JJBS

Volume 16, Number 2,June 2023
ISSN 1995-6673
Pages 297 – 305
https://doi.org/10.54319/jjbs/160214

Jordan Journal of Biological Sciences

Peptides from Casein Extend the Survival Rate and Protect *Drosophila melanogaster* from Oxidative Stress Via Interacting with the Keap1-Nrf2 Pathway

Idris Zubairu Sadiq[1,*], Babangida Sanusi Katsayal[1], Rashidatu Abdulazeez[2], Odunola Safiyyah Adedoyin[1], Yakubu Fatimat Omengwu[1], Abdullahi Garba Usman[3,4]

[1]*Department of Biochemistry, Faculty of life sciences, Ahmadu Bello University, Zaria;* [2]*Drosophila and Neurogenetics laboratory, Department of Zoology, Faculty of life sciences, Ahmadu Bello University, Zaria;* [3]*Operational research Centre in healthcare, Near East University, Nicosia, Turkish Republic of Northern Cyprus;* [4]*Department of Analytical Chemistry, Faculty of Pharmacy, Near East University, Nicosia, Turkish Republic of Northern Cyprus*

Received: August 11, 2022; Revised: September 25, 2022; Accepted: December 23, 2022

Abstract

It is generally established that oxidative stress has a role in the etiology of diseases linked to the lifestyle, such as cancer, aging, diabetes mellitus, and neurological diseases, among others. This research aims to evaluate the effect of casein on the survival rate and oxidative stress markers in *Drosophila melanogaster*. 360 flies were divided into four groups, each containing 90 flies (30 flies in triplicate). The groups include:1) the control group; 2) the H_2O_2 treated group; 3) the H_2O_2 induced + 1% Casein group; 4) the 1% Casein only group. Oxidative stress was induced in the flies and 1% Casein was used to protect the flies through enrichment of their diet and the effect of stress and Casein treatment was monitored on the mortality rate of the flies and selected oxidative stress markers. The results revealed that H_2O_2 significantly decreases the survival rate, GSH, total thiol and total protein while it increases the level of MDA, SOD and CAT. However, treatment with 1% Casein significantly reverses the effect of H_2O_2 on the oxidative stress biomarkers and increases flies' survival rate. In conclusion, Casein may increase the survival rate of the fruit flies under stress and modulate stress biomarkers via possible binding of the peptides AS29, AS14, K009, and K010 to the Keap1/Nrf2 signaling pathway.

Keywords: Antioxidants, fruit flies, hydrogen peroxide, casein, Keap1/Nrf2

Practical application

The oxidation process is thought to be the primary cause of human aging, degenerative diseases, and stress related diseases. Casein is a protein found in mammalian milk that accounts for roughly 20 to 60% of the proteins in human milk and 80% of the proteins in cow's milk. It represents a good source of antioxidants and bioactive peptides making it a promising candidate for preventing harmful stress, aging, immune boosting and preventing/treating disorders associated with oxidative stress.

1. Introduction

Oxidative stress is a typical situation in which the biological buffering capacity of the system is exceeded by production of reactive oxygen/nitrogen species (Tabima *et al.*, 2012). This reactive species often damages biological macromolecules (carbohydrates, DNA, proteins and lipids), thereby modifying the functions of these molecules (Roberts and Hubel, 2004). The genesis and/or progress of numerous diseases, including cardiovascular diseases, atherosclerosis, diabetes, cancer and metabolic disorders were linked to oxidative stress (Taniyama and Griendling, 2003). Life of living organisms encounters reactive oxidants from internal metabolisms and environmental exposure to chemicals. The body's defense system responds via the Nrf2, which controls the expression of an array of antioxidant response element–dependent genes (Ma, 2013). As free radicals are extremely reactive, macromolecules in nearly all the cells are often prone to attack, causing irreversible damage. Therefore, the breakdown of excessive H_2O_2 is a prerequisite for survival of the living cells (Droge, 2002).

Natural antioxidants derived from foods have been studied and believed to have protective biological functions in preventing stress induce diseases and cell damage due to free radicals (Balsalobre-Fernández *et al.*, 2017). Milk, in particular, contains proteins with varied biological functions, and caseins are the major protein of ovine and bovine milks, which is present in the form of macromolecular aggregates.

Milk proteins and dairy products were reported to have antioxidant activity which can scavenge reactive oxygen species (Khan *et al.*, 2019). Casein possesses several bioactivities including antimicrobial, antioxidative, antithrombotic, antihypertensive, anti-carcinogenic, and immunomodulatory process (Shahidi and Zhong, 2008).

* Corresponding author. e-mail: izsadiq@abu.edu.ng.

The basic structure of casein molecules works as a scavenger for free radicals since free individual amino acids may not quench these radicals *(*Suetsuna *et al.*, 2000). Owing to their chemical antioxidant capabilities, bioactive peptides have attracted a lot of attention these days. The properties of these bioactive peptides are demonstrated by their well-characterized properties, which include chelating transition pro-oxidant metals and hydrogen atom transfer (Aguilar-Toalá and Liceaga, 2021), inhibiting the lipoxygenase-catalysed lipid oxidation (Suetsuna *et al.*, 2000) as well as the ability to attract free radicals (Rocha-Decker, 2004). These well characterized properties make these peptides as potential antioxidants present in milk and dairy products.

2. Materials and methods

2.1. Chemicals and reagents

Casein from bovine milk and hydrogen peroxide were purchased from Sigma Aldrich. All other reagents were of analytical grades.

2.2. Drosophila melanogaster stock and culture

D. melanogaster wild type (Harwich strain) flies was obtained, maintained and reared from the Drosophila laboratory, Department of Zoology, Ahmadu Bello University, Zaria on a cornmeal medium at constant temperature and humidity (23 - 25^0C) under 12 hrs dark/light cycle conditions.

2.3. Experimental protocol

Thirty (30) flies consisting of ten (10) males and twenty (20) females were segregated into twelve (12) vials each, and divided into four (4) groups, with each group in triplicate. The experimental design is presented in figure 1.

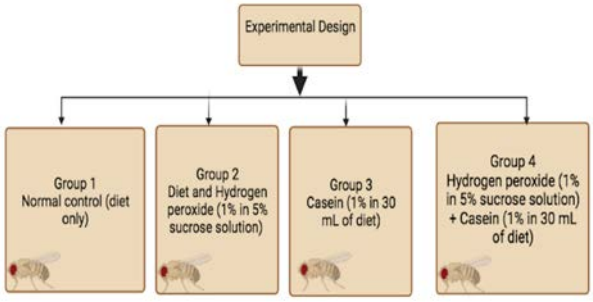


Figure 1. Experimental design

2.4. Stress induction in Drosophila melanogaster

Wild type (Harwich strain) of *D. melanogaster* was used for *in vivo* studies. The flies were induced by exposing 3 days old flies (both sexes) to a filter paper moistened with 1% H_2O_2 (in 5% sucrose solution) for 4 hours. The exposed flies were then placed in a culture media containing Casein for 3 days before subjecting the flies to biochemical analysis as described elsewhere (Subramanian *et al*., 2014) with minor modifications. The control flies were only provided with the normal diet without exposure to H_2O_2 or administration of casein.

2.5. Survival rate of flies

The survival rate was determined as modified from (Chattopadhyay *et al.*, 2015). 10 male and 20 female (single-sex) flies were segregated into vials diet with the treatment of Casein after exposure with H_2O_2. The flies were transferred into new vials with fresh diet every 5 days.

2.6. Preparation of samples and biochemical assays

2.6.1. Sample Preparations

Thirty (30) flies were anesthetized on ice for the biochemical experiments, weighed, homogenized in 0.1M phosphate buffer (PBS), and centrifuged for 10 min at 4,000 rpm and 4°C. Assays for total protein, GSH, catalase, superoxide dismutase, malondialdehyde, and total thiol (TSH) levels were performed using the supernatants that were obtained after separating the pellets from the supernatants.

2.6.2. Determination of Biochemical Parameters

A modified Lowry method was used to measure the protein concentration (Peterson, 1977). Glutathione and total thiol levels were estimated using the Ellman method (Ellman, 1959). Catalase activity was measured using the Aebi-reported technique (Aebi, 1984). Malondialdehyde level was measured using the method described by Niehaus & Samuelson (Niehaus & Samuelson, 1968), while superoxide dismutase activity was analyzed using the method described by Fridovich (Fridovich, 1989).

2.7. In silico *Digestion and Bioactivity Prediction*

Gastrointestinal enzymatic hydrolysis of casein was accomplished using BIOPEP Web server by simulating the action of different proteases found in the digestive tract of *Drosophila* such as chymotrypsin (EC3.4.21.1), trypsin (EC 3.4.21.4) and (pepsin, pH = 1.3) (Kose, 2021). The potential antioxidant bioactivities of the peptides were analyzed using FeptideDB and published research papers (http://www4g.biotec.or.th/FeptideDB/)(Panyayai *et al.*, 2019). FeptideDB represent bioactive peptide database which contain records from both available research articles and about 12 public bioactive peptide databases including BIOPEP-UWM, APD, BACTIBASE, CAMP, PenBase, RAPD, Hmrbase, PhytAMP, PeptideDB, ACEpepDB, Amper, and BAGEL3.

2.8. Molecular Docking Studies

The 3-Dimensional structure of Keap1 was retrieved from protein data bank with PDB Code 6TYM, and the selected bioactive peptide sequence was converted to 3D structure using a 3D peptide generator. By eliminating solvent molecules, the crystal structures of Keap1 and the peptides were formed, and then Chimera 1.11 was used to optimize them to mimic physiological conditions (Pettersen *et al*., 2004). The standard residue was given polar hydrogen, and Gasteiger partial charge—which presumes that all hydrogen atoms are clearly represented—was used to assign partial charges to the standard residue. Utilizing AutoDock Vina v.1.1.2, molecular docking studies were used to identify the most advantageous binding interactions. With the assistance of Molegro Molecular Viewer 2.5, the interactions of the docked complexes were virtually examined.

3. Results

The result shows a significant decrease in the survival of flies exposed to hydrogen peroxide due to oxidative stress compared to those administered 1% casein. The survival assay graph shown below (Fig.2) indicates the survival rate of the flies as percentage survival against number of days.

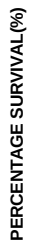


Figure 2: Effect of Hydrogen peroxide and Casein on survival of *Drosophila melanogaster*. Flies exposed to hydrogen peroxide had a 100% decreased survival at day 28, while 30%, 25% and 20% decrease in survival were observed in Casein-treated, control and casein only flies, respectively.

Essentially, we noted that oxidative stress significantly ($p < 0.05$) lowered the amount of total protein in the stress flies, but no statistical significant differences were observed in the 1% casein-treated, normal control, and negative control (1% casein alone) groups (Fig. (3a). We also observe that the activity of SOD considerably ($p < 0.05$) increased in the flies exposed to H_2O_2, but no statistically significant difference ($p < 0.05$) was noted in the case of the flies treated with 1% casein and the control group (Fig. 3b). Flies subjected to hydrogen peroxide also showed a significant rise ($p < 0.05$) in the level of malondialdehyde (MDA), whereas in the 1% casein treatment and negative control (1% casein alone) groups, the MDA concentration did not differ significantly ($p < 0.05$) from that of the control group (Fig. 3c). In the stressed flies also, total thiol levels also decreased significantly ($p < 0.05$). However, neither the group treated with 1% casein nor the negative control (1% casein only) group showed a statistically significant decrease ($p< 0.05$) in comparison to the control (Fig. 3d). Additionally, stress was discovered to significantly ($p<0.05$) lower the level of GSH in the flies (Fig. 3e), whereas the catalase activity in flies exposed to hydrogen peroxide considerably ($p < 0.05$) increased in comparison to the other groups (3f).

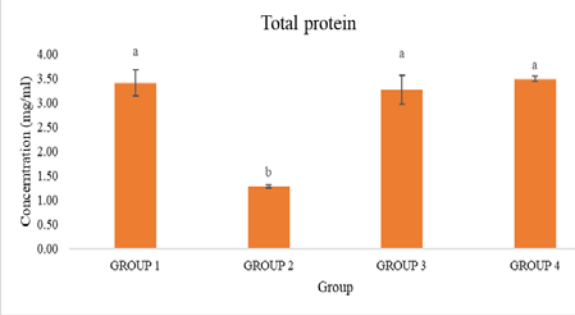


Figure 3a: Effect of Casein on total protein in hydrogen peroxide induce oxidative stress in *Drosophila melanogaster*. [All results are expressed as mean ± standard deviation, and superscripts containing different letters (a, b) indicate significantly different results when at $p < 0.05$].

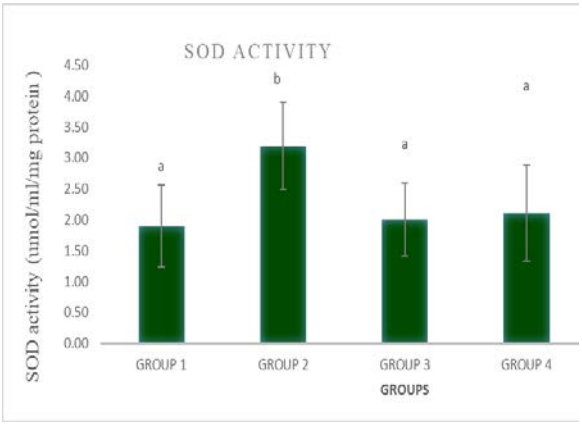


Figure 3b: Effect of Casein on SOD in hydrogen peroxide induces oxidative stress in *Drosophila melanogaster*. [All results are expressed as mean ± standard deviation, and superscripts containing different letters (a, b) indicate significantly different results when at $p < 0.05$].

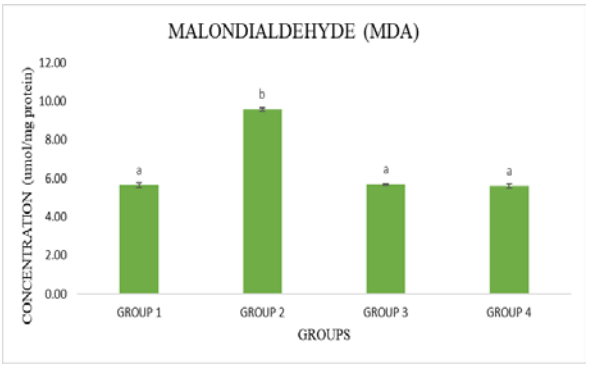


Figure 3c. Effect of Casein on MDA in hydrogen peroxide induced oxidative stress in *Drosophila melanogaster*. [All results are expressed as mean± standard deviation, and superscripts containing different letters (a, b) indicate significantly different results when at $p < 0.05$]

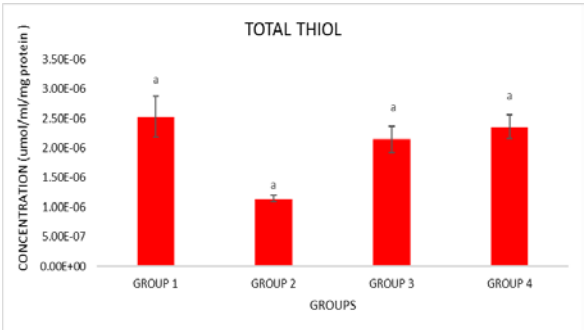


Figure 3d: Effect of Casein on total thiol in hydrogen peroxide induced oxidative stress in *Drosophila melanogaster*. [All results are expressed as mean ± standard deviation, and superscripts containing different letters (a, b) indicate significantly different results when at $p < 0.05$].

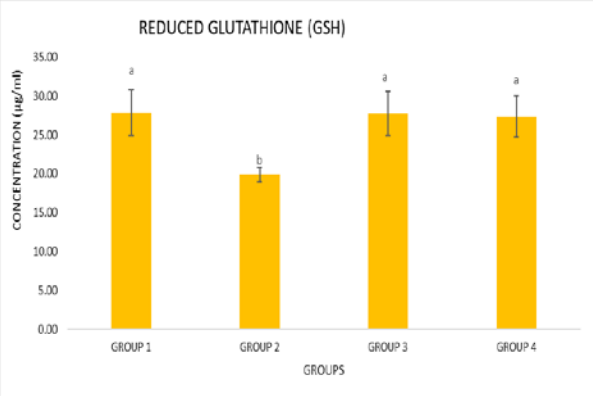


Figure 3e: Effect of Casein on GSH in hydrogen peroxide induced oxidative stress in *Drosophila melanogaster*. [All results are expressed as mean± standard deviation, and superscripts containing different letters (a, b) indicate significantly different results when at $p < 0.05$].

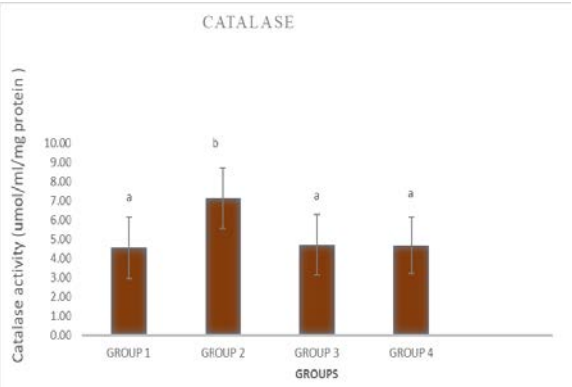


Figure 3f: Effect of Casein on CAT in hydrogen peroxide induce oxidative stress in *Drosophila melanogaster*. [All results are expressed as mean ± standard deviation, and superscripts containing different letters (a, b) indicate significantly different results when at $p < 0.05$].

3.1. In silico Digestion and Bioactivity Prediction

Hydrolysis of casein proteins with cysteine/aspartic/serine type endopeptidases was accomplished using the BIOPEP website. These enzymes were reported to play most important role in producing bioactive peptides of 3-30 amino acid chains in the gastrointestinal tract of *Drosophila melanogaster* (Lemaitre & Miguel-Aliaga, 2013). The enzymes generate about 90 peptides 3-50 amino acid chains with different molecular weight and isoelectric points (Table 1). Four bioactive peptides were selected based on their antioxidant properties predicted from FeptideDB and literature search (written in red in Table.1) . The selected bioactive peptides were converted into a 3D structure for molecular docking interaction with Keap1.

Table1. Digestion Product

S/N	Mass	PI	Peptide	Mass	PI	Peptide	Mass	PI	Peptide	Mass	PI	Peptide
	Alpha S2			Alpha S1			Beta Casein			Kappa Casein		
1.	348.46	8.80	AMK	351.4	5.52	LGY	407.51	5.52	LLY	402.45	8.75	QQK
2.	381.43	5.15	TVY	524.62	8.76	HIQK	388.46	6	IEK	330.43	8.75	IAK
3.	392.46	5.52	LNF	493.61	8.76	HPIK	373.45	8.75	INK	348.36	5.55	SDK
4.	422.48	5.52	LQY	667.76	4	YPELF	512.61	6.74	IHPF	418.41	4.37	DER
5.	403.44	6.00	QEK	614.76	8.76	LHSMK	750.85	4	YPVEPF	378.44	5.99	CEK
6.	490.60	5.49	VIPY	747.91	5.19	TTMPLW	747.91	6.1	EMPFPK	408.57	8.5	MMK
7.	484.60	8.75	IQPK	636.66	3.8	TDAPSF	645.77	6.1	EAMAPK	503.51	5.84	NQDK
8.	539.59	8.76	QHQK	770.93	6	LEQLLR	741.93	5.52	GPFPIIV	465.51	5.52	GLNY
9.	502.57	9.75	ISQR	688.78	5.97	VNELSK	820.96	3.8	DMPIQAF	528.56	5.52	YPSY
10.	488.54	5.84	DQVK	776.89	5.49	VPLGTQY	829.95	8.79	AVPYPQR	473.57	9.72	VLSR
11.	553.62	5.52	FPQY	830.85	4.14	EDVPSER	779.98	8.72	VLPVPQK	512.61	10.18	PAAVR
12.	501.58	6.10	EVVR	910	6.85	EGIHAQQK	994.16	6	QEPVLGPVR	651.76	5.52	LPYPY
13.	590.68	5.57	ALPQY	905.06	4	VAPFPEVF	1087.3	6	LQPEVMGVSK	632.76	5.52	IPIQY
14.	747.80	4.25	LTEEEK	1107.19	3.8	QLDAYPSGAW	1438.88	8.22	VLILACLVALALAR	655.85	8.5	MAIPPK
15.	689.76	4.21	ITVDDK	1363.55	3.8	EPMIGVNQELAY	1834.82	3.77	QSEEQQQTEDELQDK	971.09	7.02	HPHPHLSF
16.	867.82	3.67	NANEEEY	1486.56	4.14	SDIPNPIGSENSEK	2735.09	4.13	TESQSLTLTDVENLHLP LPLLQSW	942.08	5.24	SPAQILQW
17.	812.89	4.00	ENLCSTF	1580.76	4.25	VPQLEIVPNSAEER	2646.84	3.83	ELEELNVPGEIVESLSSS EESITR	1015.18	5.96	PVALINNQF
18.	948.04	4.00	ALNEINQF	1468.9	8.25	LLILTCLVAVALAR	2912.42	8.54	MHQPHQPLPPTVMFPP QSVLSLSQSK	1056.23	8.75	QVLSNTVPAK
19.	874.02	8.75	NMAINPSK	1759.98	5.4	HQGLPQEVLNENLLR	3755.37	5.57	AQTQSLVYPFPGPIPNS LPQNIPPLTQTPVVVPPF	1193.36	7.96	SCQAQPTTMAR
20.	1023.20	5.52	QGPIVLNPW	1767.84	3.71	DIGSESTEDQAMEDIK				1299.66	5.52	LVVTILALTLPF
21.	1157.26	3.57	TVDMESTEVF	2321.5	3.98	QMEAESISSSEEIVPNSVEQ K				1510.63	4.53	LGAQEQNQEQPIR
22.	1002.28	7.89	TCLLAVALAK							5455.92	3.28	TEIPTINTIASGEPT STPTTEAVESTVA TLEDSPEVIESPPEI NTVQVTSTAV
23.	251.27	4.25	EQLSTSEENSK									
24.	1195.38	9.75	NAVPITPTLNR									
25.	1838.90	3.98	SIGSSSEESAEVA TEEVK									
26.	2171.27	4.09	NTMEHVSSSEES IISQETY									
27.	880.86	4.53	STSEENSK									
28.	752.69	3.79	GSSSEESA									

AS29 = Alpha S2 peptide 9, AS14 = Alpha S1 peptide 4, K009 = Kappa casein peptide 9 and K010 =Kappa casein peptide 1

3.2. Molecular Docking Studies

As presented in the Figures 4-7 below, the four bioactive peptides selected from table1 bind to Nrf2 binding pocket in Keap1 with a very strong binding energy (Table2). The interactions resulted from both polar and nonpolar amino acid residues found within the binding cavities of the proteins which include Tyr10, Arg36, Phe253, Tyr248, Gly231, Ala232, Val280, Gly275, Gly9, Ser39, Arg53, and Asn90 among many others (Fig.4-7).

Table 2. Binding energy of the selected bioactive peptides on Keap1.

Ligand/Peptides	Binding Energy (Kcal/mol)
AS29	-8.0
AS14	-8.4
K009	-8.6
K010	-9.7

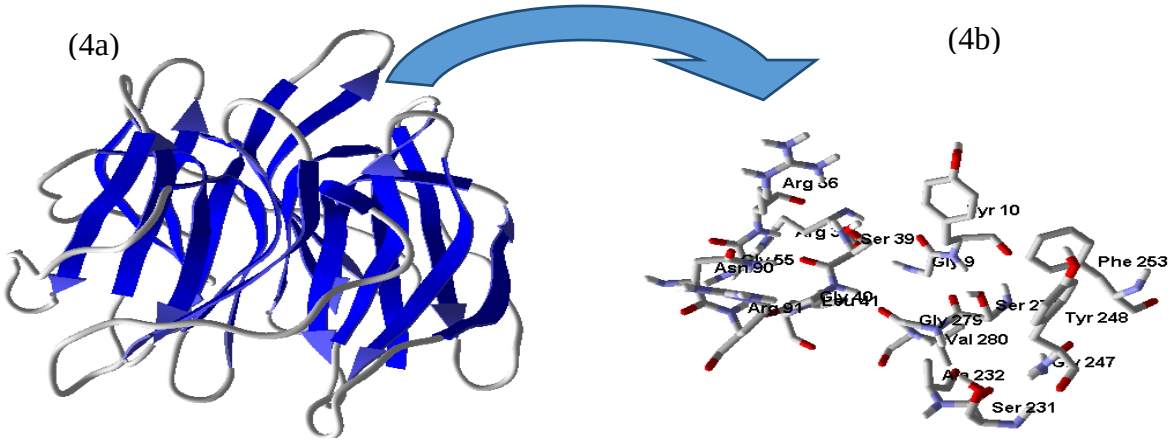


Figure 4. Keap1 (4a) cartoon structure (4b) 3D pocket amino acids residues.

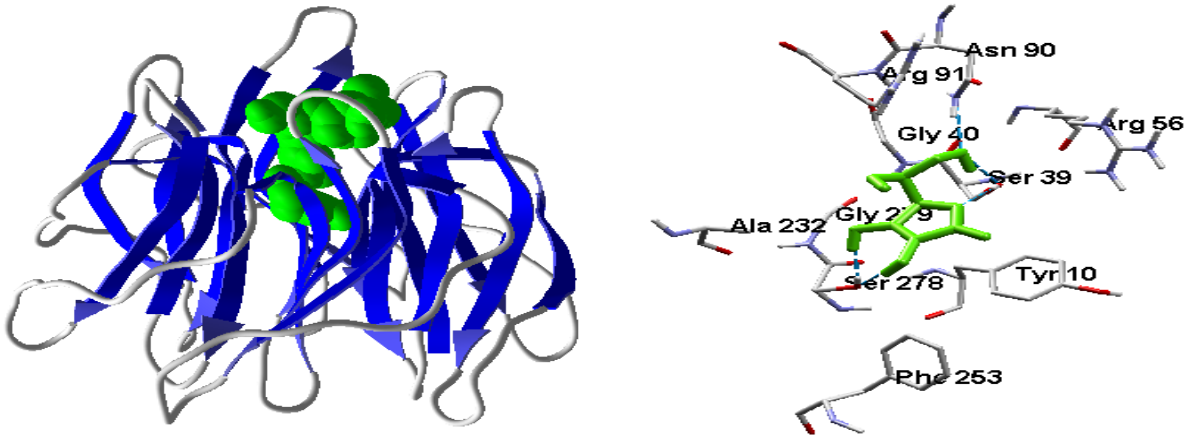


Figure 5. Keap1 bound to AS29 (5a) cartoon structure (5b) 3D interaction residues.

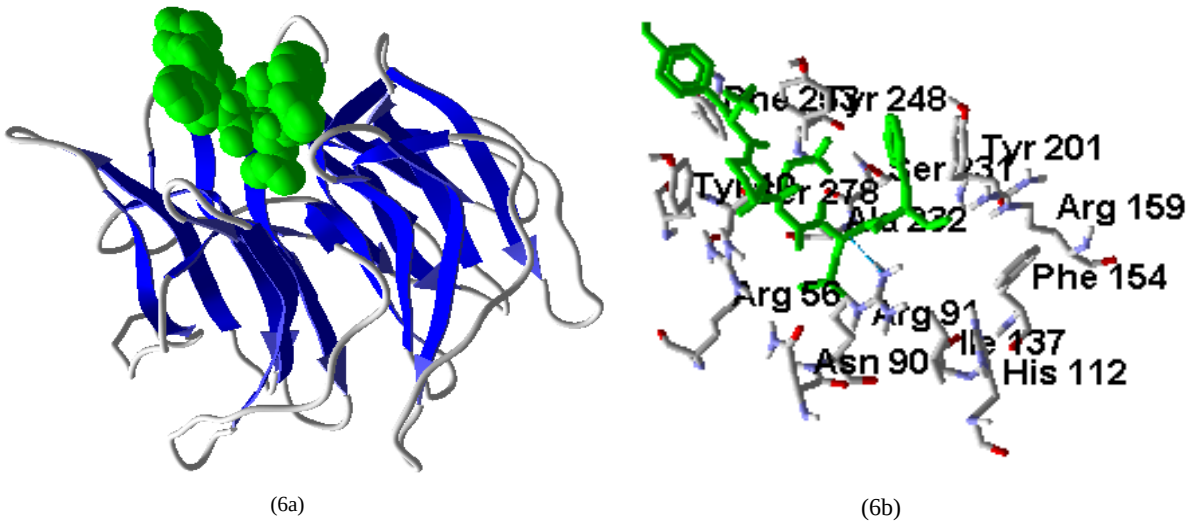
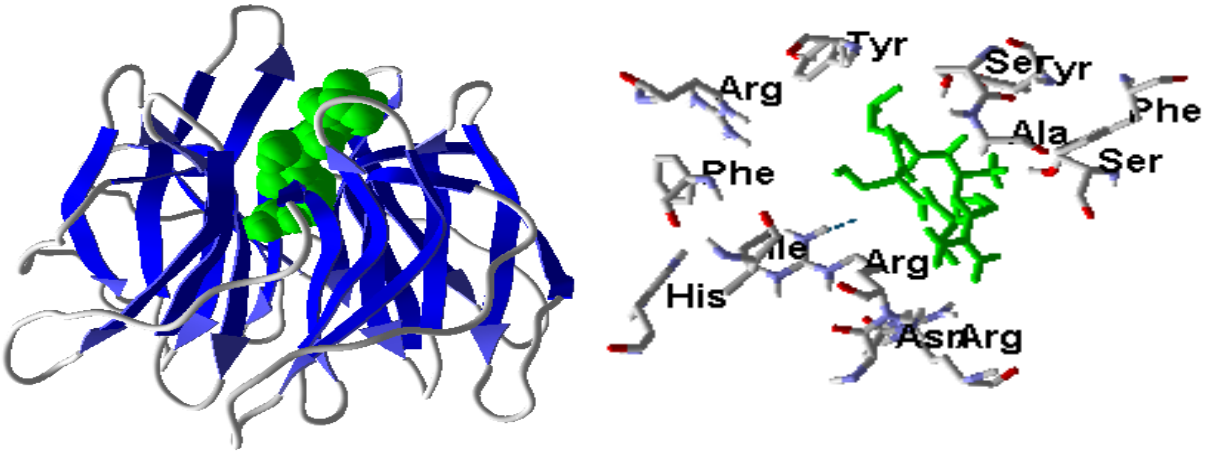


Figure 6: Keap1 bound to AS14 (6a) cartoon structure (6b) 3D interaction residues.



(7a) (7b)

Figure 7: Keap1 bound to K010 (7a) cartoon structure (7b) 3D interaction residues.

4. Discussion

This research is aimed at exploring the possible use of peptides as potentially antioxidant agents for enhancing longevity as well as the amelioration of oxidative stress that is common in both aging process and stress related illnesses. In the cellular models, exposure to hydrogen peroxide (H_2O_2) is a common approach for inducing oxidative damage or stress (Gille & Joenje, 1992).Previous study indicated that casein had a clearly unfavorable effect on longevity at an acid pH, contrary to the finding obtained at a neutral pH(Van Herrewege, 1974).Essentially, the current study uses a neutral pH and 5% sucrose which may likely play role in flies longevity(Van Herrewege, 1974).Our survival assay results indicated that casein increase the survival rate of the flies by protecting the flies from oxidative stress. Our data thus showed that stress substantially reduced the level of some oxidative stress markers including total protein, total thiol, and reduced glutathione while increasing the level of malondialdehyde, superoxide dismutase and catalase ($p< 0.05$) when compared to the control. The increase in SOD activity in stressed fruit flies suggests that this enzyme is involved in reducing the presence of hydrogen peroxide free radicals. The critical antioxidant activity of SOD is to catalyzes the dismutation of superoxide anion radicals to hydrogen peroxide as well as involving in chain breaking reactions that protect against harmful effects of lipid peroxidation (Wang *et al.*, 2018). CAT safeguard cells by converting hydrogen peroxide to molecular oxygen and water, a crucial process for the development of tolerance and adaptive cellular response to oxidative stress (González-Párraga *et al.*, 2003). The increase in the activity of catalase in the stress flies indicates the presence of oxidative stress and antioxidant response by the system (Perkhulyn *et al.*, 2017).

A significant source of necessary amino acids that also function as intracellular antioxidants is dietary protein and a study has linked arginine and histidine oxidative stress and inflammation in obese and non-obese women (Niu *et al.*, 2012). Treatment with 1% casein to stress flies considerably increased the survival of *D. melanogaster* under stress conditions and reversed H_2O_2 induced oxidative stress by modulating the levels of the oxidative stress biomarkers. According to a study, casein enrichment in the diet of flies can dramatically reduce oxidative damage and undo alterations in the activity of antioxidant enzymes (Venkareddy, 2015). Milk containing A1β-casein was linked to slower and less accurate cognitive functioning, worsened PD3 symptoms, delayed transit and increased gastrointestinal inflammation (Jianqin *et al.*, 2016). Therefore, taking milk that solely contains the A2 form of beta casein could prevent those unwanted effects (Jianqin *et al.*, 2016).

A study has also evaluated the antioxidant activity of casein phosphoprotein in caco-2 cells and confirmed its cytoprotective effects (Laparra *et al.*, 2008). Another study also indicated the protective effects of casein and dairy products from breast, prostate and colon cancer (Khan *et al.*, 2019). The existence of a larger quantity of glutathione, which is widely known for its antioxidant activity, could be responsible for casein's anticancer properties (Khan *et al.*, 2019). Mammalian milk contains casein that makes up roughly 20% to 60% of the proteins in human milk and 80% of the proteins in cow's milk. Considering the exceptional properties of this peptide, casein may function as a co-adjuvant in conventional treatment for conditions like cardiovascular disease, metabolic disease, asthma and other stress-related diseases (Khan *et al.*, 2019). Casein could also be hydrolyzed using different enzymes to produce a vast number of bioactive peptides having physiological activities in the endocrine, cardiovascular, immunological, and neurological systems (Mohanty *et al.*, 2016). Antioxidant peptides have become increasingly popular as they have the ability to remove excess free radicals (Zhao *et al.*, 2021). From this study, we observed that four bioactive peptides from casein, AS29, AS14, K009, and K010 had antioxidant activity and may help the flies live longer by reversing oxidative stress markers.

The Nrf2 is a master transcription factor tasked with guarding against oxidative cell damage by controlling the production of antioxidant proteins (Zhang, 2006). An essential signaling pathway for controlling the antioxidant system is provided by the interaction between Keap1 and Nrf2, which also maintains the cell's redox balance and metabolism (Zhang, 2006). When under normal

circumstances, Nrf2 is located within the cytoplasm and rapidly broken down by a group of proteins. However, when under conditions of oxidative stress, Nrf2 binds to the antioxidant response element (ARE) in the nucleus and starts the transcription of antioxidant genes such as catalase, glutathione S-transferase, superoxide dismutase, heme oxidase (heme oxygenase), thioredoxin reductase, glutathione reductase/peroxidase, NAD(P)H dehydrogenase, thioredoxinreductase, heme oxygenase, glutathione S-transferase. As numerous conditions were linked to oxidative stress, including cancer, Alzheimer's disease, Parkinson's disease, and diabetes, study have shown that the Keap1-Nrf2 signaling pathway to be involved (Tkachev *et al.,* 2011). Selected bioactive peptides from casein were reported in the literature to conferred antioxidant activities by stimulating the activities of CAT, GSH, SOD and GSH-Px (Xiao et al., 2021). According to a recent study, bioactive peptides derived from milk have an antioxidant effect through activating the Nrf2 pathway (Tonolo *et al.,* 2020). Molecular docking from this study reveals that the selected bioactive peptides (AS29, AS14, K009, and K010) fits exactly in the binding pocket of Keap1 (Fig. 4-7), and these will facilitate its binding to the antioxidant response element and subsequent activation of antioxidant enzymes.

5. Conclusion

Casein increased the survival rate of *Drosophila melanogaster* under oxidative stress conditions. Casein was also found to modulate the selected oxidative stress markers by acting as an antioxidant.

Conflict of interest

None

Acknowledgement

The team appreciates the assistance of Mal. S. Muhammad, the research assistant of the *Drosophila* and Neurogenetics laboratory, Department of Zoology, A.B.U. Zaria. We thank Prof. D.M. Shehu, Department of Zoology A.B.U. Zaria for his mentorship during this research.

Authors Contributions

IZS. Conception, supervision, financial contribution and manuscript writing. RA. Supervision of the lab work and edited the manuscript. O. S. A. and Y. F. O. carried out the research and manuscript writing. BSK. Supervision, molecular docking and manuscript writing. AGU. Statistics, graphs, and final manuscript version.

References

Aebi, H. (1984). Catalase in vitro. *Meth Enzymol., 105*, 121–126.

Aguilar-Toalá, J. E., & Liceaga, A. M. (2021). Cellular antioxidant effect of bioactive peptides and molecular mechanisms underlying: beyond chemical properties. *International Journal of Food Science & Technology*, ***56*(5)**, 2193–2204.

Balsalobre-Fernández, C., Marchante, D., Baz-Valle, E., Alonso-Molero, I., Jiménez, S. L., & Muñóz-López, M. (2017). Analysis of wearable and smartphone-based technologies for the measurement of barbell velocity in different resistance training exercises. *Front Physiol., 8*, 649.

Chattopadhyay, D., James, J., Roy, D., & Sen, S. (2015). Effect of semolina-jaggery diet on survival and development of Drosophila melanogaster. *Fly (Austin)*, ***9*(1),**16-21. https://doi.org/10.1080/19336934.2015.1079361

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiol Rev*, *82*(1), 47–95.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Arch Biochem Biophys*, ***82*(1),** 70–77.

Fridovich, I. (1989). Superoxide dismutases: an adaptation to a paramagnetic gas. *J Biol Chem*, *264*(14), 7761–7764.

Gille, J. J. P., & Joenje, H. (1992). Cell culture models for oxidative stress: superoxide and hydrogen peroxide versus normobaric hyperoxia. *Mut Res/DNAging*, ***275*(3–6),** 405–414.

González-Párraga, P., Hernández, J. A., & Argüelles, J. C. (2003). Role of antioxidant enzymatic defences against oxidative stress (H2O2) and the acquisition of oxidative tolerance in Candida albicans. *Yeast*, ***20*(14),** 1161–1169.

Jianqin, S., Leiming, X., Lu, X., Yelland, G. W., Ni, J., & Clarke, A. J. (2016). Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk. *Nutr J.*, ***15***, 35. https://doi.org/10.1186/s12937-016-0147-z

Khan, I. T., Nadeem, M., Imran, M., Ullah, R., Ajmal, M., & Jaspal, M. H. (2019). Antioxidant properties of Milk and dairy products: a comprehensive review of the current knowledge. *Lipids Health Dis.*, *18*(1), 1–13.

Kose, A. (2021). In Silico Bioactive Peptide Prediction from The Enzymatic Hydrolysates of Edible Seaweed Rubisco Large Chain. *Turkish J Fish Aquat Sci.*, ***21*(12)**, 615–625.

Laparra, J. M., Alegra, A., Barber R., & Farr, R. (2008). Antioxidant effect of casein phosphopeptides compared with fruit beverages supplemented with skimmed milk against H2O2-induced oxidative stress in Caco-2 cells. *Food Res Int.*, ***41*(7),** 773–779.

Lemaitre, B., & Miguel-Aliaga, I. (2013). The digestive tract of Drosophila melanogaster. *Ann Rev Genet.*, ***47***, 377–404.

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Ann Rev Pharmcol Toxicol*, ***53***, 401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Mohanty, D. P., Mohapatra, S., Misra, S., & Sahu, P. S. (2016). Milk derived bioactive peptides and their impact on human health – A review. *Saudi J Biol Sci.*, ***23*(5)**, 577–583. https://doi.org/https://doi.org/10.1016/j.sjbs.2015.06.005

Niehaus, W. G., & Samuelson, B. (1968). Formation of malondialdehyde from and glucose 6-phosphate dehydrogenase from fermenting yeast and phospholipids arachidonate during microsomal lipid peroxidation. *Eur J Biochem.*, ***6***, 126–130.

Niu, Y.-C., Feng, R.-N., Hou, Y., Li, K., Kang, Z., Wang, J., Sun, C.-H., & Li, Y. (2012). Histidine and arginine are associated with inflammation and oxidative stress in obese women. *British J Nutr.*, ***108*(1),** 57–61.

Panyayai, T., Ngamphiw, C., Tongsima, S., Mhuantong, W., Limsripraphan, W., Choowongkomon, K., & Sawatdichaikul, O. (2019). FeptideDB: A web application for new bioactive peptides from food protein. *Heliyon*, ***5*(7),** e02076.

Perkhulyn, N. V, Rovenko, B. M., Lushchak, O. V, Storey, J. M., Storey, K. B., & Lushchak, V. I. (2017). Exposure to sodium molybdate results in mild oxidative stress in Drosophila melanogaster. *Redox Rep.*, ***22*****(3),** 137–146.

Peterson, G. L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal Biochem.*, ***83*****(2)**, 346–356.

Roberts, J. M., & Hubel, C. A. (2004). Oxidative stress in preeclampsia. *American Journal of Obstetrics & Gynecology*, ***190*****(5)**, 1177–1178.

Rocha-Decker, M. S. (2004). *The development and validation of the Proactive Assessment of Social Skills for preschool children*. University of Oregon.

Shahidi, F., & Zhong, Y. (2008). Bioactive peptides. *J AOAC Int.*, ***91*****(4),** 914–931.

Subramanian, P., Prasanna, V., Jayapalan, J. J., Rahman, P. S. A., & Hashim, O. H. (2014). Role of Bacopa monnieri in the temporal regulation of oxidative stress in clock mutant (cryb) of Drosophila melanogaster. *J Insect Physiol.*, ***65***, 37–44.

Suetsuna, K., Ukeda, H., & Ochi, H. (2000). Isolation and characterization of free radical scavenging activities peptides derived from casein. *J Nutr Biochem.*, ***11*****(3),** 128–131.

Tabima, D. M., Frizzell, S., & Gladwin, M. T. (2012). Reactive oxygen and nitrogen species in pulmonary hypertension. *Free Rad Biol Med.*, ***52*****(9),** 1970–1986.

Taniyama, Y., & Griendling, K. K. (2003). Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension*, ***42*****(6),** 1075–1081.

Tkachev, V. O., Menshchikova, E. B., & Zenkov, N. K. (2011). Mechanism of the Nrf2/Keap1/ARE signaling system. *Biochem (Moscow)*, ***76*****(4)**, 407–422.

Tonolo, F., Folda, A., Cesaro, L., Scalcon, V., Marin, O., Ferro, S., Bindoli, A., & Rigobello, M. P. (2020). Milk-derived bioactive peptides exhibit antioxidant activity through the Keap1-Nrf2 signaling pathway. *J Funct Foods*, ***64***, 103696. https://doi.org/https://doi.org/10.1016/j.jff.2019.103696

Van Herrewege, J. (1974). Nutritional requirements of adult drosphila melanogaster: The influence of the casein concentration on the duration of life. *Exp Gerontol.*, ***9*****(4)**, 191–198.

Venkareddy, L. K. (2015). Potential of casein as a nutrient intervention to alleviate lead (Pb) acetate-mediated oxidative stress and neurotoxicity: first evidence in Drosophila melanogaster. *Neurotoxicol.*, ***48***, 142–151.

Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol.*, ***217*****(6)**, 1915–1928. https://doi.org/10.1083/jcb.201708007

Zhang, D. D. (2006). Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metab Rev.*, ***38*****(4)**, 769–789.

Zhao, X., Cui, Y.-J., Bai, S.-S., Yang, Z.-J., Megrous, S., Aziz, T., Sarwar, A., Li, D., & Yang, Z.-N. (2021). Antioxidant activity of novel casein-derived peptides with microbial proteases as characterized via Keap1-Nrf2 pathway in HepG2 cells. *J Microbiol Biotechnol.*,***31*****(8)**:1163-1174.