafunso MA(1998). Antimicrobial and phyto-toxic activities of saponins extracts from two Nigerian edible medicinal plants. *Biokemistri.* 8 (2): 69-77
- Njoku OU, Joshua PE, Agu CV, Dim NC(2011). Antioxidant properties of *Ocimum gratissimum*. *New York Sci. J.* 4(5): 98-103
- Nwanjo HU(2006). Lipid profile of rats treated with *Gongronema latifolium* in Albino rats. *J. Biosci.* 17: 23-27
- Orafidiya LO, Oyedele AO, Shittu AO, Elujoba AA(2001). The Formulation of an effective topical antitubercular product containing *Ocimum gratissimum* leaf essential oil. *Int. J. Pharmacology.* 244: 177-184
- Oze G, Nwanjo H, Oze R, Akubugwo E, Orisakwe E, AKA P(2007). Reproductive Impairment Associated with the Ethanolic Extract of *Alstonia Boonei* (De wild) stems bark in male rats. *The Int. J. 3rd World Med.* 6: 1.
- Ozulua RI, Alonge P(2008). Aqueous and Ethanol leaf extracts of *Phlogothima thoningii* (Schum) increase in locomotor activity in Sprague Dawley rats. *Afr. J. Biotech.* 7(2):73 – 76.
- Raji Y, Udoh US, Mewoyeka OO, Ononye FC, Bolarinwa AF (2003). Implication of reproductive endocrine malfunction in male antifertility efficacy *Azadirachta indica* extract in rats. *Afri. J. Med. Sci.* 32: 159-165.
- Schneider BL, Sherman PM (2008). *Pediatric Gastrointestinal Disease*. Connecticut: PMPH-USA. Pp.751
- Simons IE, Berman E(1995). *Environ. Health Perspective.* 103: 67-71.
- Ugochukwu NH, Babady NE, Cobourne M, Gasset SR(2003). The effect of *Gongronema latifolium* leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. *J. Biosci.* 28: 1-5
- Ugochukwu NH, Babady NE(2002). Anti-oxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. *Fitoterapia.* 73: 7-8.