

Exploring the effects of a multicomponent phytoceutical health product in a *Drosophila melanogaster* ageing and repetitive concussion model

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Abstract

Objectives: This study aims to investigate the long-term effects of multiple concussions on physical function and oxidative stress, as well as the potential protective effects of a novel phytoceutical product (CONKA1) on ageing and brain health of *Drosophila melanogaster*.

Methods: Adult female *D. melanogaster* flies were exposed to repeated concussive events using a high-impact trauma (HIT) device. The effects of these impacts were assessed on lifespan, motor activity, and levels of reactive oxygen and nitrogen species (RONS) in both the brain and body. The flies were administered a multicomponent supplement (CONKA1), comprising *Withania somnifera*, *Curcuma longa*, *Melissa officinalis*, *Rhodiola rosea*, and *Vaccinium myrtillus*, and outcomes were compared to untreated controls.

Key findings: CONKA1 improved the lifespan and motor activity and reduced oxidative stress in ageing flies. Repetitive concussion hits significantly increased long-term mobility deficits, shortened lifespan, and exacerbated oxidative stress in both the brain and body, above and beyond normal ageing. CONKA1 demonstrated mitigation of extra motor deficits, reduced lifespan, and exacerbated oxidative stress in both the brain and body of the flies.

Conclusions: The findings validate that *D. melanogaster* is a suitable system to mimic the effects of repetitive concussions and evaluate the effects of novel health products.

Keywords: flies; *Drosophila melanogaster*; brain injury; concussions; neurodegenerative diseases

Introduction

Concussions or traumatic brain injuries (TBIs) are a major cause of morbidity and mortality worldwide that disproportionately impacts all ages [1]. Recently, there has been growing interest in investigating the effects of concussions on physical and mental health [2–4]. One of the health challenges is that concussions may have implications for the brain and can generate progressive defects that are difficult to detect until considerable time has elapsed [1]. The pathophysiology of TBI/concussion involves primary and secondary injuries and causes cell damage and death in the brain. Primary injuries occur during the initial impact and are triggered by external mechanical forces that deform the brain, whereas secondary injuries are triggered by cellular and molecular responses that occur over time in response to the primary injuries [5, 6]. The outcomes of concussions are heterogeneous in the human population owing to variations in the location and strength of primary injuries as well as genetic and environmental factors that affect the severity of primary and secondary injuries [5].

Injured neurons, glial cells, and vascular endothelium [7] lead to the generation of reactive oxygen and nitrogen species (RONS) [8].

To date, there is no effective natural product available on the market to manage concussion implications. A way to look at this issue is through the use of a combination of different plant products, chosen based on the available scientific evidence relevant to both physical and mental health consequences of concussion. The phytoceutical product, termed “CONKA1,” a combination of five plant powders, including *Withania somnifera*, *Curcuma longa*, *Melissa officinalis*, *Rhodiola rosea*, and *Vaccinium myrtillus*, was investigated in this present study. The rationale for selecting these components for contact sport physical recovery and mental health well-being maintenance is based on many previous studies that associate these species with physical and mental health attributes. For example, it was found that ashwagandha (*W. somnifera* root) has anxiolytic effects [9], improves sleep parameters [10], increases muscle mass and strength [11], is

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antineuroinflammatory [12], and has positive memory effects [13]. Turmeric (*C. longa* root) has potent anti-inflammatory effects [14], is an antioxidant [15], and has positive cardiovascular effects [16]. Lemon balm (*M. officinalis* leaves) has calming, analgesic, and antiseizure effects, maintains attention [17–20], and has anti-inflammatory effects [21]. Roseroot (*R. rosea* root) has positive effects on fatigue [22], particularly mental fatigue, and exercise performance (restorative) [23] improves life-stress symptoms [24], and there is some evidence it may improve mental health performance [25]. Finally, it has been shown that bilberry (*V. myrtillus* fruit) is anti-inflammatory and antioxidant [26], has positive effects on fatigue [27], and may improve visual deficits [28].

To investigate the behavioural consequences of concussion as well as testing the phytoceutical product, we recently developed a *D. melanogaster* model [29]. The fruit fly is a versatile model organism that has been used in biomedical research for over a century to study a broad range of phenomena [30]. Fruit flies share evolutionarily conserved genes and signaling pathways with vertebrates, including humans [31]. *Drosophila* can exhibit a large variety of complex social behaviours while having a relatively small number of neurons (~128 000) [32], which makes it an ideal experimental system for understanding concussions. The main benefits of using flies include the ability to quickly and affordably analyse large numbers of animals to establish causation between injuries and outcomes, the availability of numerous molecular and genetic tools to explore the molecules and pathways underlying injuries, and the ease with which outcomes can be assessed over the course of an animal's lifespan [5]. *Drosophila* has a compact brain, which is advantageous in research related to neurodegenerative diseases [33]. Moreover, fruit flies can be interesting in the process of drug testing, with simple oral drug delivery via dissolving the drug in food. Therefore, the test of the phytoceutical product in *D. melanogaster* in this project was both achievable and some results could be transferrable to humans.

The abrupt movement that causes human concussion can be simulated in *Drosophila* using a “high-impact trauma” (HIT) device [5, 29, 34]. When a HIT is administered, flies show neurological phenotypes homologous to those observed following TBI in mammals, including temporary incapacitation, disorientation, the fencing response (abnormal posturing; wing splay), innate immune gene expression changes in the brain, and gradual recovery of mobility [5, 34]. This paper aims to test two fundamental hypotheses. The first one is that concussions in adult *Drosophila* would lead to motor deficits, a short lifespan, and increased oxidative and nitrosative stress in the brains and bodies. The second hypothesis is that the phytoceutical product can have a positive effect on the physical and mental health and behaviour of fruit flies.

Materials and methods

Plant powders

All the plant powders were sourced from Herbal Apothecary, UK. All the powders were analysed separately by liquid chromatography–mass spectrometry (LC–MS) to detect and verify compounds known to occur in the plants investigated. The LC–MS method is described in the online supplementary material and the assigned compounds detected in each of the plant powders are shown in [Supplementary Tables 1–5](#).

Water extract

To run the individual experiment, each component of the phytoceutical product was prepared separately (100 mg/ml of each component), while the whole phytoceutical product stock solution (3 mg/ml) was prepared based on the following ratio of plant powders: 1.05 g *W. somnifera*, 1.05 g *C. longa* (combined with 5 mg black pepper), 1.05 g *M. officinalis*, 350 mg *R. rosea*, and 350 mg *V. myrtillus*, which is as used in a human tolerance health trial. The plant powder mixture or individual components were dissolved in distilled water and vortexed vigorously, before aliquoting into fly food.

Fly stocks and culturing conditions

Drosophila melanogaster female wild type (WT) was used for this experiment. Female flies were used in these experiments due to their greater responsiveness compared to males [35], which facilitated the generation of more reliable data. Due to their relatively short lifespan of ~5 weeks [36], *Drosophila* serves as an efficient model for rapid data generation, making it well suited for this study. Flies were divided into three groups: concussed, concussed plus an individual phytoceutical component, and control groups ($n = 40$ per group). Flies were kept in an incubator with a 12-hour day–night cycle at 25°C. Fly food was prepared by mixing 7.6 g of instant medium (Jazz-Mix *Drosophila* Food, Thermo Fisher Scientific, MA, USA) with 23 ml of deionized water per bottle. A few grains of baker's yeast were added to each bottle, and each bottle was plugged with a foam plug and left to set for at least an hour at room temperature before transferring the flies (progenies) into them. Fresh food was prepared every 2 weeks, and flies were flipped into new bottles every 2 to 3 days.

Fly concussion model induction

The creation and evaluation of the concussion model uses the HIT device constructed in the lab that is based on a TBI model device in flies previously described ([Fig. 1](#)) [5, 29]. Flies from a given vial were transferred into an empty vial and attached to the free end of the spring. The spring was lifted by the metal bracket, with the horizontal midpoint of the spring aligned at the 45° angle that delivered a force of 1.8 N. After being released, the vial rapidly contacted the foam pad, with flies hitting the vial walls, eliciting TBIs. Hits were repeated five times with 48–96-hour intervals, on Days 11, 13, 18, 20, and 25 post-eclosure (into adulthood) in all experiments. For each experiment, the control group was not exposed to hits.

Product concentration

A concentration of 0.1 mg/ml of the water-extracted ground powder product(s) (*W. somnifera*, *C. longa*, *M. officinalis*, *R. rosea*, and *V. myrtillus*) was tested to investigate the phytoceutical product (whole or individual components) in flies. The 0.1 mg/ml concentration was chosen to reflect the one used in a previously performed tolerance human trial and is currently under exploration in an efficacy trial. The acute safety and toxicity of this concentration were validated on flies (not shown).

Fly treatment

At the start of each experiment, flies were allowed to lay eggs on food, and once the larvae appeared, the adult flies were released from the bottles (which was counted as Day 0 post-eclosure). After 6 days, the progenies were transferred to

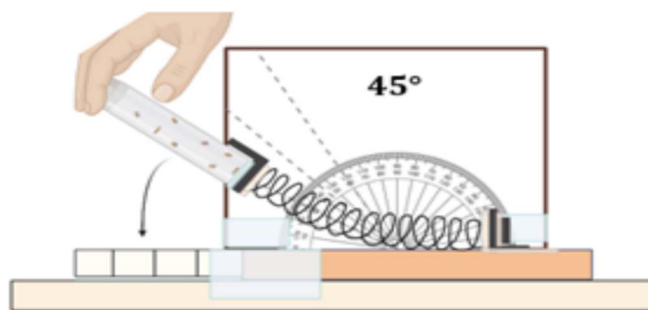


Figure 1. Schematic of the HIT device used to induce the concussion model in flies, with a 45° angle of release. It consists of a metal spring clamped at one end to a wooden board with the free end positioned over a pad.

fresh food. Female flies were selected for the experiment and were differentiated from males under a microscope based on phenotypic sex differences using CO₂. The phytoceutical drug administration started on Day 4 post-eclosure and continued throughout the experiment. Flies were put into vials for the experiment with 1.2 g of food. For concussed and control groups, 4 ml of distilled water was added to the food. For the treated groups, 3.6 ml of distilled water plus 0.4 ml of an individual phytoceutical component was added to the food. A few grains of baker's yeast were added to all the vials.

Survival rate (lifespan assay)

The Kaplan–Meier survival curve is also commonly used to evaluate the health of flies during a period of time. Every 2 days flies were counted and recorded. The survival rate per day was calculated by determining the percentage of surviving flies relative to Day 0. The experiment was carried out until 50% of the control group died, which allowed us to investigate the effects of multiple concussions on the lifespan of flies as well as having sufficient survivors to carry out the RONS assay. On the other hand, we investigated the effect of the whole phytoceutical drug, in which the experiment duration was until every fly in each group died, and this was a full lifespan experiment.

Climbing assay

The climbing assay is the most frequently used approach for measuring the motor function of flies [33]. For the motor activity, performed every 6 to 7 days, all the groups were tested at random [36]. Flies were transferred into an empty 100 ml (Pyrex graduated cylinder with a foam plug, with a 10 cm horizontal line drawn on the cylinder, without using CO₂ as this can have effects on fly behaviour [37]. Flies were left 15 min in the cylinder to acclimate. The flies were then gently tapped down and allowed to climb up past the 10 cm mark (in 15 s) on the cylinder and, afterward, tapped down again. A digital camera was used to record the flies at 25 cm from the paper. The total number of flies that crossed the 10 cm line was recorded as the “flies that pass” the line. This was repeated six times. The ability of flies to survive per day (%) was calculated by dividing the number of flies that climbed over the 10 cm line by the total number of surviving flies multiplied by a hundred. The mean of each group was calculated using the data from the six replicate tests.

Biochemical assays in fly brains and bodies

At key dates of the phytoceutical treatment, 10 flies from each group, the concussed, control, and treated groups (PBS

only and drug), were anesthetized with CO₂, and flies were desiccated to separate heads from the body under a microscope. The fly heads and bodies were put separately in Eppendorf filled with a given volume of 0.1 M PBS pH 7.0 and then homogenized using a small homogenizer. Eppendorf was stored at –80°C until further use in the assay.

RONS level assay

To determine the RONS levels, oxidation was measured as an index of oxidative stress using a commonly used 5(6)-carboxy-2',7'-dichlorofluorescein diacetate (DCFHDA) assay [38]. Homogenized head and body samples of each group were pipetted into a 96-well plate, with distilled water, 0.1 M PBS (pH 7), and 200 μM DCFH-DA. The solution of 200 μM DCFH-DA was wrapped in foil to protect it from light. To each well of a transparent 96-well plate, 185 μl of PBS, 10 μl of samples (for blank wells, 10 μL of PBS was added), and 5 μL of DCF-DA were added. The fluorescence product was measured for 10 minutes (at 30 s intervals) using a Synergy H4 hybrid multimode microplate reader (excitation set at 488 and 525 nm emission) (Tecan Trading AG, Switzerland). The rate of DCF formation was expressed in percentage of the control group.

Statistical analyses

Data were processed in Microsoft Excel 2023 and GraphPad Prism software version 10 was used for all statistical analyses, including means, standard deviations (SDs), and *P*-values (where * significant *P* < .05; **highly significant *P* < .01; and n.s. denotes nonsignificant). To determine significant differences in climbing ability, lifespans, and the RONS levels, an adjusted two-way multiple measures analysis of variance (ANOVA) with post-test Bonferroni correction was performed. All quantitative data are expressed as mean values ± SD of the mean.

Results

Whole product effects

The analysis and comparison of fruit flies treated with the whole product revealed an improvement in climbing ability compared to the control group (Fig. 2). Moreover, the product significantly extended the lifespan of treated flies relative to the control (**P* < .05). Interestingly, the complete phytoceutical product significantly reduced RONS levels (***P* < .01) in the treated flies compared to the control group (Fig. 2).

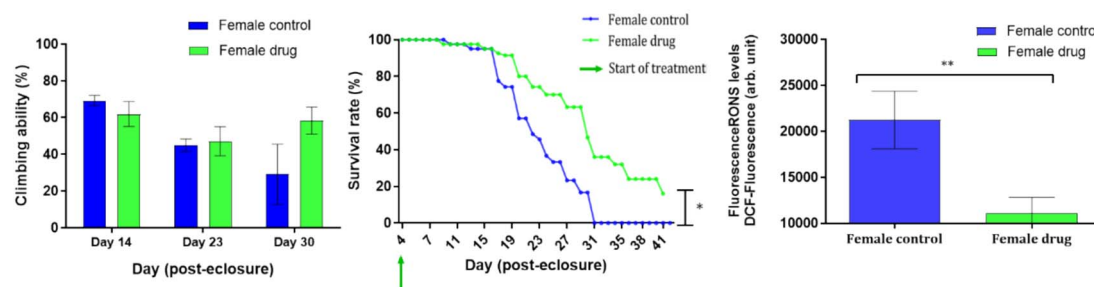


Figure 2. Effects of whole product on lifespan, motor activity, and oxidative stress of female flies. Climbing ability: mean averages of climbing ability (%) are shown, considering six repeats in the climbing assay day per group. Error bars show ± 1 SD between percentages in each group. RONS: lines and bars present mean averages, after subtracting sample-free blank averages and correcting to 0 arbitrary units at 0 s while maintaining line gradients. Error bars show ± 1 SD, accounting for blank and sample well replicates. * $P < .05$; ** $P < .01$; $n = 40$ flies per group.

Table 1. The average percentage of the climbing ability of flies: individual components vs whole phytoceutical.

No.	Individual components vs total phytoceutical product	Climbing ability (%) Averages of six climbing days (SD)
1	Ashwagandha— <i>Withania somnifera</i>	84.2 (± 3.6)
2	Turmeric— <i>Curcuma longa</i> + black pepper— <i>Piper nigrum</i>	83.5 (± 7.6)
3	Lemon balm— <i>Melissa officinalis</i>	92.6 (± 1.9)
4	Roseroor— <i>Rhodiola rosea</i>	89 (± 6.1)
5	Bilberry— <i>Vaccinium myrtillus</i>	92.3 (± 7.0)
Total phytoceutical product		87.0 (± 5.2)

Individual component effects

Motor ability assay

A progressive decline was observed in the motor activity of flies who received five nonconsecutive concussions with increasing age (Fig. 3). The flies exposed to three and five concussions and simultaneously treated with the total phytoceutical product and individual components displayed a significant improvement in motor ability. For example, a highly significant increase in motor activity was seen in flies treated with lemon balm (Fig. 3C) and roseroot (Fig. 3D) compared with the concussed groups (** $P < .01$) on Day 28. Furthermore, a significant elevation in motor activity was seen in flies treated with ashwagandha (Fig. 3A) and bilberry (Fig. 2E) compared with the concussed groups (* $P < .05$). It should be mentioned that flies treated with turmeric plus black pepper showed significantly improved motor ability compared with other groups (Fig. 3B). This experiment was previously carried out with only turmeric, which showed modest improvement (not shown). Finally, the average of the total phytoceutical product for each figure (Fig. 3F) indicates that the motor ability of the treated flies' group was gradually improved, and there was a highly significant difference between them and the concussed group on Day 28 (** $P < .01$).

To compare flies' climbing ability to each component and the whole phytoceutical product, Table 1 shows the mean percentages of climbing ability for the six experimental days. While all of the five components resulted in improved climbing ability in flies following repetitive concussions, the two components (lemon balm and bilberry) resulted in the largest improvement in motor activity.

Lifespan

Similar to the effect on motor activity, the concussed flies (red lines) have shorter survival rates compared with the other two groups (controls and concussed plus treated groups). There

was a significant increase in the survival rate of concussed flies treated with the total product and all the individual components compared with the concussed and control groups (Fig. 4A–F, * $P < .05$). Notably, a highly significant improvement was observed in the survival numbers of concussed flies treated with lemon balm (Fig. 4C) compared with the concussed and control group (** $P < .01$).

RONS level assay

The RONS production levels were calculated in the brains and bodies of the flies from the three groups (concussed, concussed plus products, and control groups). The treatment with ashwagandha (Fig. 5A), turmeric plus black pepper (Fig. 5B), lemon balm (Fig. 5C), and bilberry (Fig. 5E) significantly reduced the levels of RONS in fly bodies, compared with the concussed group (** $P < .01$). Moreover, the treatment with ashwagandha (Fig. 5A) and with lemon balm (Fig. 5C) significantly lowered the RONS levels in the brains of flies (* $P < .05$ and $.01$, respectively). However, it should be mentioned that flies treated previously with only turmeric did not significantly reduce RONS levels in the heads or bodies of flies.

Discussion

The biological effects following concussions vary across individual species, which also applies to flies that respond differently to head and body trauma. This could be due to the fact that the location or strength of concussions plays an important role. *Drosophila* is extensively used as an *in vivo* paradigm to detect the function of genes involved in multiple human neurodegenerative diseases [1]. Therefore, we hypothesized that *Drosophila* could be a useful model to study concussion outcomes and investigate a novel product designed to manage head and body trauma.

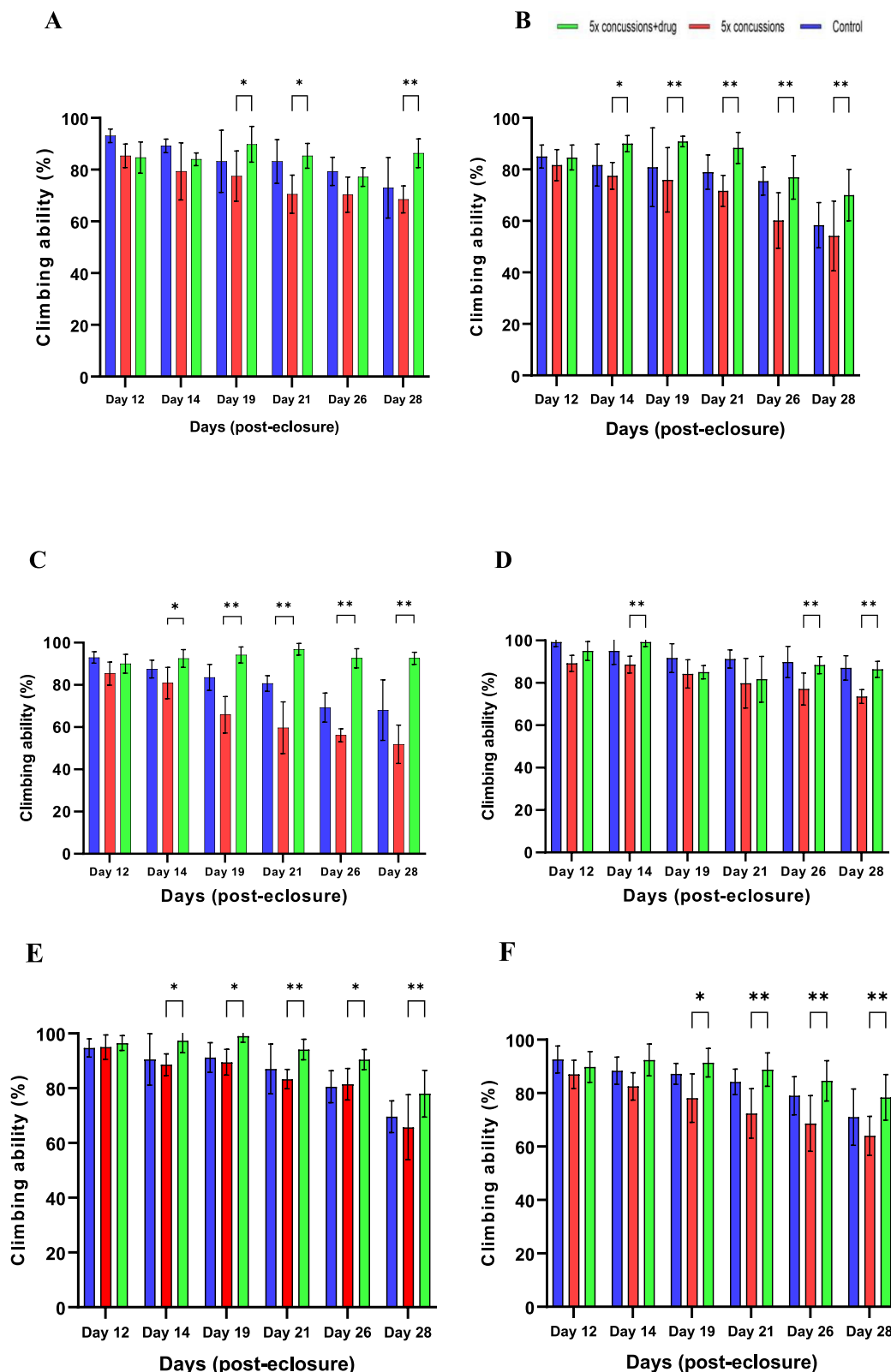


Figure 3. The percentage of fly climbing ability in female WT flies. The red columns represent the concussed groups, and the blue columns indicate the controls. (A) Hits were repeated five times with 48–96-hour intervals, on Days 11, 13, 18, 20, and 25 post-eclosure (into adulthood) in all experiments. The green columns represent the flies treated with ashwagandha, (B) turmeric, (C) lemon balm, (D) roseroor, and (E) bilberry. (F) Whole product. Mean averages of climbing ability (%), considering six repeats in the climbing assay day per group. Error bars show ± 1 SD between percentages in each group. * $P < .05$; ** $P < .01$; $n = 40$ flies per group.

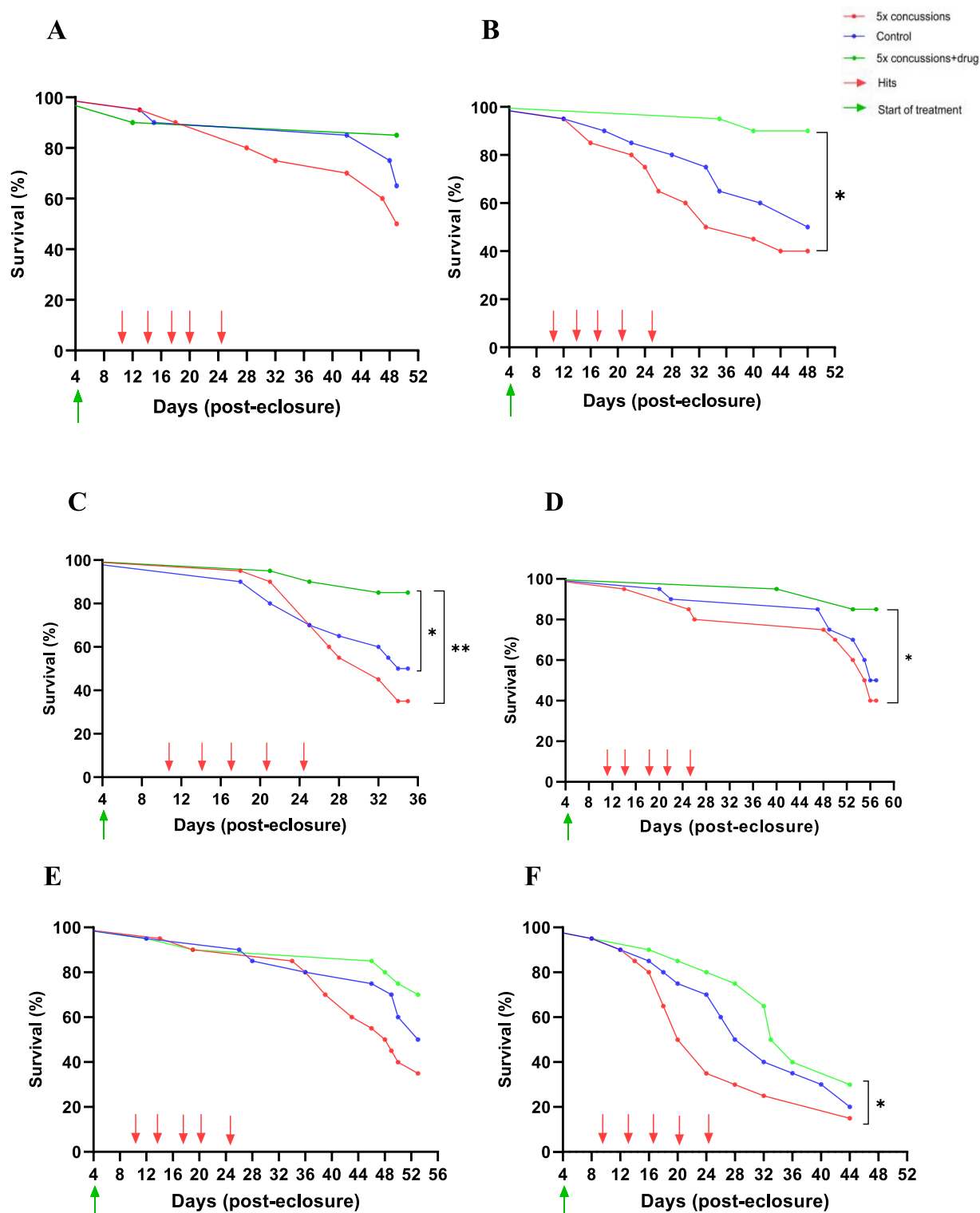


Figure 4. The percentage of fly survival in lifespan studies in female WT flies. The red line represents the concussed group and the blue line indicates the control group. The green lines represent the concussed fly groups treated with (A) ashwagandha, (B) turmeric, (C) lemon balm, (D) roseroot, and (E) bilberry. (F) The green line is the concussed fly group treated with the whole phytochemical product. The green arrow is the start of the treatment, while the red arrows are the concussion hit days for the fly groups that received hits (red and green line). * $P < .05$. Hits were repeated five times with 48–96-hour intervals, on Days 11, 13, 18, 20, and 25 post-eclosure (into adulthood) in all the experiments. ** $P < .01$; $n = 40$ flies per group.

Previous studies have demonstrated that flies can be used as a model to better understand the outcomes of traumatic brain injuries (TBIs). Barekat and coworkers [1] developed a TBI model using adult *Drosophila*. They observed that control

flies and flies exposed to mild repetitive TBI displayed similar climbing indexes, while flies exposed to severe TBI exhibited a modest, but significant, reduction in climbing abilities, indicating that they had sustained a modest level of damage to

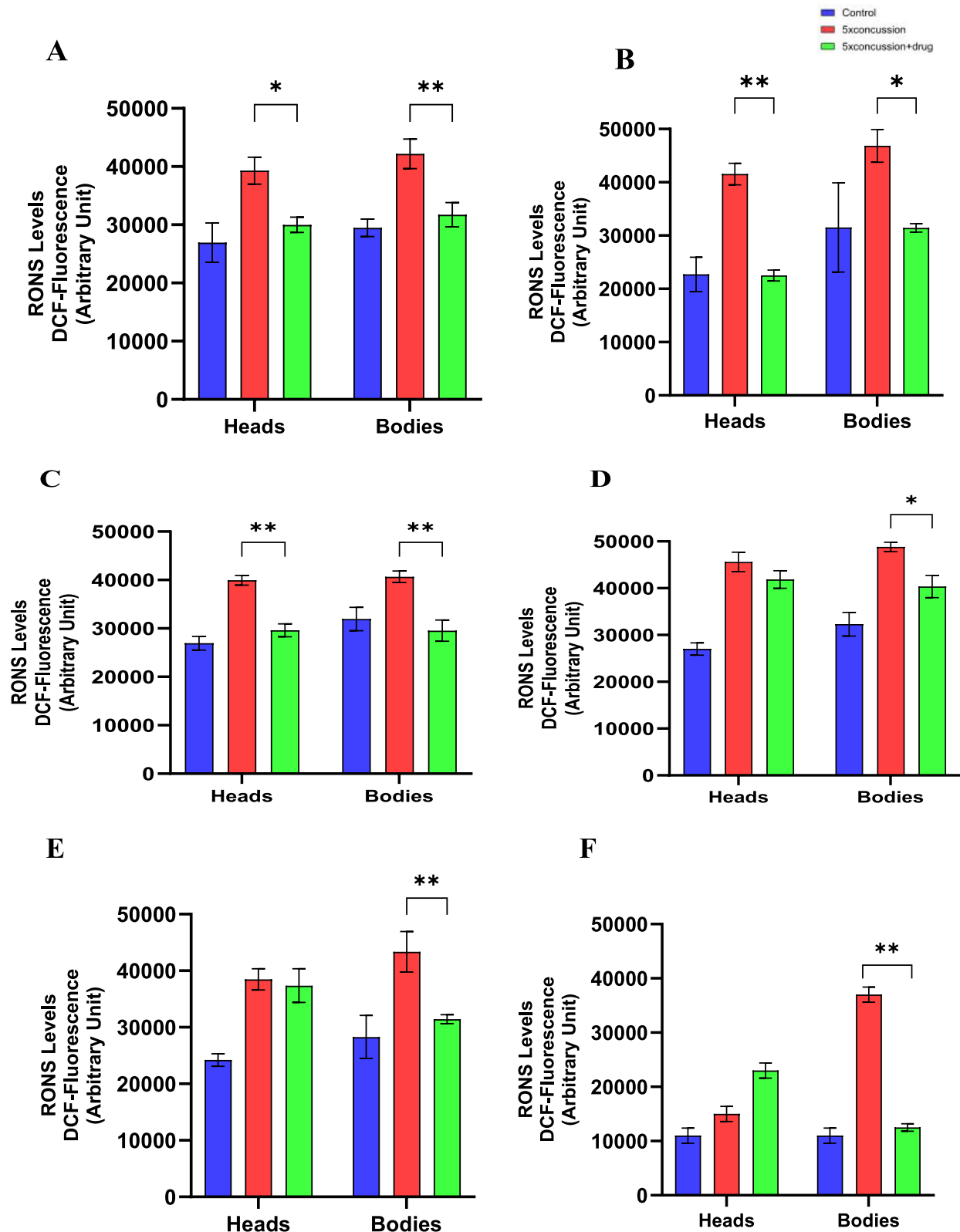


Figure 5. Effect of the individual and whole phytoceutical on the RONS levels in flies (heads and bodies). The red columns represent the concussed group and the blue columns indicate the control group. Hits were repeated five times with 48–96-hour intervals, on Days 11, 13, 18, 20, and 25 post-eclosure (into adulthood) in all the experiments. (A) The green columns represent the concussed group flies treated with (A) ashwagandha, (B) turmeric, (C) lemon balm, (D) roseroot, and (E) bilberry. (F) The green column represents the flies treated with the whole phytoceutical product. Mean averages, after subtracting sample-free blank averages. Error bars show ± 1 SD, accounting for blank and sample well replicates. * $P < .05$; ** $P < .01$; $n = 3$ replicates.

internal structures and tissues. They also reported that flies exhibited increased mortality following a single severe TBI compared to mild TBI [1]. To investigate the mechanisms underlying TBI pathologies [5], they developed a model of TBI in *D. melanogaster*. They observed that, similarly to humans with TBI, flies exposed to TBI exhibited temporary incapacitation, ataxia, activation of the innate immune response, neurodegeneration, and death. They reported that TBI results in death shortly after a primary injury only if the injury exceeds a certain threshold and that age and genetic background, but not sex, substantially affect this threshold. Furthermore, this threshold also appears to be dependent on the same cellular and molecular mechanisms that control normal longevity [5]. These results are consistent with the findings of this study as repetitive concussions result in motor deficits and increased mortality of flies.

To evaluate the effects of TBI *in vivo*, a mild trauma and a severe trauma were applied to *Drosophila* and it was found that both forms of TBI lead to the induction of stress granules in the brain. The degree of stress granule induction directly correlates with the level of trauma. Furthermore, they observed that the level of mortality is directly proportional to the number of traumatic hits. TBI in animals expressing amyotrophic lateral sclerosis (ALS)-linked genes increased mortality and locomotion dysfunction, suggesting that mild trauma may aggravate symptoms associated with ALS [39]. Using the HIT device, this hypothesis was tested [40]. Closed-head TBI in young adult *Drosophila* induced motor deficits, associated with increased oxidative and nitrosative stress in the brain. It was found that HIT causes severity-dependent increases in phenotypic acute behavioural deficits and mortality. They also denoted that several measures of oxidative stress, including *Drosophila* nitric oxide synthase expression, protein nitration, and hydrogen peroxide production, were significantly decreased in female flies [40]. These findings agree with our results that multiple concussions lead to motor deficits, shorten lifespan, and increase the RONS levels in heads and bodies.

Recently, a TBI model in *D. melanogaster* was developed to understand concussion, postconcussion syndrome (PCS), and concussive trauma encephalopathy (CTE). Fly motor ability was not significantly different acutely or in the long term following HIT device impacts. Similarly, with 1.8 N impacts, lifespan was not significantly different compared with nontrauma controls. Meanwhile, RONS levels increased in both fly heads (brains) and bodies (periphery) with five 1.8 N impacts, representative of physiological stress in contact sportspeople with PCS [29]. Sun and Chen [41] developed a model of CTE in *Drosophila*. They observed that the mTBI-treated group showed reduced walking activity and distance travelled compared with the sham group. Furthermore, compared with the sham group ($n = 129$), treated flies ($n = 100$) had a substantially reduced median lifespan and significantly reduced maximum lifespan. They emphasized that the ongoing characterization of the model will generate important mechanistic insights into disease prevention and therapeutic approaches [41]. Using the TBI *Drosophila* model, Shah *et al.* [42] evaluated and compared biological sex between males and females focusing on gene transcription changes. They found that following TBI, females of *Drosophila* showed more gene transcript changes than males. Female flies also exhibited upregulated expression changes in immune response and mitochondrial genes across all time

points. Although both males and females showed similar changes in mitochondrial oxidation and negative geotaxis, locomotor activity was found to be more weakened in males compared with females. They suggested that sex variations not only impact the response to TBI but also contribute to varied outcomes postinjury [42]. Behnke *et al.* [43] developed a *D. melanogaster* head impact model to look into the long-term effects of mTBI on the structure and function of the brain and underlying mechanisms. They discovered that flies subjected to repetitive head impacts develop long-term deficits, including impaired startle-induced climbing, progressive brain degeneration, and shortened lifespan, all of which are substantially exacerbated in female flies. Furthermore, head impacts elicit an elevation in neuronal activity and its acute suppression abrogates the detrimental effects in female families [43].

Alphen *et al.* [44] developed the *Drosophila* closed head injury (dCHI) model, which involves the delivery of preset, nonpenetrating strikes to the heads of unanesthetized flies using the forward movement of a brass block. They observed that the dCHI model induces analogous TBI phenotypes, including increased motor deficits, neuronal cell death, mortality, and an altered sleep/wake cycle [44]. In a similar vein, Saikumar *et al.* [45] constructed a modified dTBI model that involves using a piezoelectric actuator that rapidly compresses the head of *Drosophila* with precision. They discovered that the dTBI led to dose-dependent and long-lasting neurological deficits, including deficits in righting reflex, climbing, and reduced lifespan. Furthermore, severe dTBI is linked with cognitive decline and transient glial dysfunction and stimulates antioxidant, proteasome, and chaperone activity. Together, these results pose a tunable, head-specific method for TBI in *Drosophila* that recapitulates mammalian injury phenotypes and underscores the ability of the stress response to mitigate TBI-induced brain degeneration [45].

Interestingly, we were able to demonstrate that the novel phytochemical product can be useful in protecting from motor deficits, improving lifespan, and maintaining lower levels of oxidative stress in the heads and bodies of flies. All five components contributed to the positive properties as predicted [11–28], eliciting effects on both physical and mental health following a series of repetitive concussions. The *C. longa* component alone did not have a full effect on the flies (not shown), which may be attributed to poor bioavailability [46]. However, the addition of black pepper to turmeric improved its bioavailability, resulting in enhanced motor function, extended lifespan, and reduced RONS levels in the heads and bodies of the flies [47]. Among the components, *M. officinalis* (lemon balm) and *V. myrtillus* (bilberry) appeared to elicit particularly strong effects. Lemon balm, known for its anxiolytic, neuroprotective, and cognition-enhancing properties, and modulation of voltage-gated sodium channels, may have contributed to profound improvements in lifespan, motor ability, and stress resilience in both the brain and body. Bilberry, rich in anthocyanins and polyphenols, has demonstrated potent antioxidant and anti-inflammatory effects in prior studies, which could explain its role in reducing RONS and supporting neuronal health [17, 26, 48]. The combination of these components, acting synergistically, likely amplified their individual benefits, underscoring the importance of multitargeted approaches in mitigating the long-term impacts of TBIs [49].

These findings may have translational value, as concussions and TBIs are closely associated with increased oxidative stress,

a hallmark of several chronic neurodegenerative conditions such as Alzheimer's disease, motor neuron disease (ALS), and CTE. By targeting oxidative stress and functional decline early, such phytochemical combinations could potentially serve as adjunct therapies in prophylactic neurodegenerative disease prevention.

Conclusions

The fly concussion model allowed us to better understand concussion outcomes and facilitate examining the proposed phytochemical components. Our findings demonstrated that recurrent head hits increased mobility deficits, shortened lifespan, and increased oxidative stress, which could increase the risk of neurodegenerative diseases. The data therefore suggested that the phytochemical product can be used as a therapeutic approach to improve motor deficits, enhance lifespan, and maintain appropriate levels of antioxidants.

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Author contributions

P.C., K.H., and D.G.: Conceptualization. P.C., N.A., A.F., E.A.E., D.G., S.Q., and M-J. H.: Methodology. P.C.: Software. N.A., E.A.E., S.Q., J.A.F. and M-J. H.: Investigation. P.C.: Resources. P.C.: Data curation. N.A.: Writing—original draft. P.C., N.A. and M-J. H.: Writing—review & editing. N.A., A.F., E.A.E., and M-J. H.: Visualization. P.C., K.H., and D.G.: Supervision. P.C., K.H., and D.G.: Project administration; P.C.: Funding acquisition.

Supplementary data

Supplementary data is available at *Journal of Pharmacy and Pharmacology* online.

Conflict of interest

The authors declare no conflict of interest.

Funding

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

Ethical approval

This study did not require ethical approval.

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