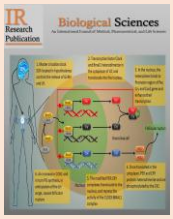
RESEARCH ARTICLE

Biological Sciences
(An International Journal of Medical, Pharmaceutical, and Life Sciences)

Journal home: https://irrespub.com/biolsciences/index.php/1/index



The Effect of *Heinsia crinita* Aqueous Leaf extract on Codeine-induced Changes of Brain Antioxidant Parameters, TNF-α and Interleukin-6 in Male Wistar rats

Oguche EE[1,2]*, Musa SA[2], Agbon AN[2], Magaji MG[3], Danborno AM[1]

[1]*Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine and Allied-health Sciences, Bingham University, Karu, Nasarawa State.*
[2]*Neuroanatomy and Neurosciences Research Unit, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University (ABU), Zaria. Kaduna State, Nigeria.*
[3]*Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Ahmadu Bello University, ABU, Zaria.*

ARTICLE HISTORY

Received: 19-06-2024
Revised: 07-08-2024
Accepted: 12-08-2024
Online: 23-08-2024

KEYWORDS

Antioxidant
Anti-inflammatory
Brain
Oxidative stress

ABSTRACT

Codeine is short-time acting narcotic opiate with widespread clinical use for over a century; as an analgesic for mild-to-moderate pain and in low doses, as an anti-tussive for suppression of nonproductive cough and diarrhoea. Although having diverse applications, codeine use requires caution due to its potential adverse effects and risk of dependence, associated to oxidative stress through various mechanisms and inflammatory responses. *Heinsia crinita* leaves are consumed either as vegetable in preparation of local cuisine or as component of alcoholic concoction for the treatment of diverse ailments including bacterial infections, diabetes, hypertension, and pharmacologically having neuroprotective benefits. This study assessed the antioxidant and anti-inflammatory properties of aqueous leaf extract of *H. crinita* (ALHC) on codeine-induced oxidative stress and inflammation in rats. Twenty-five (25) adult male Wistar rats were obtained and divided into five groups (groups 1 – 5; n= 5). Group 1 (control) received distilled water (1 ml/ kg), group 2 received only codeine (10 mg/kg), groups 3, 4 and 5 received codeine (10 mg/kg) and afterwards ALHC (100 mg/kg, 200 mg/kg and 300 mg/kg, respectively). The antioxidant and anti-inflammatory properties of ALHC were determined by biochemically assessing for oxidative stress bio-markers (MDA, SOD and CAT) and inflammatory markers (TNF-α and IL-6) in brain homogenates of the rats. Results revealed increased ($p<0.05$) MDA and inflammatory markers (TNF-α and IL-6) levels and reduced SOD and CAT activities in codeine-treated group. However, ALHC-treatment restored the system by decreased levels of MDA and IL-6, especially ($p<0.05$) in ALHC (300 mg/kg)-treated group; TNF-α decreased ($p<0.05$) in groups ALHC (200 and 300 mg/kg)-treated groups; SOD and CAT increased ($p<0.05$) in groups ALHC (200 and 300 mg/kg)-treated groups. In conclusion, findings are suggestive of the antioxidant and anti-inflammatory properties of ALHC following oxidative stress and inflammation induced by exposure to codeine in rats

*Address for correspondence
Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine and Allied-health Sciences, Bingham University, Karu, Nasarawa State. Neuroanatomy and Neurosciences Research Unit, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University (ABU), Zaria. Kaduna State, Nigeria
Email: Oguche.emmanuel@binghamuni.edu.ng
DOI: https://doi.org/10.55006/biolsciences.2024.4302

Introduction

Drug abuse is surfacing as a global public health pandemic that is eating into the fabrics of our societies. According to the world drug report – 2019 of the United Nations Office of Drugs and Crime (UNODC) estimated 271 million (5.5%) of the World population (aged between 15 and 64 years) had used drugs in the previous years [1]. The report projected that 35milion individuals will be experiencing drug use disorders [1]. Also, the Global Burden of disease study 2017 estimated that there were 585,000 deaths due to drug use globally in 2017[1]. The burden of drug abuse, use and trafficking has also been related to areas of concern. These are organized crime, illicit financial flows, corruption and terrorism/insurgency [2]. Therefore, effort is ongoing to combat drug abuse which has become a menace, and also the need for quick intervention on possible ways of ameliorating the impact of drug abuse on the health of individuals.

Codeine is an opiate, it is a short time acting narcotic and naturally occurring methylated morphine that has seen widespread clinical use for more than a century as an analgesic for mild-to-moderate pain and in low doses, as an antitussive for suppression of nonproductive cough [3]. Codeine (3-methylmorphine) is an alkaloid prepared from the methylation of morphine derived from *Papaver Somniferum* also known as poppy seeds [4].

A small amount of codeine is metabolized in the body to form morphine (its active metabolite) and binds to receptors in the brain known as opioid receptors and induce signaling processes throughout the brain and rest of the body [4].

Codeine is a medicinal drug with a rich history and diverse applications. It belongs to the opioid family and is widely used to manage pain, cough, and diarrhea [5]. Its diverse applications make it a versatile drug, but its use also requires careful consideration due to potential side effects and the risk of dependence. Codeine is primarily used for the management of mild to moderate pain. Codeine is commonly combined with these non-opioid analgesics to enhance pain relief through a synergistic effect [6]. It is a common ingredient in many prescription cough syrups and cold medications [7]. Codeine suppresses cough by acting on the brain's cough centre in the medulla, reducing the urge to cough. This makes it particularly useful in managing coughs that are persistent and disruptive, such as those associated with bronchitis, pertussis, or the common cold [8].

Codeine decreases pain perception and stimulate the intense sensation of euphoria or well-being when it interacts with receptors in the brain. These feelings of euphoria and sedation in users are usually sought after, leading to the abuse of opioids [9]. Given latent for abuse of codeine, at least 25 countries, including Germany, Japan and the US have banned over-the-counter codeine sales [10].

Codeine decreases pain perception and stimulate the intense sensation of euphoria or well-being when it interacts with receptors in the brain. These feelings of euphoria and sedation in users are usually sought after, leading to the abuse of opioids [9]. Given latent for abuse of codeine, at least 25 countries, including Germany, Japan and the US have banned over-the-counter codeine sales [10].

Inflammation is a response of oxidative stress induced by codeine. Inflammatory markers, such as interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), increase in response to infection and tissue damage and inactive diseases states [14]. These inflammatory cytokines including IL-6 and TNF-α, are cytokines involved in inflammation, which could be released quickly under pathological conditions, causing an inflammatory response in the central nervous system [15, 16]. According to [17], the utilization of these biomarkers is beneficial due to their ease of administration and reduced processing time. Drugs have the ability to activate the consequences of its use or misuse on the neurological system, which makes them possible stress mediators [18].

Heinsia crinita with the name "atama" is a vegetable which belongs to the family known as Rubiaceae [19]. The leaves are consumed either as vegetable in preparation of local cuisine or as component of alcoholic concoction for the treatment of some diseases such as bacterial infections, diabetes, hypertension and infertility [20; 21]. *H. crinite* extract had been postulation to exhibited neuroprotective properties [22]. *H. crinita* leaves has been shown to contain some phenolic acid and flavonoid with 50% enzyme/antioxidant activity (EC50) with inhibitory effect on monoamine oxidase and acetylecholinesterase, inhibit Fe^{2+} induced lipid peroxidation in isolated rat brain and stimulated Na+/K+-ATPase activity and show great potential in defending the body against oxidative stress reaction (22; 23; 24). The study aim was to evaluate the effect of *H. crinita* aqueous leaf extract on codeine-induced changes on brain antioxidant parameters, TNF-α and interleukin-6 in male adult Wistar rats

Materials and Methods

Animal Source and Handling

Twenty five (25) male Wistar rats weighing between 110g and 150g were used for the experiment. The rats were obtained from National Veterinary Research Institute (NVRI) in Vom, Plateau State. They were allowed to acclimatize for a period of 14 days with daily feeding and care in the Animal Care Unit of Faculty of Basic Medical Sciences, College of Medicine, Ahmadu Bello University, Zaria, under standard laboratory condition before commencement of administration. Ethical approval was obtained from Ahmadu Bello University, Zaria Committee on Animal Use and Care and registered as ABUCAUC/2023/113.

Plant Collection and Extraction

H. crinite leaves was collected from Kerawa in Igabi Local Government Area of Kaduna State. It was then identified and assigned a vourcher specimen number ABU0900275 in the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University (A.B.U), Zaria, Nigeria. The *H. crinita* leaf was extracted using the method of [25].

Drug Preparation and Treatment

Dihydrocodeine (30mg) manufactured by PL Holder, Bristol Laboratory Ltd, United Kingdom with batch number FDC004 was used for the experiment. The stock solution for dihydrocodeine solution was prepared by dissolving the dihydrocodeine tablet (30 mg) in 10 mL of distilled water. Each reconstituted dihydrocodeine solution was prepared daily. The dihydrocodeine solutions were administered to the animals according to their body weight via gastric intubation for 21 days (3 weeks) after which they were administered aqueous leave extract of *H. crinita* for 7days (1 week).

Experimental Design

The experimental animals were randomly assigned to 5 groups (n = 5). Group A was administered distilled water (1.0 mL), group B received 10mg/kg of dihydrocodeine group C received 10mg/kg of dihydrocodeine and 100mg/kg of *H. crinita* aqueous leave extract, group D received 10mg/kg of dihydrocodeine and 200mg/kg of *H. crinita* aqueous leave extract, and group E received 10mg/kg of dihydrocodeine and 300mg/kg of H crinita aqueous leave extract. All administrations were done via gastric intubation. The dosage of dihydrocodeine was according to [26] and that of *H. crinita* was according to [27]. At the end of the study period, animals were anesthetized with chloroform. The brains were quickly dissected out, rinsed with normal saline, and the tissue processed for the study.

Sample Preparation

At the end of the experiment, animals were anaesthetized with chloroform. The brain was harvested and then 1 g of tissues immediately homogenized. The tissue homogenates was centrifuged at appropriate centrifugal force for l0 min and the supernatant was utilized for different estimations according to the method of [28]. The homogenate was used to determine oxidative stress markers malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase and inflammatory markers TNF- α and Interleukin 6.

Oxidative Stress and Inflammatory Markers Assay

MDA was determined using the widely used thiobarbituric acid (TBA) method for measuring lipid peroxidation as the amount of thiobarbituric acid reactive substance TBARS generated during lipid peroxidation. MDA binds to TBA at 60°C and low PH producing a pink complex (TBS and 2-malondialdehyde adduct) with maximum absorbance at 532nm [29; 30].

Activity of SOD enzyme were evaluated by the method of [31]. The analysis of SOD was based upon the principle in which xanthine reacts with xanthine oxidase to produce superoxide radicals. The SOD activity was measured by the level of suppression of this reaction. Results were expressed as U/mg protein.

The Aebi technique for measuring catalase activity was used [31], where a reference solution of 50 mM potassium phosphate buffer, pH 7.0, was used to adjust the spectrophotometer's wavelength to 240 nm (1 000 µL). In addition, 950 mL of working assay buffer and 50 mL of sample (tissue homogenate) were pipetted into a clem cuvette and immediately mixed. A catalase standard was produced by mixing 950 µl of the assay buffer, 460 µl of 30 mM H2O2 and 50 µl of diluted catalase. For 5 minutes, the rate of which H2O2 was broken down was monitored at 240 nm every 15 seconds, a standard curve was plotted using catalase as the reference. The rate of decomposition in the Sample is given by its (A240nm/min) micro-average. Measurements of catalase activity were made against a standard curve and reported in units of activity per millilitre (Uml).

Glutathione peroxidase concentration was measured by calculating the content of non-protein sulfhydryl groups by technique described by [33]. 400 µL of the homogenate was added to 320 µL of distilled water and 80 µL of trichloroacetic acid, mixture was centrifuge for 15 min at 3000 rpm and then 400 µL of supernatant were taken and added

to 800 µL of Tris-HCl buffer (0.4 M, pH 8.9) and 20 µL of 5, 5'- dithiobis (2- nitrobenzoic acid) 0.1 M and after 1 min the reading were made at 412 nm.

Tumor Necrotic Factor-Alpha (TNF-α) was determined utilizing the approach outlined by Zang, TNF-α activity was determined. The TNF-α ELISA kit uses a technique called sandwich ELISA. A pre-coated macro ELISA plate that has been immunosensitized with a rat TNF-α specific antibody is included in this kit. Both the standard or sample to be tested and the appropriate antibody combined in individual wells of micro-ELISA plates. After incubation, each well received an addition of an Avidin-horseradish peroxidase (HRP) combination and a biotinylated detection antibody against rat TNF-α. Substrate solution was this applied to each well. A blue colour was formed only in wells containing Rat TNF- α, Avidin-HRP conjugate, detection antibody. The colour changes back to yellow when the enzyme-substrate reacting is halted. The OD is measured at a wavelength of 450 nm 2 nm using a spectrophotometer. The optical density (OD) value is proportional to the concentration of Rat TNF- α. In order to qualify the concentration of rat TNF-α in a sample, the optical density (OD) is compared to that of a standard curve [34].

For Interleukin-6 (IL-6), the method published by [35] for measuring IL-6 activity was used. The Sandwich-ELISA method is and for the determination of interleukin-6 in this study. Included in this kit is a micro-ELISA plate that has been pre-coated with an antibody specific to Rat IL-6. All that was done is mix the standard or sample with the appropriate antibodv and put it to a well on a micro-ELISA plate.

Data analysis

Data were statistically analyzed using SPSS 21 and the data were reported as Mean ± SEM. Data were analyzed One-way analysis of variance (ANOVA) and Turkey Post hoc test for multiple comparism. A p value of less than 0.05 will be considered to be statistically significant.

Results

After codeine exposure there was increased ($p<0.05$) MDA level in the brain tissue homogenate of the animals when compared to the control. After treatment with *H. crinite* aqueous leaf extract there was significant decreased ($p<0.05$) in MDA level in the group that received the dose of 300 mg/kg of the extract. The SOD level decreased significantly ($p<0.05$) after administration of codeine when compared with the control. There was an increase

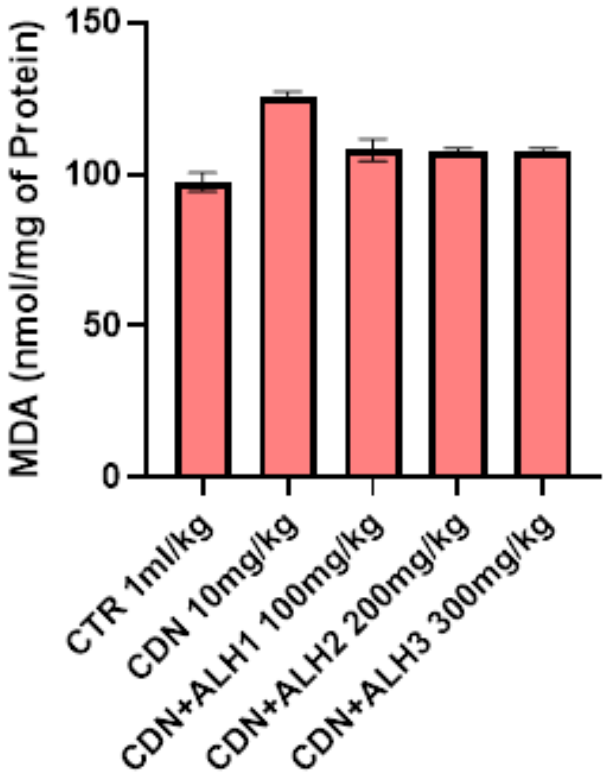


Figure 1. Effect of *H. crinite* on Malondialdehyde (MDA) after codeine exposure in Wistar rats. n =5, mean ± SEM, One-way ANOVA, $p<0.005$, CTR: Control, CDN: Codeine, ALH: Aqueous leaf extract of *H. crinite*

after treatment with codeine *H. crinite* aqueous leaf extract at a dose of 300mg/kg, which was not significant when compare to the control. CAT level in the brain tissue homogenate of the animals after codeine treatment was decreased when compared to the control, though the decrease was not significant. After treatment with *H. crinite* aqueous leaf extract there was significant increase ($p<0.05$) in CAT level in the group that received the dose of 300 mg/kg of the extract. The level of TNF-α increased significantly ($p<0.05$) after codeine administration when compared with control but decreased after administration of aqueous leaf extract of *H. crinite* but the decrease was only significant ($p<0.05$) in groups that received 200 mg/kg and 300 mg/kg of the extract. IL-6 increased significantly ($p<0.05$) after codeine administration when compared with control but decreased after administration of aqueous leaf extract of H. crinite. The decrease was only significant ($p<0.05$) in groups that received 300 mg/kg.

Discussion

This result agrees with the report of [26] who reported increase in MDA after codeine consumption, an indication of lipid peroxidation as a result of oxidative stress. In addition, codeine

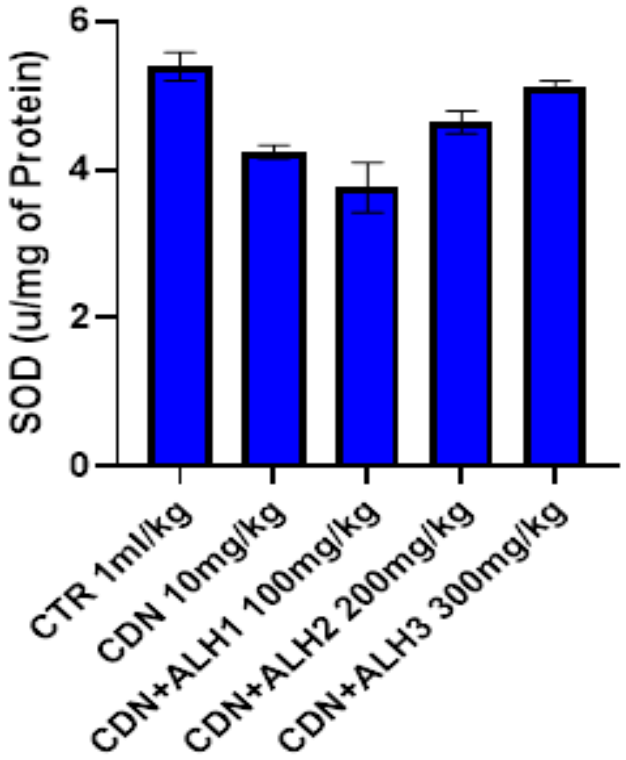


Figure 2. Effect of *H. crinite* on Superoxide Dismutase (SOD) after codeine exposure in Wistar rats. n =5, mean ± SEM, One-way ANOVA, p<0.005, CTR: Control, CDN: Codeine, ALH: Aqueous leaf extract of *H. crinita*.

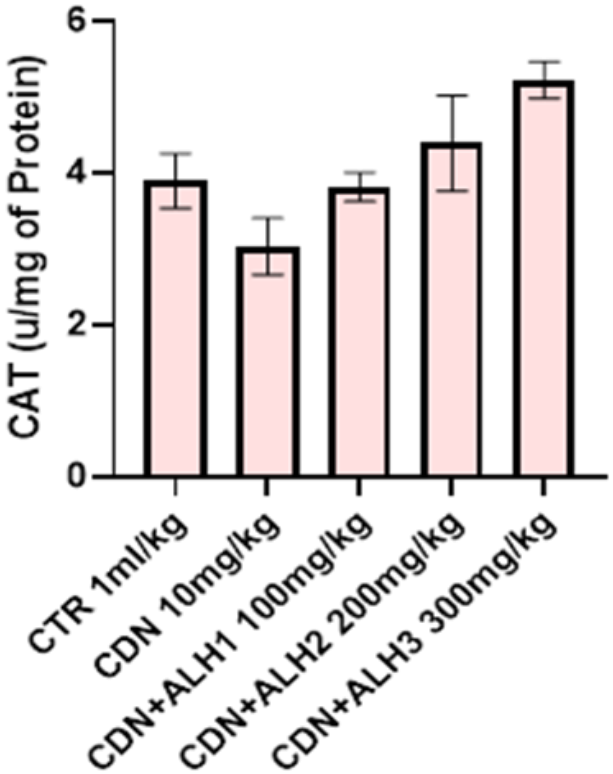


Figure 3. Effect of *H. crinite* on Catalase (CAT) after codeine exposure in Wistar rats. n =5, mean ± SEM, One-way ANOVA, p<0.005, CTR: Control, CDN: Codeine, ALH: Aqueous leaf extract of *H. crinita*.

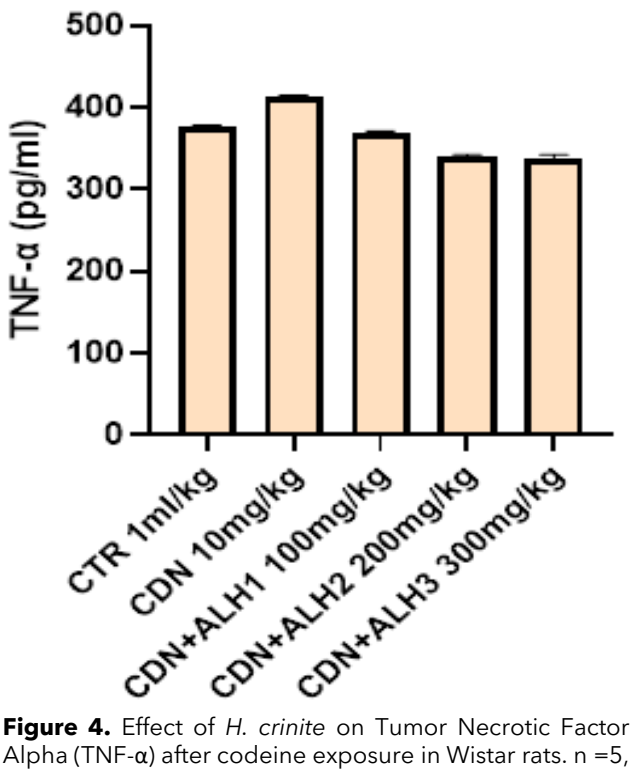


Figure 4. Effect of *H. crinite* on Tumor Necrotic Factor Alpha (TNF-α) after codeine exposure in Wistar rats. n =5, mean ± SEM, One-way ANOVA, p<0.005, CTR: Control, CDN: Codeine, ALH: Aqueous leaf extract of *H. crinita*.

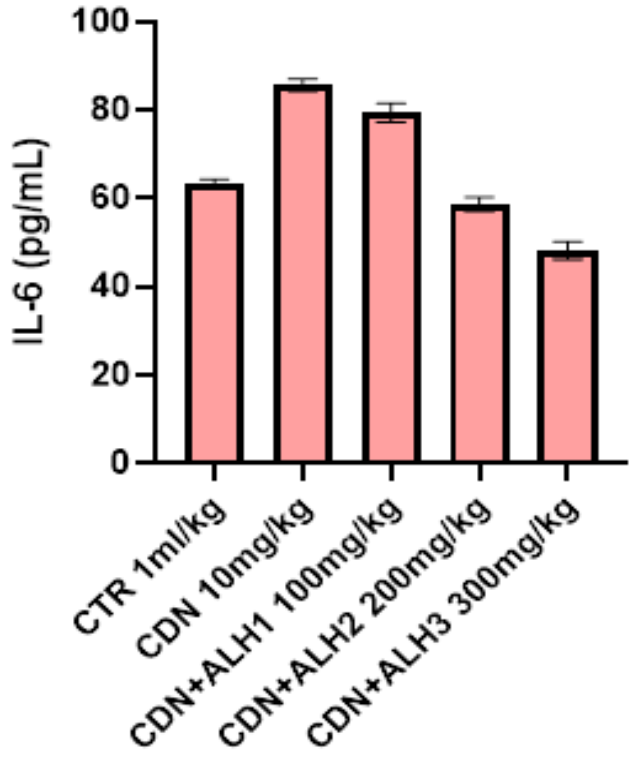


Figure 5. Effect of *H. crinite* on Interleukin 6 (IL-6) after codeine exposure in Wistar rats. n =5, mean ± SEM, One-way ANOVA, p<0.005, CTR: Control, CDN: Codeine, ALH: Aqueous leaf extract of *H. crinita*.

treatment caused significant decrease in the activities of enzymatic antioxidants SOD and catalase. [36; 37], reported that redox dysregulation in the brain is an independent risk factor that is strongly associated with neurodegenerative diseases supporting earlier studies [38; 39], opioids induce oxidative brain damage evident in elevated MDA level and reduced enzymatic antioxidant activities.

The reduction in MDA and increase in the enzymatic antioxidant SOD and catalase is an indication of the ameliorative effect of the extract, though it is only indicative at a dose of 300 mg/kg. Lipid peroxidation inhibitory effect of *H. crinite* aqueous leaf extract an evidence of its neuroprotective properties and increased the activity of SOD. Which agrees with this studies [22; 24]. The increase SOD will form a first line of defence, scavenging superoxide radicals and converting them to H_2O_2 which will be converted to H_2O and O_2.

The increased level of inflammatory markers as compared to control is an indication of inflammation in the brain. Inflammation is triggered to fight and control an injury, infection or other stimulus and it involves many cell types, as well as the secretion of soluble factors. In a redox balance, the inflammatory response is self-regulated, and is able to repair tissue damage and eliminate pathogenic elements. However, when the response is chronic, it causes an inflammatory environment that leads to progressive tissue damage [40]. Neuroinflammation itself at the first initial stage is a protective response in the brain, but excessively inappropriate inflammatory responses are detrimental, and in fact, it diminish the neuronal regeneration thereby leading to neurodegenerative diseases and other neurological disorders [41; 42].

Conclusion

Codeine induced oxidative stress as seen in the increase in the level of MDA and under-regulation of SOD and catalase. This is evident in the production of inflammatory markers TNF-α and IL-6. Aqueous leaf extract of *H. crinita* has shown neuroprotective potential by down regulating MDA, TNF-α and IL-6 and up regulating SOD and catalase.

Contribution of authors

Oguche E.E; conceived the idea of the study and carried out the animal experimentation, Musa S.A., Agbon A.N., Magaji M.G., and Danborno A.M.; participated in its design and supervision. Oguche E.E; analyzed the data, drafted the manuscript and all authors read and approved the final manuscript.

Acknowledgements

The Department of Human Anatomy, Faculty of Basic Medical Sciences, Bingham University, Karu, Nigeria, and Neuroanatomy and Neurosciences Research Unit, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University (ABU), Zaria. Kaduna State, Nigeria made their laboratory available to the authors for this study. We therefore wish to express our gratitude to the department for their support. The Authors did not receive any funds from any funding agency for this study. We wish to state that the study was self-sponsored by all the authors

Conflict of interest: The authors declare no conflicts of interests regarding this manuscript.

Funding

This work was supported by Bingham University Karu, Nasarawa State, Nigeria.

References

1. UNODC. UNODC-SMART: Almost 900 NPS Reported to UNODC from 119 Countries and territories. Vienna: United Nations Office on Drugs and Crime. February 2019.
2. Merz F. United Nations Office on Drugs and Crime: World Drug Report 2017. 2017. SIRIUS -Zeitschrift für Strategische Analysen. 2018; 2(1): 85-86.
3. Ewah BN, Robb PJ, Raw M. Postoperative pain, nausea and vomiting following paediatric day-case tonsillectomy. Anaesthesia. 2006 Feb; 61(2):116-22.
4. Pratiwi R, Noviana E, Fauziati R, Carrão DB, Gandhi FA, Majid MA, Saputri FA. A review of analytical methods for codeine determination. Molecules. 2021 Feb 4; 26(4):800.
5. Ademikanra A, Olayiwola A, Oyewole O. Introduction to Opioids: A Review. Biomedicine and Chemical Sciences. 2023 Jan 1; 2(1):01-6.
6. Van Hout M, Bergin M, Foley M, Rich E, Rapca AI, Harris R, Norman I. A scoping review of codeine use, misuse and dependence. CODEMISUSED Project. European Commission 7th Framework Programme, EU. Brussels. 2014.
7. Vora A, Nadkar MY. Codeine: a relook at the old antitussive. Journal of the Association of Physicians of India. 2015 Apr 1; 63(4):80-2.
8. Lam SH, Homme J, Avarello J, Heins A, Pauze D, Mace S, Dietrich A, Stoner M, Chumpitazi CE, Saidinejad M. Use of antitussive medications in acute cough in young children.

Journal of the American College of Emergency Physicians Open. 2021 Jun; 2(3):e12467.
9. Kayode AA, Alonge AJ, Adewoye EO. Codeine induced hematological, hepatic alterations, lung and brain damage in mice. Advances in Toxicology and Toxic Effects. 2022 Jul 26; 6(1):001-7.
10. Mikhael V, Ghabi R, Belahmer A, Kadi R, Guirguis N, Gutzeit A, Froehlich JM, Ferreira E, Higgs T, Dufour MM, Theoret V. Intimate partner violence: Defining the pharmacist's role. Canadian Pharmacists Journal/Revue des Pharmaciens du Canada. 2023 Mar; 156(2):63-70.
11. Akhigbe RE, Dutta S, Hamed MA, Ajayi AF, Sengupta P, Ahmad G. Viral infections and male infertility: a comprehensive review of the role of oxidative stress. Frontiers in Reproductive Health. 2022 Feb 3;4:782915.
12. Ajayi AM, John KA, Emmanuel IB, Chidebe EO, Adedapo AD. High-fat diet-induced memory impairment and anxiety-like behavior in rats attenuated by peel extract of Ananas comosus fruit via atheroprotective, antioxidant and anti-inflammatory actions. Metabolism open. 2021 Mar 1; 9:100077.
13. El-Missiry MA, Othman AI, Alabdan MA. Melatonin for protection against ionizing radiation. INTECH Open Access Publisher; 2012 Mar 9.
14. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Bmj. 2000 Jul 22; 321(7255):199-204.
15. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, Pollmächer T. Cytokine-associated emotional and cognitive disturbances in humans. Archives of general psychiatry. 2001 May 1; 58(5):445-52.
16. Sparkman NL, Buchanan JB, Heyen JR, Chen J, Beverly JL, Johnson RW. Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers. Journal of Neuroscience. 2006 Oct 18; 26(42):10709-16.
17. Stucken C, Olszewski DC, Creevy WR, Murakami AM, Tornetta III P. Preoperative diagnosis of infection in patients with nonunions. JBJS. 2013 Aug 7; 95(15):1409-12.
18. Kousik SM, Napier TC, Carvey PM. The effects of psychostimulant drugs on blood brain barrier function and neuroinflammation. Frontiers in pharmacology. 2012 Jun 29; 3:121.
19. Etukudo, I. Ethnobotany: conventional and traditional uses of plants, Verdict Press, Uyo, Akwa Ibom, Nigeria; 2003:191
20. Ebong PE, Igile GO, Mgbeje BI, Iwara IA, Odongo AE, Onofiok UL, Oso EA. Hypoglycemic, hepatoprotective and nephroprotective effects of methanolic leaf extract of Heinsia crinita (Rubiaceae) in alloxan-induced diabetic albino Wistar rats. IOSR Journal of Pharmacy. 2014; 4(1):37-43.
21. Enyi-Idoh, K. H., Ikpeme, E. M., Iwuh, G. C., Egeonu, S. U., Mandor, B. I., & Ofor, U. A. Antibacterial activity of gnetum africanum and heinsia crinita on diarrhoeagenic bacteria stool isolates from children in Calabar south Lga, cross river state, Nigeria. Transnational Journal of Science and Technology; 2013: 3(3), 28-36.
22. Oboh G, Nwanna EE, Oyeleye SI, Olasehinde TA, Ogunsuyi OB, Boligon AA. In vitro neuroprotective potentials of aqueous and methanol extracts from Heinsia crinita leaves. Food Science and Human Wellness. 2016 Jun 1; 5(2):95-102.
23. Abolaji AO, Olaiya CO, Oluwadahunsi OJ, Farombi EO. Dietary consumption of monosodium L-glutamate induces adaptive response and reduction in the life span of Drosophila melanogaster. Cell biochemistry and function. 2017 Apr; 35(3):164-70.
24. Oboh G, Oladun FL, Ademosun AO, Ogunsuyi OB. Anticholinesterase activity and antioxidant properties of Heinsia crinita and Pterocarpus soyauxii in Drosophila melanogaster model. Journal of Ayurveda and integrative medicine. 2021 Apr 1; 12(2):254-60.
25. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and phytochemistry. 2014; 2(5):115-9.
26. Ajayi AF, Akhigbe RE. Apoptotic inducement of neuronal cells by codeine: possible role of disrupted redox state and caspase 3 signaling. Heliyon. 2021 Jul 1; 7(7).
27. Karimi S, Karami M, Zardooz H, Salimi SH, Sahraei H. Biphasic effects of naloxone in the rats receiving morphine overdose a place preference study. Iranian Journal of Pharmaceutical Research: IJPR. 2011; 10(3):605.
28. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976 May 7; 72(1-2):248-54.
29. Akhigbe R, Ajayi A. Testicular toxicity following chronic codeine administration is via oxidative DNA damage and up-regulation

of NO/TNF-α and caspase 3 activities. PLoS One. 2020 Mar 13; 15(3):e0224052.
30. Hamed MA, Aremu AO, Akhigbe RE. Concomitant administration of HAART aggravates anti-Koch-induced oxidative hepatorenal damage via dysregulation of glutathione and elevation of uric acid production. Biomedicine & Pharmacotherapy. 2021 May 1; 137:111309.
31. Sun YI, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical chemistry. 1988 Mar 1; 34(3):497-500.
32. Aebi H. Catalase in vitro. InMethods in enzymology 1984 Jan 1 (Vol. 105, pp. 121-126). Academic press.
33. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry. 1968 Jan 1; 25:192-205.
34. Zang J, Zhu Y, Zhou Y, Gu C, Yi Y, Wang S, Xu S, Hu G, Du S, Yin Y, Wang Y. Yeast-produced RBD-based recombinant protein vaccines elicit broadly neutralizing antibodies and durable protective immunity against SARS-CoV-2 infection. Cell Discovery. 2021 Aug 18; 7(1):71.
35. Faulkner SH, Spilsbury KL, Harvey J, Jackson A, Huang J, Platt M, Tok A, Nimmo MA. The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise. European journal of applied physiology. 2014 Jun; 114:1207-16.
36. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology. 2007 Jan 1; 39(1):44-84.
37. Archibong AE, Inyang F, Ramesh A, Greenwood M, Nayyar T, Kopsombut P, Hood DB, Nyanda AM. Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo (a) pyrene. Reproductive Toxicology. 2002 Nov 1;16(6):801-8.
38. Atici S, Cinel L, Cinel I, Doruk N, Aktekin M, Akca A, Camdeviren H, Oral U. Opioid neurotoxicity: comparison of morphine and tramadol in an experimental rat model. International journal of neuroscience. 2004 Jan 1; 114(8):1001-11.
39. Faria J, Barbosa J, Leal S, Afonso LP, Lobo J, Moreira R, Queirós O, Carvalho F, Dinis-Oliveira RJ. Effective analgesic doses of tramadol or tapentadol induce brain, lung and heart toxicity in Wistar rats. Toxicology. 2017 Jun 15; 385:38-47.
40. Morales I, Farías GA, Cortes N, Maccioni RB. Neuroinflammation and neurodegeneration. Update on dementia. 2016 Sep 28; 13.
41. Gresa-Arribas N, Viéitez C, Dentesano G, Serratosa J, Saura J, Solà C. Modelling neuroinflammation in vitro: a tool to test the potential neuroprotective effect of anti-inflammatory agents. (2012): e45227.
42. Kempuraj D, Thangavel R, Natteru PA, Selvakumar GP, Saeed D, Zahoor H, Zaheer S, Iyer SS, Zaheer A. Neuroinflammation induces neurodegeneration. Journal of neurology, neurosurgery and spine. 2016; 1(1).