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## Evaluating the effect of an aqueous extract of *Pistia stratiotes* Linn (Araceae) on tear secretion and tear film stability in ICR mice

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### ABSTRACT

Management of allergic conjunctivitis with conventional anti-allergic drugs usually cause the discomfort of dry eyes. This study, therefore, sought to evaluate the effect of *Pistia stratiotes*, a herbal remedy with anti-allergic properties, on tear secretion and tear film stability. ICR mice were grouped and treated with 5 mg/kg cetirizine, 10 mg/kg prednisolone, 100 mg/kg leaf extract of *P. stratiotes* (LEPS), or 2 ml/kg normal saline *per os* for 7 consecutive days. With the phenol red thread test and the determination of fluorescein tear film break-up time, tear secretion and tear film stability before and after drug interventions were determined. Results recorded indicated that tear secretion and tear film stability declined significantly with cetirizine treatment ( $1.188 \pm 0.4369$  mm;  $P \leq 0.05$ , and  $2.688 \pm 0.6185$  s;  $P \leq 0.001$  respectively), while only tear secretion reduced significantly ( $P \leq 0.05$ ) in mice treated with prednisolone ( $0.9380 \pm 0.4422$  mm). Treatment with LEPS showed no significant effect ( $P > 0.05$ ) on tear secretion and tear film stability ( $0.7050 \pm 0.4236$  mm, and  $0.9790 \pm 0.6145$  s respectively); comparable to normal saline treatment. Per the findings, *Pistia stratiotes* does not affect tear secretion and tear film stability and hence is not likely to exhibit the adverse effect of dry eyes, as conventional anti-allergic drugs do, in the management of allergic conjunctivitis.

### INTRODUCTION

Drugs prescribed are of invaluable therapeutic benefit when associated adverse effects with its use are minimal. Conventional management of allergic conjunctivitis (AC) involves the use of antihistamines, mast cell stabilizers, steroids and decongestants (Duvall and Kershner 1998; Rosa *et al.*, 2013). Treatment with these drugs usually is targeted at managing the symptoms (aftermaths of the allergic response). AC is therefore recurrent, demanding sufferers to continuously rely on these medications. AC management with these drugs, however, may present with the challenge of adverse effects including cataract, corneal ulcers, glaucoma, and dry eyes (Ousler *et al.*, 2004; Allen *et al.*, 1989; Mohan and Muralidharan 1989). Abokyi *et al.*, (2012) in earlier studies, on epidemiology of AC, drug-prescription patterns and adverse effects, established that dry eyes was the most

common ocular disorder associated with systemic antihistamine use. Currently, the recommend therapy for AC involves the use of anti-allergic medications; that have mast cell stabilization and antihistaminic effects, and have longer duration of action (Abelson *et al.*, 2007; Beauregard *et al.*, 2007; Torkildsen and Shedden, 2011). It has, however, been reported by some researchers that the newer orthodox anti-allergic drugs that meet the above criteria also adversely impair tear secretion or decrease tear film stability, resulting in dry eyes. Studies have indicated that some herbs possess most of these qualities (Makino *et al.*, 2001; Takano *et al.*, 2004; Tachibana *et al.*, 2001). Recent studies have revealed *Pistia stratiotes*, a traditional medicinal plant, to possess potent anti-inflammatory and anti-allergic properties, and have been used in the management of uveitis and arthritis (Kyei *et al.*, 2012; Koffuor *et al.*, 2012). A preliminary investigation conducted on the anti-allergic properties of *P. stratiotes* has shown that the plant is effective in the management of a murine model of ovalbumin-induced AC. It was observed that the herb effectively lowered the serum IgE concentration and also significantly reduced signs of acute allergic inflammation, suggesting its useful in the therapeutic

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management of AC in humans. Other studies have also indicated the beneficial role of this *P. stratiotes* in the management of asthma (Alexander and Singh, 2011).

While efforts are being channeled into exploring the potentials of this herb in the management of ophthalmic disorders, it is important to study the effect on tear secretion and tear film stability as a way of evaluating its tendency to causing dry eyes (as seen with conventional antihistamines) when used in the management of AC.

## MATERIALS AND METHODS

### Animals

ICR mice of either sex (10–12 weeks old) weighing 26–30 g obtained from the Department of Pharmacology, KNUST, Animal House were used in this study. All mice were housed in a constant room temperature  $27 \pm 5$  °C and relative humidity  $60 \pm 10\%$  with ambient light and dark cycle. Mice were fed with normal commercial mice chow pellet from Agricare Ltd, Tanoso, Kumasi, and water *ad libitum*.

### Ethical considerations

All procedures performed were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. Laboratory study was carried out in a level 2 biosafety laboratory. All the technical team observed all institutional biosafety guidelines for protection of personnel and laboratory.

### Preparation of the aqueous leaf extract of *Pistia stratiotes* (LEPS)

Fresh leaves of *Pistia stratiotes* were picked, washed and air dried. The dried leaves were powdered using a hammer mill (Schutte Buffalo, New York, USA). A 700 g quantity of the powder was soaked in a liter of water for 24 h. Reflux filtration was performed at 80 °C. The collected filtrate was lyophilized into powder with a Hull freeze dryer /lyophilizer 140 sq ft (model 140FS275C, USA) and labeled LEPS i.e. an aqueous leaf extract of *Pistia stratiotes*. This was stored at 4°C until during experimentation when powder was constituted into the required concentrations with normal saline.

### Drugs Used

Cetirizine Hydrochloride (Kinapharma Limited, Accra, Ghana), Prednisolone (Ernest Chemists Limited, Accra, Ghana), ketamine (Laboratorio Sanderson SA, Santiago, Chile), Fluorescein solution (Sigma-Aldrich, St. Louis, MO), phenol red thread (Tianjin Jingming New Technological Development Co., Ltd., China).

### Experimental procedure

#### Randomization and treatment

ICR mice were randomly assigned into 4 experimental groups (n=7) and treated as follows: Group1 (Normal saline: 2

ml/kg), Group 2 (Cetirizine: 5 mg/kg); Group 3 (Prednisolone: 10 mg/kg), Group 4 (LEPS: 100 mg/ kg). Treatment were given consecutively for 7 days. Tear film secretion and tear film stability test were carried out before and after treatments using the phenol red thread test and the fluorescein tear break-up time. Animals were given intramuscular injection of 80 mg/kg ketamine to immobilize them for the assessment of tear film parameters. Tests were carried out at same time (12 pm) of the day in an air-controlled environment.

#### Phenol red thread (PRT) test

The amount of aqueous tear produced was measured with a phenol red thread. For each mouse in a group, one eye was randomly selected for the test. The lower eyelid was pulled down slightly, and a 1 mm portion of the thread was inserted at the conjunctival cul-de-sac at a point approximately one-third of the distance from the lateral canthus of the lower eyelid. A time interval of 15 seconds was allowed after insertion of the thread for the wetting and color change to red. The wetting length was measured under a microscope, using a micron-scale digital ruler. This procedure was repeated three times for that eye and the average was considered as the final score. After the test, eyes were turned close to avoid excessive exposure and irritation of ocular surface.

#### Fluorescein tear film break-up time (FTBUT)

The stability of the tear film on the ocular surface was assessed by the FTBUT. This started with the instillation of 1 microliter of 1% sodium fluorescein solution was instilled into the conjunctival cul-de-sac. Mice were allowed to blink three times following instillation of the solution, after which the lids were held open under a SL500 Shin Nippon Slit Lamp (Ajinomoto Trading Inc., Tokyo, Japan) for the measurement of the FTBUT using the cobalt blue filter. This procedure was repeated three times for each eye and the average of these measurements was recorded as the final score.

#### Data Analysis

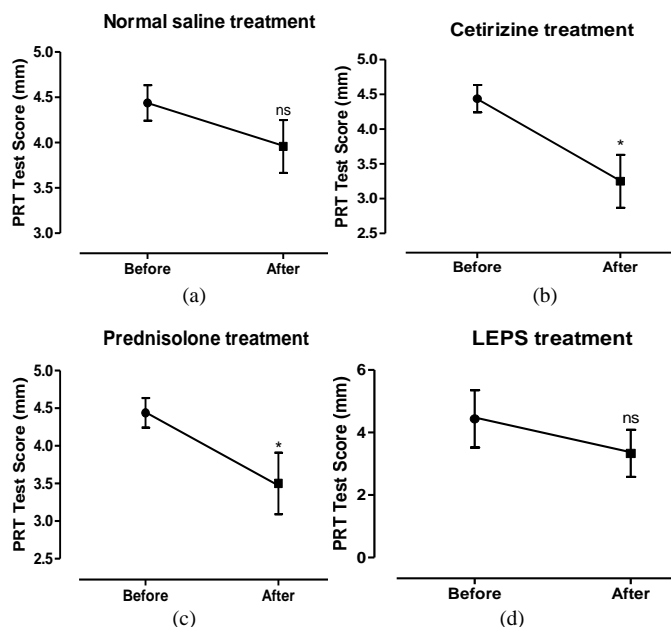
Data obtained from tests was presented as mean  $\pm$  SD. Baseline values for the amount of tear secretion and tear film stability were compared between groups using one-way ANOVA. Paired t-test was used to determine changes between the baseline measurements and post-treatment measurements of tear film parameters for each group.  $P \leq 0.05$  was considered statistically significant.

## RESULTS

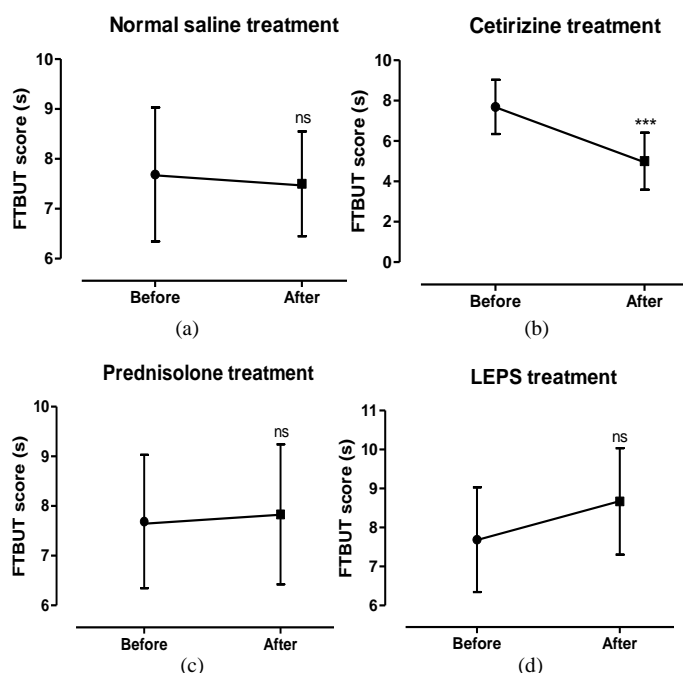
#### Phenol red thread (PRT) test

Comparison of the PRT scores at baseline and post-treatment showed that mice treated with cetirizine and prednisolone had a significant decline in tear secretion i.e.  $1.188 \pm 0.4369$  mm;  $P \leq 0.05$ , and  $0.9380 \pm 0.4422$  mm;  $P \leq 0.05$  respectively (Figure 1B and 1C), while no significant changes ( $P > 0.05$ ) were observed following treatment with LEPS and normal

saline i.e.  $0.4800 \pm 0.4211$  mm, and  $0.7050 \pm 0.4236$  mm respectively (Figure 1D and 1A).



**Fig. 1:** Tear secretion before, and after one week of treatment with (A) 2 ml/kg normal saline, (B) 5 mg/kg Cetirizine, (C) 10 mg/kg Prednisolone, (D) 100 mg/kg LEPS in a phenol red thread test. ns  $P > 0.05$ , \*  $P \leq 0.05$ ; paired t-test (two-tailed). PRT=Phenol red thread.



**Fig. 2:** Fluorescein tear film break-up time before, and after one week of treatment with (A) 2 ml/kg normal saline, (B) 5 mg/kg Cetirizine, (C) 10 mg/kg Prednisolone, (D) 100 mg/kg LEPS in a phenol red thread test. ns  $P > 0.05$ , \*\*\*  $P \leq 0.001$ ; paired t-test (two-tailed). FTBUT=fluorescein tear film break-up time.

### Fluorescein Tear Film Break-Up Time (FTBUT)

No significant changes in tear film stability ( $P > 0.05$ ) were observed with normal saline ( $0.1880 \pm 0.5909$  s), prednisolone ( $-0.1450 \pm 0.6182$  s), and LEPS ( $-0.9790 \pm 0.6145$  s)

treatment in mice comparing FTBUT values at the baseline to post-treatment values (Figures 2A, 2C, and 2D). Cetirizine-treated mice however showed a significant decline ( $2.688 \pm 0.6185$  s;  $P < 0.001$ ) in the stability of tear film (Figure 2B).

### DISCUSSION

The precorneal tear film is a three-layered physiological secretion by lacrimal glands in the eyelids and conjunctiva, and is found overlying the corneal surface. Each layer plays a peculiar role. For instance, the mucin layer which is closest to the cornea is involved in the wetting of the corneal surface; the middle aqueous layer is involved in the supply of oxygen to the cornea epithelium, washing away of debris and also has bactericidal activity; and lastly, the lipid layer involved in retarding evaporation of the tear film to about ten percent (Mishima and Maurice, 1961; Kanski, 2003). Scientists have shown that a deficiency in one or more layers of this three-layered film underlies cases of dry eyes, but in most individuals (80% of all cases) it is attributable to a lipid layer deficiency (Dausch *et al.*, 2006). A deficient lipid layer would result in an evaporative dry eye (EDE), in which case the volume of tear produced is enough but there is a significant decline in the stability of the tear film, while a deficient aqueous layer results in a tear deficient dry eye (TDDE) (DEWS, 2007). Phenol red thread test is used in the assessment of the quantity of tear secretion and provides information on the aqueous layer as this layer makes up over 90% of tear volume. Fluorescein tear film break-up time (FTBUT) on the other hand indicates the stability of tear film which is largely dependent on the integrity of the lipid layer. Mice treated with normal saline did not show any significant decline in tear secretion and tear film. This is because normal saline treatment has no pharmacological activity on tissues, and is useful as placebo for comparisons purposes in most experimental studies. Cetirizine treatment resulted in significant decline on both tear secretion and stability of tear film while LEPS had no significant influence on tear secretion and decrease tear film. This study, therefore, further corroborates reports of earlier studies indicating that histamine antagonists (such as cetirizine) inhibited the secretion of tears (Abokyi *et al.*, 2012; Ousler *et al.*, 2004). Histamine, an autacoid synthesized from the amino acid histidine, exerts its effects on the body through the activation of four histamine receptor subtypes labelled H1, H2, H3 and H4. The existence of histamine receptors on the human conjunctiva has been confirmed by several investigators (Bielory and Ghafoor, 2005; Li *et al.*, 2012). Histamine possesses stimulant effect on exocrine glands, which include the lacrimal gland and accessory lacrimal glands responsible for the secretion of aqueous tears (Danowski and Kmiec, 2002). Hence, histamine antagonists by competitively binding to histamine receptors, inhibit histamine from binding to these receptor molecules (Van Dyke and Woodfork, 2004) on the conjunctiva decreasing the tear secretion and consequently a decline in tear film break up time. LEPS did not adversely impair tear secretion, an indication that its anti-allergic properties may differ from the mechanism exhibited by ordinary histamine

antagonists. Prednisolone in this study also, showed a significant decline in aqueous tear secretion, but did not affect tear film stability. This could have been due to the compensatory action of an increased lipid-tear layer secretion by the meibomian glands. Generally, steroids (such as prednisolone) are known for their anti-inflammatory effect on the meibomian glands and are therefore crucial in managing meibomian gland dysfunctions (Ehler and Shah, 2008). Apart from the anti-inflammatory effect of steroids on the meibomian glands, some steroids (sex steroids) influence lipid secretion by meibomian glands through suppression of genes associated with keratinization and stimulation of those genes involved in lipogenesis (Sullivan *et al.*, 2009; Schirra *et al.*, 2006). Compared to treatment with prednisolone, LEPS showed a better improvement on the tear film stability despite an insignificant decline in tear secretion. Since the tear-lipid layer is largely responsible in retarding tear evaporation (i.e. increasing tear film stability), an increase of this layer will further stabilize tear evaporation. Various anti-inflammatory agents have been shown to be effective in the management of dry eyes (i.e. increasing tear film stability) (Avni *et al.*, 2010; Nagelhout, 2005; Perry *et al.*, 2006). It has also been revealed that specific anti-bacteria agents have the tendency to decrease bacterial lipolytic enzymes involved in the breakdown of normal meibum lipids into potentially inflammatory free fatty acid fragments, thereby improving tear film stability (Dougherty *et al.*, 1991). Hence, this increase in the tear-lipid layer by the LEPS-treated mice could be attributed to the anti-inflammatory and anti-bacterial properties of *P. stratiotes*. It is noteworthy to indicate that preliminary phytochemical investigations conducted on LEPS indicated that this plant was enriched with several secondary plant metabolites including steroids, flavonoids, glycosides, tannins etc, as reported by other researchers (Aliotta *et al.*, 1991). It is documented that these numerous phytochemicals confer the anti-inflammatory and antibacterial activities among other medicinal properties of *P. stratiotes* (Conti *et al.*, 2013; Rathee *et al.*, 2009). Overall, *P. stratiotes* was safer on the precorneal tear film, manifesting in the maintenance of adequate tear secretion and tear film stability.

## CONCLUSION

An aqueous leaf extract of *Pistia stratiotes* doesn't reduce tear secretion, and does not affect tear film stability hence its use in the management of AC is not likely to be accompanied with the adverse effect of dry eyes as conventional antihistamines do.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest whatsoever .

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