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Exploring the Effects of the CONKA Nutraceutical Health Product in a *Drosophila melanogaster* Repetitive Concussion Model

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Abstract: There is growing interest in understanding the connection between concussions on physical and mental health acutely and, in retirement, on neurodegenerative diseases. In this study, we utilised a "high-impact trauma" (HIT) device to investigate the effects of multiple concussions on the motor activity, and lifespan of the adult female *Drosophila melanogaster* as well as reactive oxygen and nitrogen species (RONS) levels in the fly brain and body. We found that repetitive hits, while not having acute physical effects, significantly increased long-term mobility deficits, and shortened lifespan, and exacerbated oxidative stress in both the brain and body. Notably, the novel CONKA product (*Withania somnifera, Curcuma longa, Melissa officinalis, Rhodiola rosea, Vaccinium myrtillus)* demonstrated promising protective effects, including mitigation of motor deficits, extension of lifespan, and reduction of oxidative stress in both the brain and body of the flies. When evaluating the contributions of individual components within the CONKA formulation, *Curcuma longa*, despite extending lifespan, did not contribute to mobility improvement or oxidative stress amelioration. This suggests that the benefits of CONKA are largely driven by its other four components, which displayed all the positive effects evaluated. The exclusion of *Curcuma longa* may streamline the formulation without diminishing its brain and body effects following a history of repetitive concussions, although this would require further study to confirm. Oral bioavailability may be an issue with *Curcuma longa.* Taken together, the findings validate that *Drosophila melanogaster* is a suitable system to mimic and investigate the effects of repetitive concussions on bodies and brains and assess the effects of health products and drug therapies.

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

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

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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 available scientific evidence relevant to both physical and mental health consequences of concussion. The CONKA nutraceutical, 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 is based on many previous studies which associate these species with physical and mental health attributes. For example, it was found that ashwagandha (*Withania somnifera* root) has anxiolytic effects [9], improves sleep parameters [10], increases muscle mass and strength [11], is anti-neuroinflammatory [12], and has positive memory effects [13]. Turmeric (*Curcuma longa* root) has potent anti-inflammatory effects [14], is antioxidant [15], and has positive cardiovascular effects [16]. Lemon balm (*Melissa officinalis* leaves) has calming, analgesic and anti-seizure effects and maintains attention [17–20], and has anti-inflammatory effects [21]. Roseroot (*Rhodiola 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 (*Vaccinium myrtillus* fruit) is anti-inflammatory and antioxidant [26], has positive effects on fatigue [27], and may improve visual deficits [28].

To investigate the behavioral consequences of concussion as well as testing the CONKA nutraceutical, we recently developed a *Drosophila melanogaster* model [29]. The fruit fly *Drosophila melanogaster* 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 behaviors while having a relatively small number of neurons (ca.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 analyze 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 it in the food. Therefore, the test of the CONKA nutraceutical in *Drosophila 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 CONKA nutraceutical product can have a positive effect on the physical and mental health and behavior of the fruit flies.

2. Results

Motor Ability Assay

An observable progressive decline in the motor activity of flies who received five nonconsecutive concussions with increasing age (Figure 1). The flies exposed to five concussions and simultaneously treated with the total CONKA nutraceutical and individual components (except turmeric, Figure 1B) displayed a significant improvement in motor ability. For example, a highly significant increase in motor activity was seen in flies treated with lemon balm (Figure 1C) and roseroot (Figure 1D) compared to the concussed groups (** $p < 0.01$), at day 28. Furthermore, a significant elevation in motor activity was seen in flies treated with ashwagandha (Figure 1A) and

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bilberry (Figure 1E) compared to the concussed groups (* $p < 0.05$). Finally, the average of the total CONKA nutraceutical for each figure (Figure 1F) indicates that the motor ability of the treated flies' group was gradually improved and there is a highly significant difference between them and the concussed group on day 28 (** $p < 0.01$).

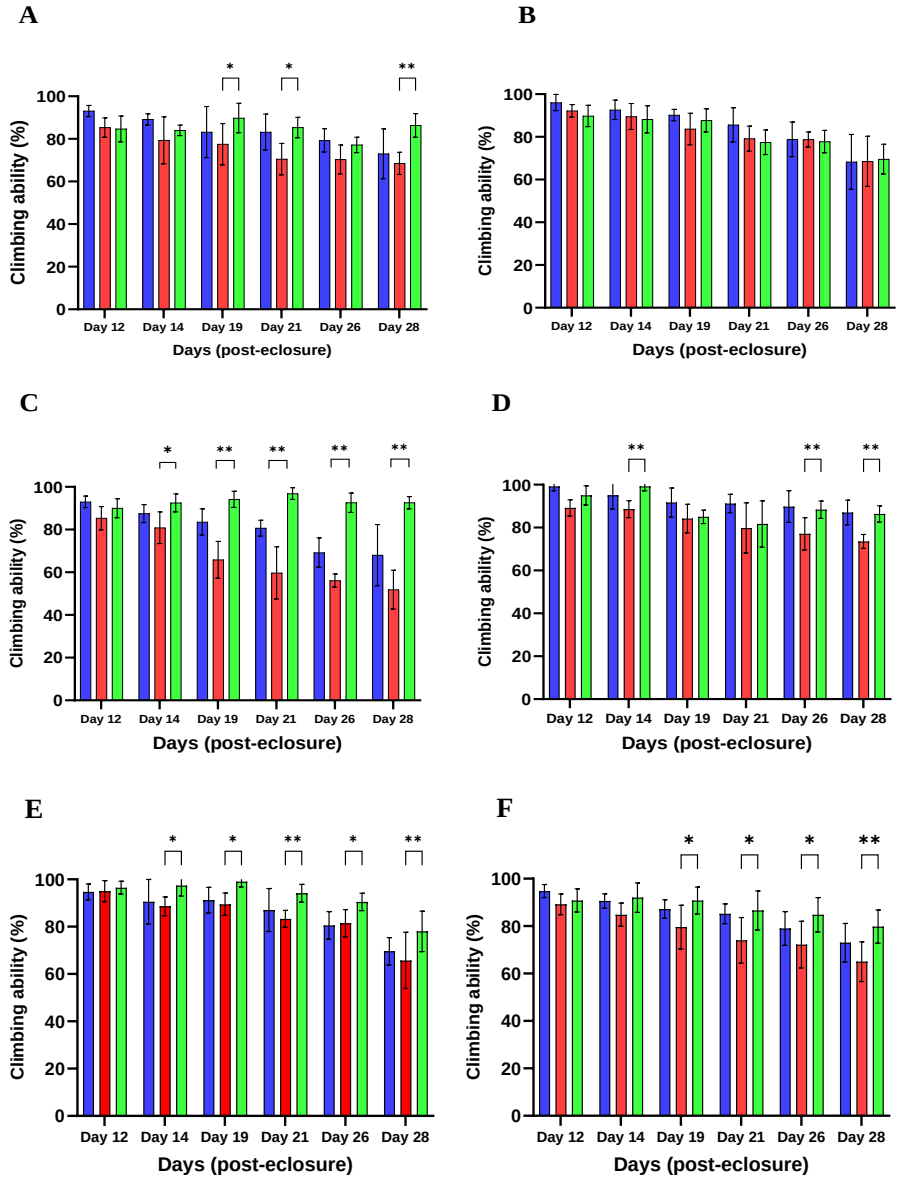

Figure 1. The percentage of fly climbing ability in female wild-type flies. The red bar lines represent the concussed groups, and the blue bar lines indicate the controls. **A**) The green bar lines represent the treated flies with Ashwagandha, **B**) Turmeric, **C**) Lemon balm, **D**) Roseroot, and E) Bilberry. **F**) Average of the total CONKA nutraceutical for each figure. The line bars represent the 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. * $p < 0.05$; ** $p < 0.01$.

To compare flies' climbing ability to each component and the whole CONKA nutraceutical, Table 1 shows the mean percentages of climbing ability for the six experimental days. It can be seen that 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.

Table 1. The average percentages of the climbing ability of flies. Individual components vs whole CONKA nutraceutical.

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No	Individual Components vs Total CONKA	Climbing ability (%) Averages of six climbing days
1	Ashwagandha – *Withania somnifera*	84%
2	Turmeric – *Curcuma longa*	81%
3	Lemon balm – *Melissa officinalis*	92%
4	Roseroot – *Rhodiola rosea*	89%
5	Bilberry – *Vaccinium myrtillus*	92%
Average total CONKA nutraceutical		87%

Lifespan

Similar to the effect on motor activity, the concussed flies (red lines) have shorter survival rates compared to 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 to the concussed group (Figures, 2A, 2D, 2E, and 2F, * $p < 0.05$). Notably, highly significant improvement was observed in the survival numbers of concussed flies treated with lemon balm (Figure 2C) compared to the concussed group (** $p < 0.01$).

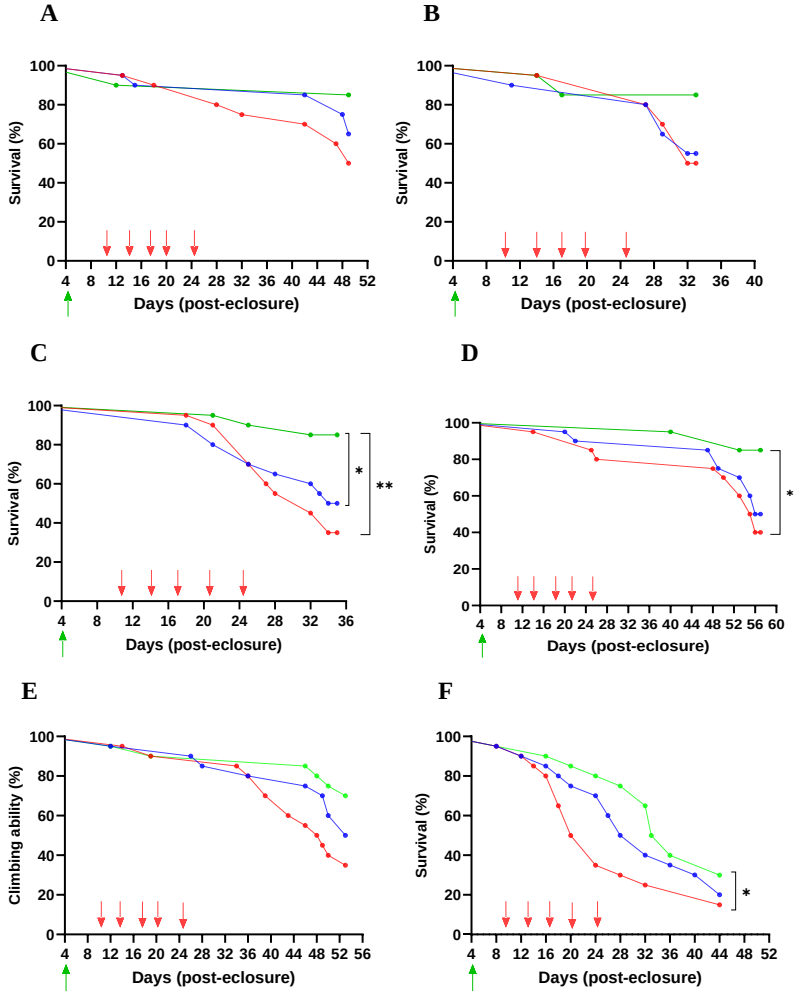

Figure 2. The percentage of fly survival in lifespan studies in female wild-type flies. The red line represents the concussed group whereas the blue line indicates the control group. **A**) The green lines represent the flies treated with Ashwagandha, **B**) Turmeric, **C**) Lemon balm, **D**) Roseroot and **E**) Bilberry. **F**) The green line is the treated flies with the whole CONKA nutraceutical. The green arrow is the start of the treatment, while the red arrows are the concussion hit days. * $p < 0.05$; ** $p < 0.01$.

Reactive Oxygen and Nitrogen Species (RONS) Levels

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The RONS production levels were calculated in the brains and bodies of the flies from the three groups (concussed, concussed + products, and control groups). The treatment with ashwagandha (Figure 3A), lemon balm (Figure 3C), and bilberry (Figure 3E) significantly reduced the levels of RONS in fly bodies, compared to the concussed group (** $p < 0.01$). Moreover, in addition, the treatment with ashwagandha (Figure 3A) and with lemon balm (Figure 3C) significantly lowered the RONS levels in the brains of flies (* $p < 0.05$ and 0.01, respectively). However, the treatment with turmeric (Figure 3B) displayed no effects on the levels of RONS either in the brains and bodies of flies. Interestingly, the whole CONKA (Figure 3F) significantly reduced the RONS levels only in the bodies of the flies' (** $p < 0.01$) compared to the concussed group (Figure 3F).

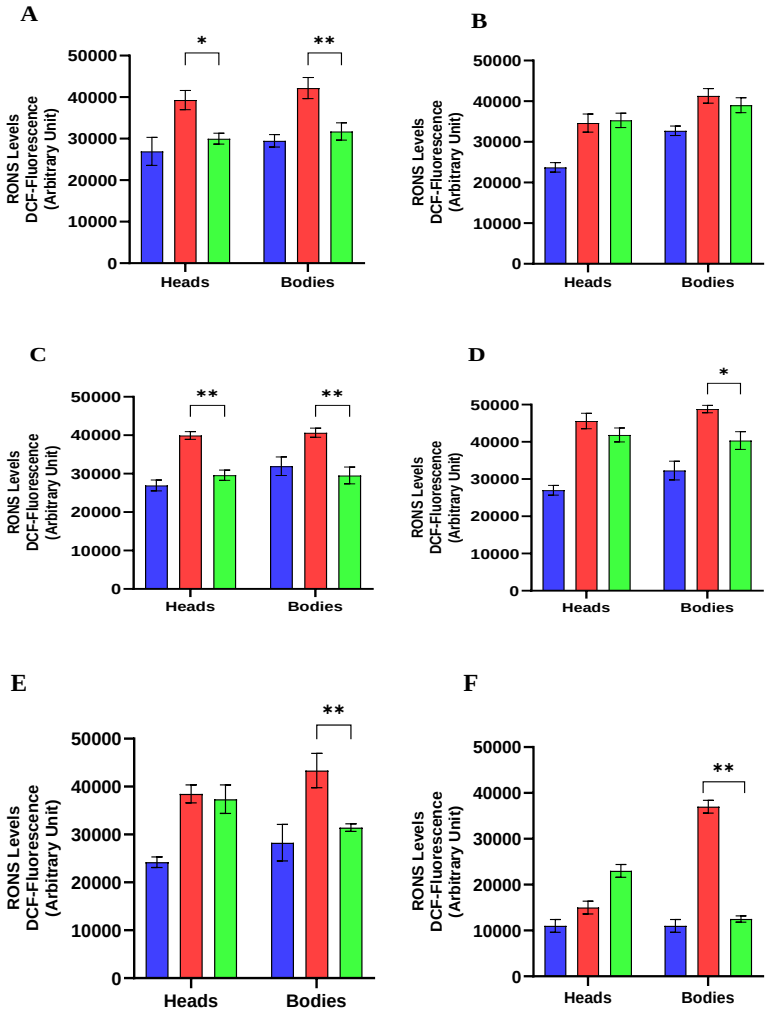

Figure 3. Effect of the individual and whole CONKA nutraceutical on the RONS levels in flies (heads and bodies). The red bar lines represent the concussed group whereas the blue lines indicate the control group. **A**) The green lines represent the treated flies with Ashwagandha –, **B**) Turmeric, **C**) Lemon balm, **D**) Roseroot and **E**) Bilberry. **F**) The green bar lines represent the flies treated with the whole CONKA nutraceutical. Lines and bars present mean averages, after subtracting sample-free blank averages and correcting to 0 arbitrary units at 0 secs while maintaining line gradients. Error bars show ± 1 SD, accounting for blank and sample well replicates. * $p < 0.05$; ** $p < 0.01$.

3. 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

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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.

Previous studies demonstrated that flies can be used as a model to better understand the outcomes of traumatic brain injuries. Barekat *and co-workers* [1] developed a TBI model using adult *Drosophila.* They observed that control and mild repetitive TBI flies displayed similar climbing indexes, while flies exposed to severe TBI exhibited a modest, but significant, reduction in climbing abilities, indicating that they sustained a modest level of damage to internal structures and tissues. They also reported that flies exhibited increased mortality following a single severe TBI compared to mTBI [1]. To investigate the mechanisms underlying TBI pathologies, [5] a model of TBI in *Drosophila melanogaster* was developed. They denoted that, similar 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 present study as repetitive concussions result in motor deficits and increased mortality of flies.

To evaluate the effects of TBI *in vivo*, a mild and severe trauma was applied to *Drosophila* and found out that TBI leads the induction of stress granules in the brain. The degree of stress granules 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 on animals expressing ALS-linked genes increased mortality and locomotion dysfunction suggesting that mild trauma may aggravate symptoms associated with ALS [35]. Using the HIT device, this hypothesis was tested [36]. Closed-head TBI in young adult *Drosophila* induced motor deficits, associated with increased oxidative and nitrosative stress in the brain. They found that HIT causes severity-dependent increases in phenotypic acute behavioral 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 [36]. 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 *Drosophila melanogaster* was developed to understand concussion, PCS, and CTE. They observed that fly motor ability was not significantly different acutely or long-term following HIT device impacts. Similarly, with 1.8N impacts, lifespan was not significantly different compared to non-trauma controls. Meanwhile, RONS levels increased in both fly heads (brains) and bodies (periphery) with five 1.8N impacts, representative of physiological stress in contact sportspeople with PCS [29]. Sun and Chen [37] developed a model of CTE in *Drosophila melanogaster*. They observed that the mTBI-treated group showed reduced walking activity and distance travelled compared to the sham group. Furthermore, compared to the sham group (n = 129), treated flies (n = 100) had a substantially reduced median lifespan and significantly reduced maximum life span. They emphasized that the ongoing characterization of the model will generate important mechanistic insights into disease prevention and therapeutic approaches [37]. Using the TBI *Drosophila melanogaster* model, Shah et al., [38] 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 weaken in males compared to females. They suggested that sex variations not only impact the response to TBI but also contribute to varied outcomes post-injury [38]. Behnke *et al*., [39] developed a head impact *Drosophila melanogaster* 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

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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 [39].

Alphen *et al*. [40] developed the *Drosophila* closed head injury (dCHI) model, which involves the delivery of preset, non-penetrating strikes to the heads of unanaesthetised 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 altered sleep/wake cycle [40]. In a similar vein, Saikumar *et al*., [41] 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 [41].

Interestingly, we were able to demonstrate that the novel CONKA product can be useful in protecting from motor deficits, improving lifespan, and maintaining lower levels of oxidative stress in the heads and bodies of the 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 lack of a full effect of *Curcuma longa* component may be due to poor bio-availability [42].

4. Methods and Materials

4.1. Plant Powders

All plant powders were sourced from Herbal Apothecary, UK. All powders were analysed separately by LC-MS to detect and verify compounds known to occur in the plants investigated. The LC-MS method is described in the Supplementary Information and the assigned compounds detected in each of the plant powders are shown in Supplementary Tables S1 – S5.

4.2. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis Method

Each powder samples was extracted in 70% ethanol (1ml per 100mg of material) at room temperature for 24h, prior to centrifugation and transfer of supernatants to LC-MS vials. Supernatants were analysed using a Thermo Scientific LC-MS system consisting of a 'Vanquish Flex' U-HPLC-PDA, and an 'Orbitrap Fusion' mass spectrometer fitted with an "Ion Max NG" heated electrospray source (Thermo Scientific, Waltham, MA, USA). Chromatography was performed on 5 µl sample injections onto a 150 mm x 3 mm, 3 µm Luna C-18(2) column (Phenomenex, Torrance, CA, USA) using the following 400µl/min mobile phase gradient of H_2O/CH_3OH/CH_3CN +1% HCOOH: 90:0:10 (0 min), 0:90:10 (60 min), 0:90:10 (70 min), 90:0:10 (71 min), 90:0:10 (75 min). Solvents were obtained from Fisher Scientific (OPTIMA LC-MS grade). The heated ESI source was operated under the manufacturer's default conditions for the flow rate employed and the mass spectrometer was set to record high resolution (60 k resolution) MS1 spectra (m/z 125–1800) in both positive and negative modes using the orbitrap; and data dependent MS2 and MS3 spectra in both modes using the linear ion trap. Detected compounds were assigned by comparison of accurate mass (ppm) and interpretation of available MSn and UV spectra, with reference to Kew's in-house libraries of ion trap MS and UV spectra (Supplementary references S1-22}.

4.3. Water Extract

To run the individual experiment, each component of the CONKA product was prepared separately (100 mg/mL of each component), while the whole CONKA product stock solution (3mg/ml) was prepared based on the following ratio of plant powders: (1.05g *Withania somnifera*, 1.05g *Curcuma longa*, 1.05g *Melissa officinalis*, 350mg *Rhodiola rosea*, 350mg *Vaccinium myrtillus*), which is as

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used in a human tolerance health trial. The plant-powder mixture was dissolved in distilled water, and vortexed vigorously, prior to aliquoting into fly food.

4.4. Flies Stocks and Culturing Conditions

Drosophila melanogaster female wild-type (WT) was used for this experiment. Flies were divided into three groups: concussed, concussed + an individual CONKA component, and control groups (n= 40 per group). Flies were kept in an incubator with a 12-h day-night cycle at 25°C. Fly food was prepared by mixing 7.6 g of instant medium (Jazz-Mix Drophila 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, 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 two weeks, and flies were flipped into new bottles every 2 to 3 days.

4.5. 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 (Figure 4) [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 1.8N. After being released, the vial rapidly contacted the foam pad, with flies hitting the vial walls eliciting TBIs. Hits were repeated five times with 48-96hr 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.

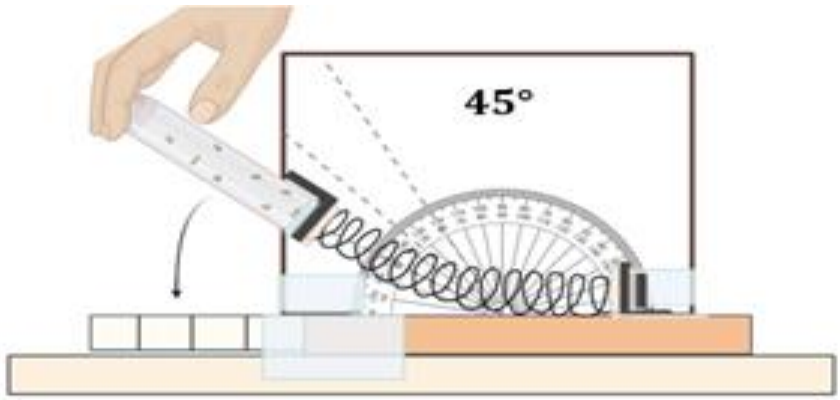

Figure 4. Schematic of the HIT device used to induce the PCS model in flies, with a 45° angle of release. It consists of a metal spring clamped at one end to a wooden board with the free end positioned over a pad.

4.6. Product Concentration

A concentration of 0.1mg/mL of the water-extracted ground powder product(s) (*Withania somnifera, Curcuma longa, Melissa officinalis, Rhodiola rosea, Vaccinium myrtillus)* was tested to investigate the CONKA 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 was validated on flies (not shown).

4.7. Flies 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 fresh food. Female flies were selected for the experiment and were differentiated from males under a microscope based on phenotypic sex

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differences using CO_2. The CONKA drug administration started on day 4 post-eclosure and continued throughout the experiment. Flies were put into vials for the experiment with 1.2g 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 CONKA component were added to the food. A few grains of baker's yeast were added to all vials.

4.8. 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 two 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 duration was carried out until 50% of the control group died, this allowed to investigate the effects of multiple concussions on the lifespan of flies as well as have sufficient survivor numbers to carry out the RONS assay. On the other hand, we investigated the effect of the whole CONKA drug in which the experiment duration was until every fly in each group died, and this was a full lifespan experiment (Figure 2F).

4.9. 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 groups were tested at random [43]. Flies were transferred into an empty 100-mL (without using CO_2), as it can have effects on fly behavior [44]. Pyrex graduated cylinder with a foam plug, with a 10-cm horizontal line drawn on the cylinder. Flies were left 15 min in the cylinder to acclimatize. 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 to survive flies 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.

4.10. Biochemical Assays in Fly Brains and Bodies

At key dates of the CONKA treatment, 10 flies from each group concussed, control, and treated groups (PBS only and drug) were anesthetized with CO_2, 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 0.1M PBS pH 7.0 and then homogenized using a small homogenizer. Eppendorf was stored at -80 °C until further use in the RONS assay.

4.11. Reactive Oxygen and Nitrogen Species (RONS) Levels Assay

To determine the RONS levels, oxidation was measured as an index of oxidative stress using a commonly used DCF-DA assay [45]. Homogenised head and body samples of each group were pipetted into a 96-well plate, with distilled water, 0.1M PBS (pH 7), and 200 μM DCFH-DA. The solution of 200 μM DCFH-DA was prepared following two steps: **1**) The solid to make a stock solution of 5 mM in 10 mL: Mass (g) = 0.005 mol/dm3 x 0.01 dm3 x 485.27 g/mol = 0.02425g DCF-DA in 10ml ethanol. **2**) Diluting the 5 mM solution to 200uM: Volume (ml) = (0.2M x 10ml)/ 5 mM = 0.4 ml of the 5mM solution + 9.6ml ethanol. Then, it was wrapped in foil to protect it from light. To each well of a transparent 96-well plate, 185 uL of PBS, 10 uL of samples (for blank wells, 10 uL of PBS was added), and 5 uL of DCF-DA were added. The fluorescence product of DFH oxidation (i.e., DCF) was measured for 10 min (at 30-sec intervals) using a Synergy H4 hybrid multi-mode 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.

4.12. Statistical Analyses

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Data were processed in Microsoft Excel 2023 and GraphPad Prism software version 10 was used for all statistical analyses, including means, standard deviations (SD), and p values (where * significant $p < 0.05$; ** highly significant $p < 0.01$; n.s. denotes non-significant). 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.

5. Conclusions

The fly concussion model allowed us to better understand concussion outcomes and facilitate examining the proposed CONKA 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 CONKA product can be used as a therapeutic approach to improve motor deficits, enhance lifespan, and maintain proper levels of RONS, in CTE, as well as other major neurodegenerative diseases (AD, PD, MND).

According to the definition given by the International Committee of Medical Journal Editors (ICMJE), the authors listed qualify for authorship based on making one or more of the substantial contributions to the intellectual content of the manuscript:

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Study conception and design [PC, KH, EAE, DG]; Acquisition of data [NA, AF,M-JH, JAF], Extraction of data [NA, AF, PC]; Interpretation of data [PC, NA, AF]; Drafting of manuscript [NA, AF]; Critical revision of manuscript [PC, M-JH].

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Data Availability Statement: Data is contained within the article and Supplementary Materials.

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Conflicts of Interest: The authors declare no conflicts of interest.

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