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MaquiBright™ standardized maqui berry extract significantly increases tear fluid production and ameliorates dry eye-related symptoms in a clinical pilot trial

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Aim. **Dry eye symptoms, resulting from insufficient tear fluid generation, represent a considerable burden for a largely underestimated number of people. We concluded from earlier pre-clinical investigations that the etiology of dry eyes encompasses oxidative stress burden to lachrymal glands and that antioxidant MaquiBright™ *Aristotelia chilensis* berry extract helps restore glandular activity.**

Methods. **In this pilot trial we investigated 13 healthy volunteers with moderately dry eyes using Schirmer test, as well as a questionnaire which allows for estimating the impact of dry eyes on daily routines. Study participants were assigned to one of two groups, receiving MaquiBright™ at daily dosage of either 30 mg (N.=7) or 60 mg (N.=6) over a period of 60 days. Both groups presented with significantly (P<0.05) improved tear fluid volume already after 30 days treatment. Schirmer test showed an increase from baseline 16.3±2.6 mm to 24.4±4.8 mm (P<0.05) with 30 mg MaquiBright™ and from 18.7±1.9 mm to 27.6±3.4 mm with 60 mg (P<0.05), respectively. Following treatment with 30 mg MaquiBright™ for further 30 days, tear fluid volume dropped slightly to 20.5±2.8 mm, whereas the improvement persisted with 60 mg treatment at 27.1±2.7 mm after 60 days treatment (P<0.05 *vs.* baseline).**

Results. **The burden of eye dryness on daily routines was evaluated employing the "Dry Eye-related Quality of life Score" (DEQS), with values spanning from zero (impact) to a maximum score of 60. Participants had comparable baseline values of 41.0±7.7 (30 mg) and 40.2±6.3 (60 mg). With 30 mg treatment the score significantly decreased to 21.8±3.9 and 18.9±3.9, after 30 and 60 days, respectively. With 60 mg treatment the DEQS significantly decreased to 26.9±5.3 and 11.1±2.7, after 30 and 60 days, respectively. Blood was drawn for safety analyses (complete blood rheology and -chemistry) at all three investigative time points without negative findings.**

Conclusion. **In conclusion, while daily supplementation with 30 mg MaquiBright™ is effective, the dosage of 60 significantly increased tear fluid volume at all investigative time points and decreased dry eye symptoms to almost a quarter from initial values after two months treatment.**

KEY WORDS: Anthocyanins – Ophthalmology - Dry eye syndromes.

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The number of people presenting with dry eyes has been suggested to rapidly increase in most parts of the world.[1] Tear fluid represents the aqueous layer on the ocular surface which has, further to lubrication of the cornea, numerous functions as part of the optical system, such as nourishing the cornea and arresting microbial growth.[2] Tear fluid provides the cornea with glucose, which has recently led to developments for utilising tear fluid for non-invasively measuring blood sugar in diabetic patients.[3] Furthermore, tear fluid contains several antioxidants: 665 µM ascorbic acid, 107 µM glutathione, 48 µM cysteine, 45 µM tyrosine and 328 µM uric acid.[4] The outermost part of tear fluid bears a fluid lipid layer which retards the evaporation of tear fluid water.[5] The antimicrobial lipocalin in tear fluid binds to microbial siderophores with high affinity and also scavenges hydrophobic, potentially harmful molecules to protect the cornea.[6] The average tear production rate from lacrimal glands is in the range of 0.5 to 2.2 µL/min with approximately 0.72 to 3.2 mL of tear fluid secreted for each eye per day.[7] When lacrimal glands release moderately insufficient tear fluid quantities, the eyes may get irritated, resulting in typical symptoms, such as a burning sensation, ocular fatigue,

temporal visual blurring, difficulties to focus at computer screens, television or mobile phones, and finding bright lights disturbing.

A considerable number of people suffer from decreased tear fluid secretion and its major symptom eye irritation, commonly referred to as "dry eye syndrome" (*keratoconjunctivitis sicca*). Several medications have a side-effect of causing dry eyes. Such medications include diuretics, certain antihypertensives, antihistamines, opiods, analgesics, antidepressants, sedatives and certain beta-blockers.[8] Several auto-immune diseases manifest in causing severe dry eye syndrome, especially Sjögren's syndrome.[9]

Other groups previously introduced the hypothesis that ageing is one of the most important factors in the pathogenesis of dry eye.[1, 10] Many people will experience signs of dry eye syndrome with increasing age. A recent survey among 672 employees of a company in Osaka showed that tear fluid secretion by Schirmer test was significantly lower in people aged ≥ 50 years (13.1 mm), than those aged <40 years (21.5 mm).[11, 12] This study also identified significantly lower tear secretion among overweight individuals presenting with metabolic syndrome (11.0 mm).

A survey among participants in the "Women's Health Study and the "Physicians'Health Studies I and II" suggested that dry eyes are generally experienced as being more common and more severe among women than men.[13] Sex hormones may play a role in the greater prevalence of dry eye syndrome in women.[14] The impact of androgens on the structure and function of the meibomian and lacrimal glands and therefore androgen deficiency may, at least in part, be associated with the etiology of dry eye syndrome during menopause.[15]

A contribution of age related increasing oxidative stress has been suggested to be involved in the pathobiology of decreased tear production.[1] Transgenic mouse models provided evidence that mitochondrial-induced oxidative stress and inflammation affects lacrimal gland function with resulting dry eye disease.[16] Recently published surveys have suggested that dry eye syndrome is a much more common phenomenon than previously thought, affecting visual performance and quality of life especially in situations requiring sustained visual attention such as reading or driving.[13] Attempts have been made to create greater awareness of the health issue with the use web-based self-screening by individuals.[17] From 980 visitors to the website who completed the self-screening questionnaire, 36% reported five or more dry eye symptoms. In support of oxidative stress involvement in the pathogenesis of dry eye syndrome is the observation that even brief passive exposure to cigarette smoke in healthy non-smokers is associated with adverse effects of reactive oxygen species on tear fluid.[18]

A research group previously described in a rodent model that anthocyanins from maqui berries are more effective for quenching of oxidative stress in lachrymal glands than black currant or bilberry species.[19] Delphinidin 3,5-O-diglucoside, an anthocyanin limited to *Aristotelia chilensis* berries, showed remarkable uptake into lachrymal glands in rats after oral administration. In a rat blink-suppressed dry eye model maqui berry extract was found to be significantly more effective for preventing tear secretion reduction than polyphenolic extracts derived from bilberry (*Vaccinium myrtillus*) and black currant (*Ribes nigrum*).

To date no investigations employing polyphenolic antioxidant supplementation for improving tear fluid generation have been undertaken in humans. We here present a first pilot trial which suggests that orally applied antioxidants derived from polyphenol-rich maqui berries may significantly improve tear fluid secretion and reduce dry eye-related symptoms.

Materials and methods

Study participants

Healthy study participants were recruited from university staff by word of mouth and university social network internet platform. Volunteers were screened for meeting inclusion criteria with moderate dryness of eyes based on the "Dry Eye-related Quality of life Score" (DEQS) [20] as described below. The DEQS represents a questionnaire with a five point Likert scale, having two domains, one related to ocular symptoms and a second related to the impact of dry eyes on daily routines. A value of zero depicts absence of dry eyes and related symptoms, the maximum value is a score of 60.

The primary inclusion criterion for this study was the DEQS questionnaire, on which suitable subjects were required to score a value of 20 or higher, yet below a value of 50. The secondary inclusion criterion

was a minimum tear fluid production by Schirmer test of 10 mm.

Inclusion criteria were a general good health as judged by a physician. A complete physical check-up was carried out which included the investigation of blood pressure and complete blood rheology and chemistry, including total bilirubin, albumin, albumin/globulin ratio, enzymes (GOT, GPT, ALP, LDH, γGTP), blood lipids (HDL, LDL, total cholesterol, triglycerides, phospholipids, free fatty acids), electrolytes (Na, Cl, K), creatine, uric acid, fasting blood glucose, HbA_{1c}, ketone bodies, complete blood cell count (leukocytes, erythrocytes, platelets), haemoglobin, haematocrit, bilirubin, ketone bodies, uric acid, BUN and creatinine.

Subjects were investigated for absence of cardiovascular disease and any other systemic disease. Subjects requiring prescription medical treatment, who had surgery, radiotherapy or chemotherapy during the past six months were excluded. Further exclusion criteria were the use of contact lenses, the consumption of dietary supplements containing flavonoids/polyphenol, EPA or DHA, as well as any supplements indicated for eye health. Lactating women or women who plan to conceive or who are pregnant were excluded. Subjects who were pregnant, breast-feeding, or planning conception were excluded. Subjects were investigated by an ophthalmologist for complete eye examination to exclude participants with ocular disorders including ocular hypertension, glaucoma, macular degeneration or any other degenerative eye disorder and severe myopia.

All subjects were informed about the aim of the investigation and treatment procedure in accordance with the Declaration of Helsinki. They were assured that they may discontinue participation in the trial at any time without having to provide reasons. Participants were required to give their written informed consent for participation in this investigation.

Investigational treatment

Patients were randomly assigned to one of two groups, receiving either 30 mg or 60 mg MaquiBright™ capsules. MaquiBright™ is a registered trademark of Oryza Japan and MNL-Group, Switzerland, for a standardised extract of maqui berry (*Aristotelia chilensis*), which is native to Chile. MaquiBright™, marketed as Delphinol® in some parts of the world, is a pure polyphenolic extract, standardised to bear no less than 25% w/w total delphinidins and no less than 35% w/w total anthocyanins. Both delphinidin and cyanidin naturally occur in maqui berry in glycosylated form, with either one or two sugar moieties bound. The sugar moieties may be glucose or sambubioside, as well as in combination thereof.[21]

Study participants were handed out bottles with 30 capsules, representing a one month supply. During their return for examination after one month treatment they were handed out another bottle with 30 capsules for the second month treatment, after which they again were investigated at the ophthalmic clinic.

Hard gelatine capsules (size 4) were used to manufacture 30 mg and 60 mg MaquiBright™ dosages, using cellulose as inactive filler. Capsules were manufactured by Oryza, Aichi, Japan. Capsules with different dosage were visibly indistinguishable.

Study participants were advised to take the capsule in the morning during breakfast time with water.

Observation and procedures

At trial start and again after one month and two months of treatment, subjects had to present at the clinic in the morning after having had fasted since 9 pm the previous evening. Subjects were gathered in an examination room under controlled temperature and humidity for having their blood pressure checked by sphygmomanometer and blood was drawn for subsequent laboratory analysis. Then they were required to fill out the DEQS questionnaire. During this occasion patients were required to return their bottle with capsules in order to check compliance. During the second visit also a new bottle with further capsules was provided to the patients.

The DEQS, initially developed by Sakane and coworkers, aims to detect multiple potential effects of dry eye disease on patients, based on two domains related to bothersome ocular symptoms and the impact on daily life.[20] The total 15 questions of both domains are self-judged by patients, based on the frequency of symptoms ranging from 0 (never) to 4 (always), as well as symptoms severity ranging from 0 (not at all) to 4 (very severe).

A set of six questions investigate the frequency and severity of ocular symptoms:

— sensation of foreign body in the eyes;

— the sensation of dry eyes;

— painful or sore eyes;
— ocular fatigue;
— the perception of eyelid heaviness;
— eye redness.

Nine questions are related to the impact on daily life:

— the difficulty to open the eyes;
— a blurred vision when focussing on an item;
— the sensitivity to bright light;
— problems with the eyes during reading;
— problems with the eyes whilst watching television or looking at computer screens or mobile phones;
— feeling distracted because of eye symptoms;
— eye symptoms affect the work;
— not feeling like going out because of eye symptoms;
— feeling depressed because of eye symptoms.

All items of the DEQS are scored on a five point Likert scale, with the Ocular Symptoms scale having maximum total score of 28, and the impact of dry eyes on daily life having maximum score of 36.

A standard Schirmer test was carried out at trial start and again after one and two months of treatment. Tear fluid production was measured in both eyes simultaneously with Schirmer test strip using a "Zone-Quick" phenol red thread, manufactured by Showa Yakuhin Kako Co Ltd (Tokyo, Japan) for five minutes without the use of anaesthetic.

Safety testing

For safety testing blood was drawn at trial start, and after one and two month treatment, respectively, for both treatment (30 and 60 mg a day) groups. Parameters investigated were identical as described above for recruitment criteria. Systolic and diastolic blood pressures were measured by sphygmomanometer.

Statistical analysis

Statistical comparison was carried using a one-way analysis of variance (ANOVA), followed by a Student's t-test. A value of $P<0.05$ was considered indicative for statistical significance. Data are presented as mean±standard error.

Results

The 13 study volunteers were randomly assigned to one of two dosage groups, one group with seven participants (two women, five mean, age range from 28 to 60 years) receiving a single 30 mg MaquiBright™ tablet a day in the morning with breakfast, the second group (two women, four men, aged 25 to 60 years), receiving a single 60 mg tablet a day. As presented in figure one, the baseline tear fluid secretion by Schirmer test was ≥15 mm, indicating that subjects presented with moderately dry eyes. Tear fluid secretion by Schirmer test showed significantly ($P<0.05$) increased gland excretory performance already after 30 days treatment for both dosages of MaquiBright™ administered.

At daily dosage of 30 mg MaquiBright™ tear fluid secretion increased significantly after 30 days by 50% from baseline 16.3±2.6 mm/min to 24.4±4.8 mm/min ($P<0.05$). Surprisingly, after a further 30 days of treatment at 30 mg daily, lachrymal gland fluid excretion presented with a slight drop to 20.5±2.8 mm/min, which no longer reached statistical significance to baseline values.

A twice as high (60 mg) daily intake of MaquiBright™ yielded a comparatively similar effect after 30 days of treatment, as did the lower dosage of 30 mg. The average tear fluid production increased significantly by 47.6% from baseline 18.7±1.9 mm/min to 27.6±3.4 mm/min. However, in contrast to the lower dosage, the achieved greater glandular performance persisted after further 30 days of treatment (Figure 1). After completion of the two months treatment period the group taking 60 mg MaquiBright™ a day presented with average tear fluid production of 27.1±2.7 ml/min, a statistical significantly greater value as compared to baseline.

The evaluation of the DEQS questionnaire provides a broader overview of the patient's health conditions, as further to the dryness of eyes the broader symptomatology is taken into consideration. The composite dry eye score across all domains of the questionnaire suggest that after 30 days treatment with 30 mg a statistically significant improvement of symptoms by 47% ($P<0.01$ *vs.* baseline) was achieved. Only minimal further improvement was recorded after further 30 days of daily treatment with 30 mg MaquiBright™. Doubling of the daily dosage yielded a dry eye symptom score reduction by 27% ($P<0.05$) after 30 days treatment, which is a much lower effect than found with the dosage of 30 mg Maqui-

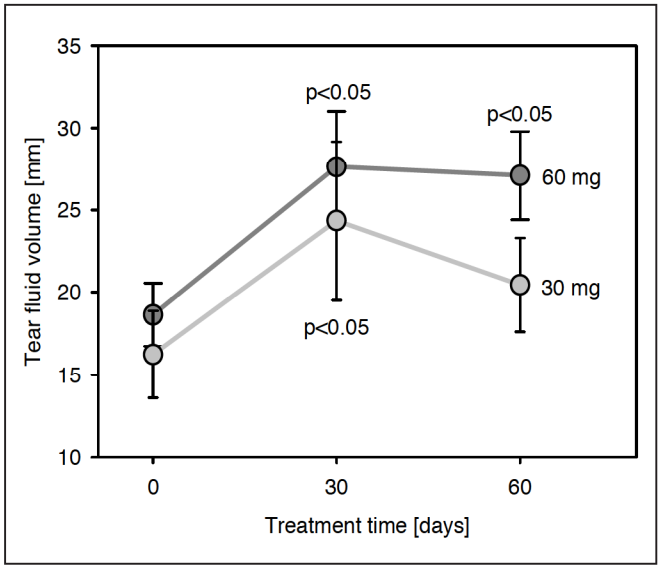


Figure 1.—The effects of treatment on tear fluid secretion volume, measured by Schirmer test, in patients treated with 30 and 60 mg Maquibright™, respectively.

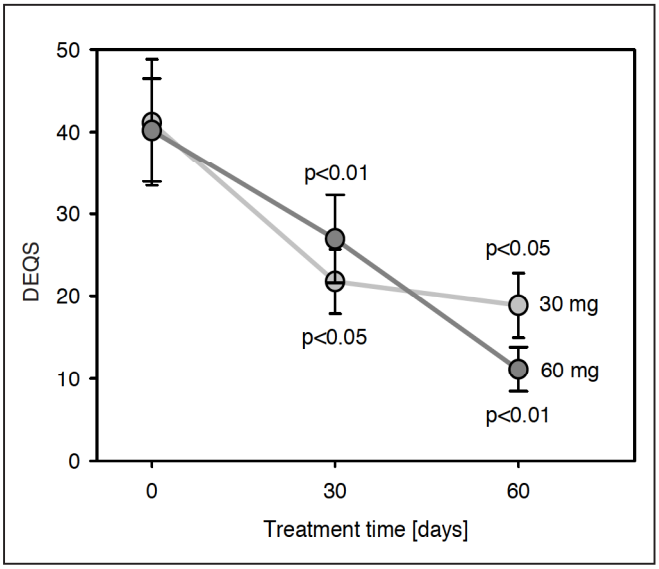


Figure 2.—The effects of treatment on tear fluid secretion volume, measured by DEQS, in patients treated with 30 and 60 mg Maquibright™, respectively.

Bright™. However, after completion of the total 60 days trial period the symptoms significantly further improved in the 60 mg-treated group, with a total dry eye symptom score reduction by 72% (Figure 2).

All patients were interviewed during their two visits to the clinic, after one and two month treatment, for observations and side effects. None of the patients reported unfavourable observations. Inspection of the capsule bottles they returned suggested very good compliance. During visits to the clinic blood was drawn from patients for a complete examination. No significant changes to any parameter tested were identified. Systolic and diastolic blood pressures were healthy at trial start and remained unaltered. Blood lipids, complete blood cell count, electrolytes, sugar (fasting, HbA_{1c}), enzymes (GOT, ALP, GPT, γGTP, LDH), haematocrit, haemoglobin, and blood chemistry, were all at healthy values throughout all three time points of investigations. All investigated safety parameters showed negligible and insignificant alterations between the three time points investigated.

In conclusion, after two months treatment 60 mg MaquiBright™ is more effective for dry eye symptomatology, based on the QOL questionnaire, than 30 mg. Moreover, only with 60 mg MaquiBright™ the tear fluid secretion volume increase reached statistical significance versus baseline after two months.

Discussion

The pilot trial presented here is in support of improving dry eyes with an antioxidant of suitable potency. It is interesting to notice that the majority of improvement is already manifest after 30 days of treatment in both, tear fluid secretion by Schirmer test, as well as by the DEQS questionnaire, with statistical significance for both parameters. During treatment for another month the improved tear fluid secretion persists in the group treated with 60 mg MaquiBright™, whereas it fades to some extent in the group given a lower dosage. This pilot is of value for a future larger study, as it suggests that a daily dosage of 60 mg MaquiBright™ may be better suitable. Nonetheless, a daily intake of 30 mg of the maqui berry extract does provide improvement for tear fluid production and dry eye symptom reduction.

Compared to research carried out with other flavonoid species, a daily applied dosage of 60 mg extract containing anthocyanins may be considered a moderate quantity in human applications. Only recently, a systematic investigation on absorption, metabolism and elimination of ^{13}C-labelled cyanidin-3-glucoside in humans suggested that anthocyanins and metabolites thereof may be considerably more bioavailable and persist much longer in the body than previously assumed.[22] Further to providing significant antioxi-

dant effects, the standardised maqui berry extract used in the study presented here was recently described to retard glucose absorption in humans with moderate glucose intolerance.[23] Berries in general have been subject to extensive research especially in ophthalmological applications.[24]

Our study is the first to point to a possible benefit of specifically maqui berry anthocyanins for improvement of dry eye in humans. The findings on lacrimal gland function shown here, extends earlier mechanistic investigation of MaquiBright™ standardised maqui berry extract in a rodent dry-eye model and acinar cells isolated from rodent lacrimal glands.[19] This group showed by HPLC analyses of lacrimal glands that a specific maqui berry delphinidin species (3,5-O-diglucoside) is better incorporated and correspondingly more potently quenches oxidative stress in glands than other anthocyanin species prevalent in other berry species. In correspondence with decreased oxidative injury in lacrimal glands, histological observations showed normal morphology and acinar cell density, identical to normal conditions.

In conclusion, the favourable outcome of the pilot trial presented here, especially in view of absence of side effects, suggests that MaquiBright™ may be useful also for preventative care, such as in sensitive individuals who expose themselves to unfavourable environments, *e.g.* computer screens, bright light, pollution or air-conditioners. The first evidence we present here on MaquiBright™ improving ocular humidity should warrant further research in a larger cohort with double-blind, placebo-controlled protocol.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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