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Original Research Article

Effects of 2'-hydroxychalcones against Oxidative Stress and Lipid Peroxidation Biomarkers in Paclitaxel-Induced Peripheral Neuropathy in *Drosophila melanogaster* (Harwich Strain)

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Abstract

Purpose: Chemotherapy-induced peripheral neuropathy (CIPN), a common adverse effect of paclitaxel treatment, significantly impacts patients' quality of life. This study investigates the potential of 2'-hydroxychalcones, natural compounds with neuroprotective properties, to alleviate paclitaxel-induced peripheral neuropathy (PIPN).
Methods: Leveraging the *Drosophila melanogaster* model, the research evaluates the compounds' effects on oxidative stress markers, Superoxide Dismutase (SOD), Reduced Glutathione (GSH) and lipid peroxidation biomarker Malondialdehyde (MDA). Flies were grouped into five groups (groups 1 to 5). Group 1 served as a positive control, group 2 the negative control exposed to Paclitaxel only, while groups 3 and 4 were served 100mg and 150mg/10ml feed 2'-hydroxychalcones, respectively, and group 5 was served Gabapentin, the standard drug. The flies were later on homogenized and the homogenates were used to assay for oxidative stress biomarkers (SOD, GSH) and lipid peroxidation (MDA) by spectrometry. Our results reveal that Paclitaxel exposure leads to significant impairments in ROS. The administration of 2'-hydroxychalcones (100mg and 150mg) mitigated this oxidative damage.
Results: These results underscore the potential of chalcones as alternative therapeutic agents for CIPN, offering a foundation for further preclinical and clinical investigations.
Conclusion: By bridging the gap between natural product pharmacology and neuropathy research, the study provides insights into novel strategies for managing CIPN.

Keywords: Chemotherapy-induced peripheral neuropathy (CIPN), Paclitaxel, 2'-hydroxychalcones, Superoxide Dismutase (SOD), Reduced Glutathione (GSH), Malondialdehyde (MDA), *Drosophila melanogaster*

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INTRODUCTION

Peripheral neuropathy refers to the damage or dysfunction of peripheral nerves, often as pain, tingling, numbness, or weakness, particularly in the extremities. Among its various causes, chemotherapy-induced peripheral neuropathy (CIPN) represents a significant clinical challenge. One of the prime adverse effects of taxanes, including paclitaxel is peripheral neurotoxicity known as peripheral neuropathy. Up to 97% of patients receiving paclitaxel will develop paclitaxel-induced peripheral neuropathy (PIPN), which turns into a chronic condition in more than 60% of cases.[1] It decreases the efficacy of the chemotherapy by causing patient discomfort and often resulting in dosage reduction or chemotherapy treatment termination. Significantly, those who suffer from chronic neuropathy have considerably poorer long-term quality of life. [1]

The clinical presentation of peripheral neuropathic pain commonly includes descriptions of burning, pins, and needles (paresthesia), tingling, numbness, electric shocks/shooting, crawling (formication), itching, and temperature intolerance. In more advanced cases, patients may describe pain arising from stimuli that are not usually painful (i.e., allodynia) or pain from normally painful stimuli that is out of proportion to what would be expected. (i.e., hyperalgesia). [2]

The mechanisms underlying paclitaxel-induced peripheral neuropathy (PIPN) are multifaceted, involving mitochondrial dysfunction, oxidative stress, inflammation, and disruption of microtubule stability. Despite extensive research, effective preventive or therapeutic interventions remain elusive. This underscores the need for novel strategies to mitigate PIPN.

Natural compounds, particularly those derived from plants, have garnered considerable attention for their potential neuroprotective properties. Chalcone is a simple chemical scaffold of many naturally occurring compounds that has a widespread distribution in vegetables, fruits, teas, and other plants.[3] The word "chalcone" is derived from the Greek word "chalcos", meaning "bronze", which results from the colors of most natural 2-hydroxychalcones.[4] 2-hydroxychalcones are intermediates in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities. These are abundant in edible plants and are considered to be precursors of flavonoids and isoflavonoids. Chalcone (and related compounds, "chalconoids") is an aromatic ketone that forms the central core for various important biological compounds, collectively known as 2'-hydroxychalcones. However, their role in alleviating PIPN remains underexplored.

MATERIALS AND METHODS

Drugs and Reagents

Paclitaxel (Sigma aldrich), Gabapentin (Sigma aldrich), 2-hydroxychalcones (synthesized) from Pharmacology ABU, Corn flour (100 g), Baker's yeast (20 g), Agar (10 g), Methyl paraben, Water (1700 ml), Phosphate buffered saline (PBS) from Bio-RTC, ELISA kits for the assessment of oxidative stress biomarkers (GSH and SOD) and lipid peroxidation biomarker (MDA), were purchased from Randox Laboratory Ltd, United Kingdom, North-West Life Science Specialities, Vancouver Canada.

Equipment and Other Materials

Hand lens (20-30x), cooking utensils (Camping gas, pots, spoon), weighing balance (LACHOI lab scale 1000*0.01 g), culture vial, treatment vials, Eppendorf's, Micropipette, Spectrometer (SP-IUV7), foam plug, glass wares, petri dish, hand towels, Whattman paper, fluorescent microscope (Olympus 8ZX16)

Experimental Animals

Drosophila melanogaster Harwich strain was obtained from the Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University, Zaria. The flies were fed with the standard formulated diet corn meal medium, which contained corn meal (52 g), brewer's yeast (5 g), glucose (3.5 g), agar (7.5 g) and Nipargin (1 g), alcohol (1 ml) and kept under 12 h dark/light cycle conditions in the Drosophila Research Laboratory, Biomedical Science Research and Training Centre (BioRTC) Damaturu, Yobe State University, Yobe State, Nigeria. The water used for making the diet was distilled water. Flies were randomly selected from vials. Caution was taken when counting the flies/larvae and an appropriate brush with soft ends was used. All the experiments were carried out with the same *D. melanogaster* strain.

Methods

Paclitaxel treatment

Paclitaxel (10 μM) was administered following the feeding regimen as described by Brazill *et al.*;(2018). Briefly, twenty female flies were mated with fifty male flies for 48–72 h, and the embryos were collected for 2–4 h on grape juice agar plates (15 ml ddH_2O, 5 ml grape juice, 0.6 g agar, 1.1 g sucrose, 0.5 ml ethanol, 0.25 ml acetic acid, supplemented with yeast paste [700 mg baker's

yeast in 1 ml ddddH$_2$O]). The embryos were grown for 72 h to develop into L3 larvae. Larvae were rinsed with distilled water and transferred to freshly made grape juice agar plates containing 10 μM paclitaxel. After which, the different assays were conducted on them.

Biochemical Assay

To determine biochemical assays, flies of both genders were divided into five groups, each having 3 vials. Each vial contained 50 flies with varying concentrations of 2-hydroxy chalcones and paclitaxel in each treatment vial for a relative period of seven (7) days, after which flies were transferred into an empty treatment vial and immobilized using ice and then kept in an empty Eppendorf tube. They were weighed and homogenized using a stick in 8.1M phosphate buffer pH7.4 and then centrifuged at 4000xg for 10 minutes at 4^0C (Allegra X-15R centrifuge, Beckman Coulter USA). The resultant supernatant was separated into Eppendorf tubes that were labelled; and used for the various biochemical assays. All the assays were carried out for the five groups, and relative absorbance was read using a Jenway spectrophotometer 7315, by Bibi Scientific Ltd, UK. Selected biomarkers (Malondialdehyde, Superoxide dismutase, and GSH) in the homogenized sample were determined.

Statistical Analysis

Statistical analyses were carried out using SPSS (Version 28), and the data obtained were expressed as mean ± SEM. Analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons were carried out. Values with $P \leq 0.05$ were considered significant.

RESULT AND DISCUSSION

From Table 1 below, Oxidative stress biomarker SOD in control and Paclitaxel-treated groups revealed the following mean values: Group I (Control) had SOD level of 82.35 ± 0.35, while Group II (Paclitaxel only) showed a decreased SOD level of 70.35 ± 0.59, and Groups III, and IV, which received 2'-hydroxychalcones at different concentrations (100 mg and 150 mg), exhibited lower levels of 71.33 ± 0.35 and 29.40 ± 0.17 respectively and group V had an increased level of (82.35 ± 0.46), 25 mg Gab the standard drug. One-way ANOVA analysis indicated a significant difference ($p > 0.05$) was seen between control group I (82.00 ± 0.35) and paclitaxel-only group II (70.35 ± 0.59), 100 mg 2'-hydroxychalcones group III (71.33 ± 0.35), and 150 mg 2'-hydroxychalcones group IV (29.40 ± 0.17). A significant difference was observed between paclitaxel-only group II (70.35 ± 0.59), with 150mg 2-hydroxychalcones group IV (29.40 ± 0.17) 2 and the 25mg Gab group V (82.35 ± 0.46). So also, a significant difference between 100mg 2'-hydroxychalcones group III (71.33 ± 0.35) and 25 mg Gab group V (82.35 ± 0.46) was also seen.

GSH in control and Paclitaxel-treated groups revealed the following mean values: Group I (Control) had a GSH level of 411.84±0.40, while Group II (Paclitaxel only) showed an increased GSH level of 458.13±0.40, and Groups III, and IV, which received 2'-hydroxychalcones at different concentrations (100 mg and 150 mg), exhibited higher levels of 426.33 ± 0.40 and 447.25 ± 0.29 respectively and group 5 had a decreased level of (382.45 ± 0.46), 25 mg Gab the standard drug. One-way ANOVA analysis indicated a significant difference ($p > 0.05$) between control group I (411.84 ± 0.40) and paclitaxel-only group II (458.13 ± 0.40), 100 mg 2'-hydroxychalcones group III (426.33 ± 0.40), 150 mg 2-hydroxychalcones group IV (447.25 ± 0.29), and 25 mg Gab group V (382.45 ± 0.46). A significant difference was observed between the paclitaxel-only group II (458.13 ± 0.40), with the 150 mg 2-hydroxychalcones group IV (447.25 ± 0.29), and the 25mg Gab group V (382.45 ± 0.46). A significant difference was also seen between the 25mg Gab group V (382.45 ± 0.46) and all the other four groups.

Lipid peroxidation biomarker MDA in control and Paclitaxel-treated groups revealed the following mean values: Group I (Control) had an MDA level of 21.76 ± 0.35, while Group II (Paclitaxel only) showed an increased MDA level of 38.64 ± 0.23, and Groups III, and IV, which received 2'-hydroxychalcones at different concentrations (100 mg and 150 mg), exhibited lower levels of 19.88 ± 0.29 and 18.48 ± 0.23 respectively and group 5 had a decreased level of (15.20 ± 0.23), 25mg Gab the standard drug too. One-way ANOVA analysis indicated a significant difference ($p > 0.05$) between control group I (21.76 ± 0.35) and paclitaxel-only group II (38.64 ± 0.23), 100mg 2-hydroxychalcones group III (19.88 ± 0.29), 150mg 2'-hydroxychalcones group IV (18.48 ± 0.23), and 25mg Gab group V (15.20 ± 0.23). A significant difference was observed between the paclitaxel-only group II (458.13 ± 0.40), with the 150mg 2'-hydroxychalcones group IV (18.48 ± 0.23), and the 25mg Gab group V (15.20 ± 0.23). A significant difference was also seen between the 25mg Gab group V (15.20 ± 0.23) and all the other four groups.

Table 1: Changes in oxidative and lipid peroxidation biomarkers

GROUP	SOD (µMol/min/mg Protein)	GSH (µMol/min/mg Protein)	MDA (µMol/mg Protein)
Control	82.35 ± 0.35	411.84 ± 0.41	21.76 ± 0.35
Paclitaxel	70.59 ± 0.29^{adc}	458.13 ± 0.40^{a}	38.64 ± 0.23^{a}
PAC + 100 mg CHAL	71.33 ± 0.35^{ade}	426.33 ± 0.40^{ac}	19.88 ± 0.29^{ac}
PAC + 150 mg CHAL	29.40 ± 0.17^{abc}	447.26 ± 0.29^{abc}	18.48 ± 0.23^{abc}
PAC + 100 mg GAB	82.35 ± 0.46^{bcd}	382.45±0.46^{abcd}	15.20 ± 0.23^{abcd}

^{a}= significant difference between grp1 and other groups, ^{b}= significant difference between grp2 and other groups, ^{c}= significant difference between grp3 and other groups, ^{d}= significant difference between grp4 and other groups, e= significant difference between grp5 and other groups ($p< 0.05$)

The effects of 2-hydroxychalcones on SOD activity can be attributed to their dual role as antioxidants and pro-oxidants, depending on the dose and context. Low-dose 2-hydroxychalcones (100 mg) likely exert a mild antioxidant effect, scavenging free radicals and reducing the oxidative burden on SOD, high-dose 2-hydroxychalcones (150 mg) may exhibit pro-oxidant behavior, generating ROS or interfering with SOD activity through enzyme inhibition or depletion of cofactors (e.g., copper or zinc required for SOD function).[5] Gabapentin's ability to restore SOD levels to normal suggests a different mechanism, possibly through indirect modulation of oxidative pathways or enhancement of endogenous antioxidant defenses.

Both doses of 2'-hydroxychalcones showed a capacity to normalize GSH levels by reducing the oxidative burden imposed by paclitaxel. The more pronounced effect at 100 mg suggests an optimal dose range, beyond which further increases might have diminishing returns. Gabapentin suppressed GSH production, possibly overcompensating and leading to a suboptimal redox state. While gabapentin effectively mitigates oxidative stress, GSH below baseline may not be ideal, as GSH depletion can make cells more vulnerable to oxidative damage.[6] In contrast, 2'-hydroxychalcones modulated GSH levels without excessive suppression, indicating a more balanced restoration of redox homeostasis. The observed effects of 2'-hydroxychalcones on GSH levels may be explained by their biochemical properties: Antioxidant activity: 2'-hydroxychalcones reduce oxidative stress, thereby lowering the demand for GSH synthesis and restoring GSH levels to near-normal, in line with previously done.[6,7] By scavenging ROS, 2'-hydroxychalcones might prevent excessive GSH consumption, promoting a balanced redox state as in previous works done,[8] through redox modulation or through a protective role where 2'-hydroxychalcones could enhance the efficiency of endogenous antioxidant systems, allowing GSH levels to stabilize without being depleted. In comparison, gabapentin may act through a different mechanism, potentially inhibiting GSH synthesis or altering oxidative pathways, leading to lower GSH levels. The study demonstrates that 2'-hydroxychalcones effectively modulate GSH levels in a *Drosophila* model of paclitaxel-induced peripheral neuropathy. By reducing the excessive GSH levels induced by paclitaxel, 2'-hydroxychalcones restore a balanced redox state, suggesting their potential as therapeutic agents for oxidative stress-related neuropathies. Compared to gabapentin, 2'-hydroxychalcones offer a more balanced approach, emphasizing their promise as safer alternatives or adjunctive treatments for PIPN.

Lipid peroxidation, reflected by elevated levels of MDA, is a hallmark of oxidative stress and a key contributor to the pathophysiology of peripheral neuropathy. Paclitaxel-induced peripheral neuropathy (PIPN) involves oxidative stress as a major mechanism, leading to neuronal damage. This study evaluates the ability of 2'-hydroxychalcones to mitigate oxidative stress in *Drosophila* larvae, a model system, by measuring

MDA levels across various treatment groups. This reflects heightened oxidative stress and lipid peroxidation, consistent with paclitaxel-induced damage. The substantial increase in MDA confirms that paclitaxel effectively induces oxidative stress, aligning with its known neurotoxic effects. This is in line with previous studies, [3,9,10,] that have explored the effect of paclitaxel on MDA levels. This significant reduction suggests that the lower dose of 2'-hydroxychalcones effectively counteracts paclitaxel-induced oxidative stress, restoring normal lipid peroxidation levels. MDA levels further decreased, suggesting a more pronounced antioxidative effect at the higher dose. However, the difference between 100 mg and 150 mg is relatively modest, indicating a potential plateau in efficacy. This aligns with previous studies done by, [11,12,13,] on the effects of chalcones and their derivatives on reducing MDA levels in peripheral neuropathy in a dependent manner. Gabapentin, a standard treatment for neuropathy, exhibited superior antioxidative efficacy compared to both doses of chalcones. This is not surprising, previous studies, [7,6,1,14,] have shown how effective Gabapentin is as a standard in reducing MDA levels in paclitaxel-induced neuropathy. Interestingly, the significant difference between Gabapentin and all other group highlights its strong ability to reduce oxidative stress, although chalcones remain promising due to their similar effects at higher doses. The lowest MDA levels in this group highlight its robust antioxidative properties, suggesting it may remain the standard of care. The trend of decreasing MDA levels in the chalcone groups suggests dose-dependent antioxidative efficacy, with both doses significantly reversing paclitaxel-induced lipid peroxidation. The reduction in MDA levels by chalcones is likely due to their antioxidant properties, which may include: Scavenging free radicals by neutralizing reactive oxygen species (ROS) and reducing lipid peroxidation as reported.[7] Or by modulating oxidative pathways by inhibiting enzymes or pathways involved in ROS production, such as NADPH oxidase and enhancing endogenous defenses by upregulate antioxidant defenses, including glutathione or superoxide dismutase as reported.[13] These mechanisms suggest that chalcones not only mitigate oxidative damage but may also prevent further neuronal injury. While chalcones are less effective than gabapentin at the doses tested, their comparable efficacy suggests potential as an alternative or adjunctive therapy. The study demonstrates that 2'-hydroxychalcones significantly reduce MDA levels in paclitaxel-induced peripheral neuropathy in *Drosophila* larvae, with dose-dependent effects observed at 100 mg and 150 mg. These results highlight the antioxidative properties of chalcones, making them promising candidates for managing neuropathy. While gabapentin showed the greatest efficacy, chalcones represent a natural, potentially safer alternative or adjunctive option. Further studies are warranted to explore their therapeutic potential and mechanisms of action.

CONCLUSION

This study demonstrates that administration of synthesized 2-hydroxychalcones looks promising in mitigating detrimental effects of Paclitaxel-induced neuropathy, suggesting its potential role as a therapeutic agent against Paclitaxel, which significantly increases nociception, ROS levels, and potentially induces peripheral neuropathy in *Drosophila Melanogaster*. Electron microscopy studies are recommended to investigate cellular changes induced by paclitaxel toxicity and the neuroprotective effects of 2'-hydroxychalcones. These studies will provide detailed insights into subcellular alterations, aiding in the identification of therapeutic targets.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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