Protective effects of skin barrier integrity against emotional stress, by an enriched Oat β-Glucan complex derived from Avena sativa L.



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ABSTRACT

The skin stress response is the activation of the endocrine, neurologic, and immune systems triggering a cascade of impacts. Cortisol is a stress hormone and prolonged exposure to elevated levels causes an increase in reactive oxygen species. Oat β-glucans have been reported to have beneficial effects on skin health.This work presents evidence of the protective effects of an enriched Oat β-glucan complex on the skin barrier integrity against emotional stress and on the effectiveness of delaying the first signs of skin-ageing. Clinical studies show that this complex protects against stress-related skin damage. Due to its high anti-oxidation and stress-response upregulation in genes, stratum corneum integrity is maintained, stress-induced skin thinning is reduced, and skin smoothness is improved.

Skin stress, results in multiple physiological impacts leading to a variety of skin disorders. A stress response is the activation of the endocrine, neurologic, and immune systems triggering a cascade of impacts, that are both systemic and cutaneous. Both the sympathetic nervous system and the hypothalamic-pituitary-adrenal system participate in the stress response.[1] The consequential release of cortisol, catecholamines and neuropeptides have multiple clinical effects including an increase in skin inflammation, impaired skin barrier function, impaired wound healing, and suppressed immunity.[2]



β-Glucans (BG) are natural cell wall polysaccharides found in yeast, fungi, seaweed, and cereal. BG possesses many health benefits[3], however, information on the skin benefits of BG derived from cereal is fragmented. Oat β-glucan (OBG), one of the major components of bran soluble fibre, is a polysaccharide made of a linear branched chain of D-glucose monosaccharides bonded by mixed β(1 → 3) and β(1 → 4) linkages. The molecular weight, in natural form, varies from approximately 65 to 3,100×10_3 g/mol, and this difference affects both viscosity and solubility.[3] Skin penetration of OBG is a query often raised, given its molecular weight, however, fluorometric microscopy studies have been able to demonstrate[4], that despite its high molecular weight, OBG does penetrate the epidermis and dermis, by permeating between cells rather than through them. In this paper, the OBG complex is obtained from oat bran subject to an enzymatic treatment and wet milling, followed by centrifugation and ultrafiltration (manufacturing process optimised to preserve the original and natural structure of oat molecules).

There is strong evidence supporting the role of BG in stressed skin. Data available indicates that BG can directly stimulate human fibroblast collagen biosynthesis through an NF-1-dependent mechanism.[4] Topical application of OBG was shown to accelerate the recovery of the damaged epidermal barrier by promoting epidermal differentiation.[4] Whilst the antioxidative effects of BG have been

reported in human disease applications, such effects in human skin are lacking. Much research has demonstrated a link between stress and skin health, and that stress impacts skin barrier function.[1]

Cortisol is a primary (psychological) stress hormone and prolonged exposure to elevated levels causes an increase in reactive oxygen species (ROS) and increased oxidative stress.[5] It affects cell turnover, reduces collagen synthesis, and creates a disordered extracellular matrix.[5] As a result, skin loses elasticity resulting in fine lines and wrinkles.[1] In this context, the objective of these studies was to investigate an enriched OBG complex (INCI: Avena sativa (Oat) Bran Extract), in terms of it's ability to infl uence the expression of gene markers in the skin, notably antioxidation, stress responses and barrier integrity. Further effects of the OBG complex were evaluated, in both ex vivo and clinical studies in counteracting the detrimental effects of stress induced skin aging.

Materials and methods

Chemical analysis

The beta-glucan content of the OBG complex, was determined using the lichenase/beta-glucosidase streamlined McCleary method and the resultant D-glucose was assayed using a glucose/peroxidase reagent. The molecular weight was determined using high-performance size exclusion chromatography.

Gene expression analysis

A 3D *in vitro* skin model composed of epidermal keratinocytes and dermal fi broblasts was used. 1% OBG complex in water was tested versus water as the vehicle control for 48 hours of treatment. After 24-hours post treatment, the tissue samples were rinsed with phosphate buffer saline (PBS) and a freshly made treatment was re-applied. 48-hours post-treatment, the tissue samples were rinsed in PBS and extracted for RNA isolation and cDNA synthesis. Gene expression analysis was performed using a qPCR-based array that contained genes regulating skin functions.

Effect on Stratum Corneum Integrity under Stress

An *ex vivo* human skin model was performed using a 35-year old female Caucasian skin explant.
Product treatment: Samples were divided into experimental groups (Table 1).

24-hours after the last series of explant treatments, a solution of fluorescent dye (Lucifer Yellow) was added onto the surface of explants and incubated for 1 hour. Finally, the skin explants were sampled, embedded in OCT for cryo-preservation and stored at minus 80°C.

Skin Barrier Integrity: To evaluate the number of corneocyte layers, cryo-fixed sections were stained with 1% Safranin-O red solution. To evaluate the stratum corneum (SC) thickness, cryo-fixed sections were stained with Haematoxylin and Eosin. Previously Lucifer Yellow treated, and cryo-fixed sections were stained with DAPI (4-,6-diamidino-2-phenylindole) for nuclear detection. The analysis of the skin barrier integrity was achieved by evaluating the specific fluorescent signal intensity at 250 µm from the explant surface.

Image Analysis: Light microscope images of stained samples, were taken with an epi-fluorescent microscope (EVOS M5000 Imaging System) and analysed with Image J software. The mean values and standard deviations were obtained per treatment.

Effects on Skin Roughness

A double-blind half-face clinical evaluation of skin roughness parameters was carried out on 20 Caucasian and Hispanic females (30-54 years). Participants were presenting visible signs of facial ageing and different skin types. Test products (vehicle control, 1% OBG complex in vehicle control) were applied twice daily for 56 days. Assessment of skin roughness was evaluated by the parameters: Sa, Stm, roughness density and area, evaluated on the cheekbone areas, with 3D image analysis (rugosity) using an AEVA-HE V4 system (Eotech). Measurements were taken at baseline (day 0), after 28 and 56 days of continuous use of the product.

Results and discussion

Chemical Analysis: The molecular weight of the OBG complex was determined to be in the region of 600 to 700 kDa. BG content (β (1 → 3)-β (1 → 4) linked) was approximately 30%; total lipids 8%; starch 30%; and tryptophan 130 ppm.

Gene Expression Analysis: Following the treatment of the 3D skin model samples with 1% OBG complex, a statistically significant change in the number of expressed genes was observed. Of those genes grouped under 'antioxidant and stress response', the most notable (+230%*) was that associated with antioxidant detoxification (NQO1).

NADPH:oxidoreductase-1 is an antioxidant stress response gene encoding for the enzyme which protects cells from oxidative damage, and can protect cells from further damage by slowing down the degradation of tyrosinase.[6] In addition, MT2A (metallothionein-2) was markedly expressed (+179%#), as is associated with lower free radical activity, oxidative

TABLE 1: SUMMARY OF THE EXPERIMENTAL GROUPS

Group	Treatment	Sampling
Control	Untreated, not stressed	Day 6
Stress	Stressed (12 strips M3 + 5 cortisol application (0.001%))	
Stress + 1% OBG Complex	Pre-treated with OBG complex diluted in water for 24h; Stressed + 5 OBG complex applications	

damage, and inflammation.[7] There was addidtionally a significant increase (+57%*) of HMOX-1 (Hemeoxygenase-1) which is involved in the protection against cytotoxic damage and the production of anti-inflammatory effects.[8] Also of note was the increased expression of the HNRNPD gene (+130%*) associated with the maintenance of 'normal' ageing and protection of telomeres; and the SIRT-1 sirtuin gene (+65%**) associated with the protection of collagens from matrix metallo-protein (MMP) degradation.[9] LCE3D (late cornified envelope), was also expressed significantly (+93%#) and is associated with the maintenance of epidermal integrity and barrier function.[10] Finally, DSG3 (Desmoglein-3) was expressed by +106%# and is associated with the promotion of epidermal and skin barrier function.[11] These observations confirm that the antioxidative potential of the OBG complex is considered significant.

Effect on Stratum Corneum Integrity under Stress: Given the findings from the gene expression analysis, particularly with referenceto the "stress" response genes, the *ex-vivo* skin explant study evaluates the protective effect of OBG complex on SC morphology under defined stressed conditions. The stress treatment in this study is considered harsh, it involved stressing the skin with cortisol and removing the SC beforehand by tape stripping. The tape stripping allows an evaluation of the rate at which the barrier recovered and the effect of cortisol on its recovery, integrity, and epidermal thickness. This highlights the negative effect of cortisol treatment on the formation and regeneration of SC and evaluates the beneficial effect of OBG complex. During the days of incubation, the cortisol treatment decreases the efficacy of SC regeneration impacting the barrier function of the skin.

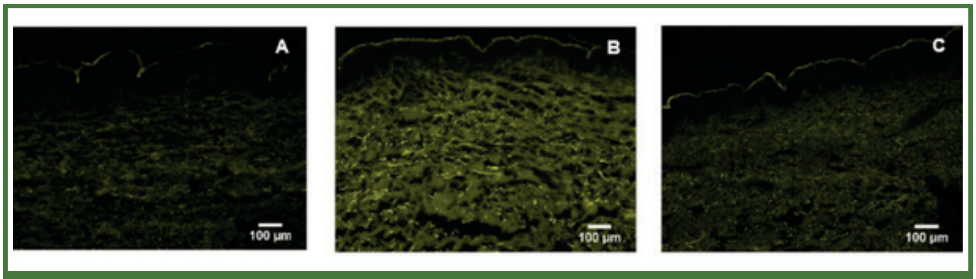


Figure 2: *In situ* visualisation of of skin barrier integrity
A = Control, B = Stress, C = Stress + 1% OBG Complex

Cell viability of the tissue samples were good and maintained the investigation (data not shown). In the assessment of SC thickness, the non-stressed and non-treated skin had a normal appearance and moderate SC thickness. During stress, the SC (and epidermis) thickness decreased indicating that cortisol is damaging the integrity of the skin. With the application of 1% OBG complex in combination with cortisol, the skin was protected from the stress-induced decrease of SC (and epidermis) thickness (Figure 1). Quantification of these observations indicated that the OBG complex can prevent the consequence of cortisol-induced oxidative stress on the SC thickness by 15%* ($p<0.1$), and the number and integrity of corneocyte layers by 31%*** ($p<0.001$).

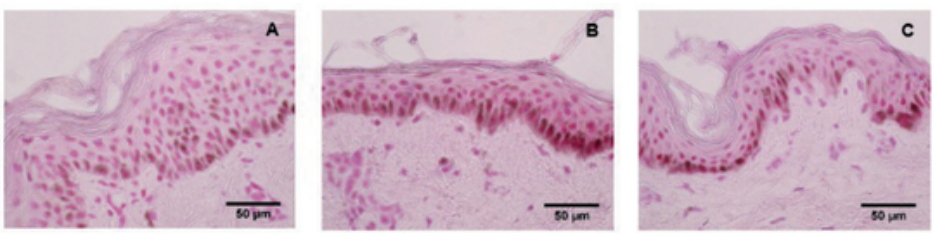


Figure 1: *In situ* visualisation of SC integrity and thickness
A = Control, B = Stress, C = Stress + 1% OBG Complex

The skin samples were assessed using Lucifer yellow staining microscopy, this revealed an increase in the fluorescent signal upon stress treatment with cortisol due to the impairment of barrier integrity and thus an increase in dye permeability. When the stress was applied with 1% OBG complex, the skin was significantly protected 82%***, ($p<0.001$) from damage to its barrier integrity and prevention of water loss (Figure 2). Wound healing studies[12] have also provided evidence that OBG promotes wound healing and repair due to the activation and proliferation of keratinocytes and fibroblasts, and the activation of immune cells.

These results suggest a potential role of OBG complex in modulating the cortisol stress response. It would be interesting to reproduce this study with more skin explants from different participants (different age and ethnicity). The cortisol levels could be measured in the below *in vivo* study to be able to validate the link between the high level of stress and the reduction of the signs of skin ageing.

Effect on Skin Roughness: 1% OBG complex significantly decreased Stm (-8%**, $p<0.01$ after 28 days and -12%***, $p<0.001$ after 56 days) and Sa (-7%*, $p<0.1$ after 28 days and -12%***, $p<0.001$ after 56 days) roughness parameters. When the vehicle control was applied, the treatment showed no relevant effects on Stm nor Sa parameters (non significant increases). The topical treatment with OBG complex displayed both smoothing and anti-roughness effects after 56 days of application, substantiated in a significant

decrease of roughness parameters (density and area), as compared to the baseline values.

Conclusions

Modern day lifestyle results in sustained stress levels that can make the skin appear visibly aged. From these findings, it has been demonstrated that topical application of 1% OBG complex is able to protect the skin from the undesirable effects of a stressful lifestyle. OBG complex prevents the consequences of cortisol induced oxidative stress, protects against stress-induced skin thinning and improves skin texture. Additional studies are in progress to further elucidate the mechanisms by which the OBG complex facilitates these protective mechanisms.

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