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OCULAR EFFECTS OF WATER EXTRACT OF *OCIMUM GRATISSIMUM* IN RABBITS

S. A. Igwe^{*1}, M. N. Ezenwaeze¹, S. O. Nweze² and D. J. Ogbonna³

Department of Pharmacology and Therapeutics¹, ESUT College of Medicine, Parklane, GRA, Enugu.

Department of Obstetrics and Gynaecology², ESUT College of Medicine/Teaching Hospital, Parklane, GRA, Enugu.

School of Optometry³, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

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Correspondence to Author:

Mr. M. N. Ezenwaeze

Department of Pharmacology and Therapeutics, ESUT College of Medicine, Parklane, GRA, Enugu.

E-mail: malachynwaeze@gmail.com

ABSTRACT: The water extract of *Ocimum gratissimum*, L Lamiaceae, used in traditional medicine and local food spice, was evaluated for its ocular effect using three ocular parameters, pupil diameter (PD), corneal reflex (CR) and pupillary light reflex (PLR). The phytochemical tests were performed on the pulverized leaves, which were used to ascertain the distribution pattern of some possible bioactive agents responsible for the presumed activity of *Ocimum gratissimum*. It was also tested for the presence of the following: glycosides, carbohydrates, alkaloids, saponins, flavonoids, tannins and proteins, and the results show the presence of fats and oil, reducing sugar and terpenes > tannins > cyanogenic glycosides > alkaloid and steroidal aglycone, while there were no traces of anthraquinones and flavonoids. The *Ocimum gratissimum* water extracts caused dilatation of the pupil (3.13%), a mydriatic effect, while the corneal reflex was not abolished, showing a lack of local anesthetic property. However, the pupillary light reflex was present throughout the study.

INTRODUCTION: The use of plant materials as spices, condiments, and medicinal purposes dates back to the history of mankind¹. The exploitation of plants for medicinal purposes has gained more acceptance in many countries as most national governments have established traditional medicine regulatory councils under the supervision of their various ministries of Science and Technology or Health to modulate the practice². *Ocimum*, commonly known as Tulsi, is a huge genus within the family Lamiaceae, comprising about 64 species of annual to perennial aromatic medicinal herbs with a long history of traditional uses³.

Ocimum gratissimum belongs to the plants known as spices and the wide use of this plant in the preparation of pig (pork) has led to the denomination of “pig spice”⁴. The essential oil has shown antioxidant activity⁵. *Ocimum gratissimum* is a herbaceous shrub that is indigenous to the tropics and grows widely in West Africa, particularly in the Savanah and the coastal areas⁶. It is a multi-branched plant with dark green leaves which are rich in essential oil⁷. When heated, the leaves give off a characteristic aroma. The leaves have been used as a general tonic and anti-diarrhoea agent in traditional medicine.

It has antibacterial activity against *E. coli* infection,⁸ and is also used to treat conjunctivitis by instilling quizzed fluid into the eyes⁹. The leaf oil, when mixed with alcohol, is applied as a lotion for skin infections⁴ and taken internally for bronchitis while the dried leaves are grounded and snuffed to alleviate headache, fever, and other ailments⁶.

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Considering the fact that *Ocimum gratissimum* is used in most dishes and foods to achieve a variety of purposes, it becomes expedient to investigate its effects on the ocular system, namely, pupil diameter, corneal reflex, and pupillary light reflex in our continued efforts to study the effects of local food spices in the ocular system and adnexa.

In our previous studies involving food spices, and edible materials consumed by people without prescription or restrictions, the ocular system and adnexa have been affected by these ethnobotanical substances. *Aframomum meleguata* (alligator pepper) caused a reduction in amplitude of accommodation (AA), increase in near point of convergence (NPC), and stimulated lacrimation¹⁰. *Xylopiya aethiopica* (African guinea pepper) caused NPC regression, an increase in AA, and a fall in intraocular pressure¹¹. Also, *Garcinia cola* constricted the pupil (miosis), improved the NPC, increased the AA, and reduced the intraocular pressure,¹² while *Cola nitida* produced an improvement in the NPC and AA¹³.

Elaeis guineensis sap (palm wine) produced a miotic effect, regression of NPC, increased the VA (visual acuity) at far, progression of AA, fall in (AC/A) accommodation convergence accommodation ratio, and a reduction in intraocular pressure¹⁴. Human eye is covered by a thin and transparent layer of tissue called the cornea, which contains the highest number of nerves in the whole body¹⁵. The cornea is a smooth, clear structure at the front of the eye¹⁶. It functions to shield the eye from foreign substances and help control visual focus^{17, 118}. Cornea reflex is affected not only by some herbal drugs but also conditions like trigeminal neuralgia-like symptoms¹⁹. Pupillary light reflex (PLR) is an involuntary response where the pupil size changes with luminance²⁰. PLR can be affected by many conditions, autism spectrum disorder inclusive and this can be detected with the aid of pupillography and eye-tracking devices^{21, 22}.

MATERIALS AND METHODS: A total of 10 rabbits, weighing 3-4 kg (3.5 + 0.9) body weight, were procured from a private farm in Olokoro, near Umuahia, Abia State, Nigeria, and quarantined for 14 days before use. The animals were fed with standard commercial feeds (Pfizer Livestock feeds), grasses and water *ad libitum*.

The rabbits were weighed daily to monitor their weights, and each had good ocular health.

Extraction of *Ocimum gratissimum*: *Ocimum gratissimum* leaves were obtained from a source in Olokoro, near Umuahia, Abia State, Nigeria. The leaves were authenticated by a Taxonomist, Dr (Mrs) Bassey of the Department of Botany, Abia State University, Uturu, where a voucher specimen was deposited in the herbarium. The leaves were washed to remove dust and impurities and were left to dry in the laboratory. The laboratory-dried leaves of *Ocimum gratissimum* were weighed, 100g, and pulverized using a blender. The blended dried leaves (powdered) were then dissolved in a liter of distilled water and allowed to stand for 48 h. The macerated mixture was separated using a separating funnel and filter paper to remove the residue. The filtrate was concentrated using a rotary evaporator at 40 °C and dried in the oven. The residue was weighed, and the yield was 1.1g.

Phytochemical Analysis: The methods described by Harbone were used to test for the presence of the active ingredients in the extract²³. The phytochemicals tested for were tannins, flavonoids, terpenes, saponins, carbohydrates, and cyanogenic glycosides.

Preparation of the Drug Solution: One gram of the yield was weighed and dissolved in 10 ml of distilled water to give a solution of 100 mg/ml as a stock solution, which was used for the study.

Ophthalmoscopy: The rabbits were further screened for ocular health using the ophthalmoscope. Any rabbit with the ocular disorder was discarded and unfit for the study. Each rabbit was used once.

Measurements:

A Pupil size: The experimental animals' pupil diameter (PD) was measured using the pupilometer to determine the initial value. The readings were taken at three different positions, and the mean was determined in mm.

Corneal Reflex: The corneal reflex was determined by flashing or shining light from a pen torch at a distance of 40 cm. The light was directed at the nose bridge of the rabbit, while the presence or absence of corneal reflex of the experimental eye

was viewed directly above the source of the light with the dominant eye. This procedure was repeated after the instillation of 2 drops of 10% solution of water extract of *Ocimum gratissimum*. Each experiment was performed three times on each rabbit's eye.

Pupillary Light Reflex: The direct pupillary light reflex method was used. The test was performed in a semi-darkened room. The ears of the rabbit were held firmly to restrict the movement of the head. The pupil was evenly and individually illuminated from an oblique direction using a pen torch at a distance of 10 cm. As the rabbit was illuminated, it was also observed for pupil reaction. The procedure was repeated after the instillation of 2 drops of 10% solution of water extract of *Ocimum gratissimum*. A period of 20 min was allowed after drug administration for proper penetration of the ocular

tissues. Each procedure was repeated three times for each rabbit's eye and recorded accordingly.

RESULTS: The results obtained from the phytochemical analysis of *Ocimum gratissimum* are presented in **Table 1**. The extract contains fats and oil in higher quantity (+++), followed by reducing sugars and terpenes (++) , while cyanogenic glycosides, saponins, alkaloids, steroidal aglycone, and certain trace materials were lowest (+).

Tannins, anthraquinones, and flavonoids were absent. On the pupil diameter, **Table 2** shows the changes obtained during the study. The mean initial pupil diameter (PD) was (9.6 + 0.4) mm, and at the end of the study, the mean pupillary diameter was (9.9 + 0.4) mm or 3.13% dilatation. However, both the pupillary light reflex and the corneal reflex remained active or present throughout the study.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *OCIMUM GRATISSIMUM* LEAF EXTRACT

Test substance	Concentrations	Remarks
Alkaloids	+	Present low concentration
Anthroquinones	-	Absent
Cyanogenic glycosides	+	Present low concentration
Fats and oil	+++	Present high concentration
Flavonoids	-	Absent
Reducing sugar	++	Present moderate concentration
Saponins	+	Present low concentration
Steroidal aglycone	+	Present low concentration
Tannins	+	Present low concentration
Terpenes	++	Present moderate concentration

TABLE 2: CHANGES IN PUPIL SIZE (PD) FOLLOWING *OCIMUM GRATISSIMUM* ADMINISTRATION

S. no.	Initial pupil size (mm)	5	10	15	20	25	30
1	9.2 ± 0.4	10.1 ± 0.2	12.4 ± 0.1	13.3 ± 0.3	13.3 ± 0.3	11.4 ± 0.4	9.2 ± 0.3
2	9.1 ± 0.3	10.2 ± 0.4	12.4 ± 0.3	13.3 ± 0.4	13.3 ± 0.3	11.4 ± 0.4	10.3 ± 0.2
3	11.2 ± 0.4	12.3 ± 0.5	13.4 ± 0.5	14.4 ± 0.4	14.4 ± 0.4	12.4 ± 0.4	11.4 ± 0.2
4	8.3 ± 0.2	9.4 ± 0.4	10.3 ± 0.3	11.4 ± 0.3	11.3 ± 0.2	9.4 ± 0.3	8.4 ± 0.2
5	10.2 ± 0.3	11.2 ± 0.5	12.4 ± 0.4	13.4 ± 0.4	13.4 ± 0.3	12.4 ± 0.2	10.3 ± 0.4
6	9.3 ± 0.3	10.4 ± 0.4	12.4 ± 0.3	13.4 ± 0.4	13.4 ± 0.3	11.4 ± 0.2	9.2 ± 0.4
7	10.2 ± 0.4	11.3 ± 0.4	12.4 ± 0.4	12.4 ± 0.3	11.2 ± 0.2	10.4 ± 0.3	10.3 ± 0.4
8	9.2 ± 0.4	11.3 ± 0.4	12.5 ± 0.4	12.4 ± 0.3	11.3 ± 0.3	10.3 ± 0.4	9.4 ± 0.4
9	11.2 ± 0.4	12.4 ± 0.4	14.4 ± 0.4	14.2 ± 0.5	13.3 ± 0.4	12.4 ± 0.4	11.2 ± 0.3
10	8.4 ± 0.2	9.4 ± 0.4	10.5 ± 0.4	11.4 ± 0.4	11.4 ± 0.4	10.2 ± 0.4	9.4 ± 0.3
Total	96.3	108	122.7	129.8	126.3	111.6	99.1
Mean	9.6 ± 0.4	10.8 ± 0.4	12.3 ± 0.4	13.0 ± 0.4	12.6 ± 0.4	11.2 ± 0.4	9.9 ± 0.4

DISCUSSION: *Ocimum gratissimum* is an innocuous edible herbaceous plant that is generally consumed without prescription or restriction. The study, which aimed at finding the effects of the plant extract on the ocular system, has demonstrated a potential for mydriasis. **Table 2** shows the effects of water extract of *Ocimum*

gratissimum on the pupil diameter (PD), causing pupillary dilatation or mydriasis. The onset of mydriasis in the rabbits was 5 min, and the time to maximum dilatation was 15 min, while the duration of mydriasis was 5 min and the recovery time was 10 min. This shows that the mydriatic effect of *Ocimum gratissimum* is short-leaved and easily

reversible; the 3.13% dilatation will be sufficient to examine the fundus. Corneal reflex is a protective mechanism for the eye resulting in automatic closing of the lids when the cornea is stimulated. This reflex action is mediated by the ophthalmic division of the 5th cranial nerve (sensory) and the 7th cranial nerve (motor) and may be used as a test of the integrity of these nerves¹⁴.

The absence of corneal reflex indicates deep coma or injury of one of the nerves carrying the reflex. On the other hand, the pupillary light reflex protects the retina from excessive illumination, damaging the photoreceptor; the pupillary light reflex involves an adjustment in pupil size that changes in the light level²⁴.

The mydriatic effects of *Ocimum gratissimum* were enough to make changes in light level hence the presence of the reflex throughout the duration of the study.

Though the pupil size in principle is controlled by both the sympathetic and the parasympathetic nervous system, the typical closure of the pupil after illumination (pupillary light reflex) is mediated by the parasympathetic innervations of the constrictor muscle of the pupil.

The presence of corneal reflex after the installation of *Ocimum gratissimum* extract shows the extract lacks local anesthetic property. The pupillary light reflex compensates for changes in illumination and is hence present throughout the duration of the study.

CONCLUSION: We conclude that *Ocimum gratissimum* has adequate potential for mydriasis but lacks the ability to abolish these reflexes; that is, it has no local anesthetic properties.

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