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In Silico evaluation of phytoconstituents from *Carica Papaya* and its anti-hyperglycemic activities on high sucrose-induced oxidative stress in *Drosophila melanogaster*

Omotade I. Oloyede[1], Seun J. Ibrahim[1], Scholastica O. Anadozie[2], Erdi C. Aytar[3], Oluwole B. Akawa[4], Isaac A. Adu[5], Oluwadunsin S. Omodaratan[1], Olabisi T. Obafemi[2,6] & Olusola B. Adewale[2]✉

Oxidative stress plays a key role in the development of metabolic disorders, such as diabetes. This study investigated the phytoconstituents present in *Carica papaya* (AECP) using an in silico model, and its anti-hyperglycemic activities on high sucrose-induced oxidative stress in *Drosophila melanogaster*. In silico molecular docking was performed to analyze the binding potential of AECP's bioactive compounds to key diabetes targets. Flies were fed a diet containing 30% sucrose to induce oxidative stress, followed by administration of AECP at doses of 50 and 100 mg/kg for five days. Biochemical assays assessed were glucose, total thiols, catalase, glutathione S-transferase (GST), and nitric oxide. In silico analysis revealed that carpaine, myricetin 3-rhamnoside, orientin 7-O-rhamnoside, and quercetin in AECP exhibited strong binding potential to key diabetes targets (alpha-amylase, beta-glucosidase, dipeptidyl peptidase 4, PPARG, and SGLT-2)). In fruit flies, sucrose-diet significantly ($p < 0.05$) reduced total thiol level, and catalase and GST activities while increasing glucose and nitric oxide levels. The AECP in a dose-dependent manner significantly ($p < 0.05$) reversed these changes, demonstrating its antioxidant and possible anti-hyperglycemic properties. These findings suggest that AECP may be a potential therapeutic agent for mitigating oxidative stress and supports its potential use in managing diabetes.

Keywords *Carica Papaya*, Diabetes, *Drosophila melanogaster*, In Silico, Oxidative stress

Oxidative stress (OS) is a common pathophysiological condition caused by an excessive production of oxidants relative to antioxidants. Oxidative stress has been implicated in the development of several metabolic disorders, including diabetes. Diabetes mellitus is characterized by persistent hyperglycemia resulting from insulin resistance, inadequate or lack of insulin secretion or both[1]. Globally, the prevalence of diabetes in adults in 2021 was 537 million, with an estimated increase to 783 million by 2045. Type II diabetes accounts for about 90% of all diabetes cases, making it the most prevalent.

Virtual screening (VS) is a powerful tool in drug discovery, enabling researchers to efficiently identify potential lead compounds for further experimental validation[2]. Molecular docking is a game-changer in virtual screening,

[1]Department of Biochemistry, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. [2]Drosophila Research/Drug Metabolism and Toxicology Laboratories, Biochemistry Program, College of Sciences, Afe Babalola University, P.M.B. 5454, Ado-Ekiti 360001, Ekiti, Nigeria. [3]Department of Horticulture, Faculty of Agriculture, Usak University, Uşak, Turkey. [4]Department of Pharmaceutical and Medicinal Chemistry, College of Pharmacy, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. [5]Department of Medical Biochemistry, College of Medical and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. [6]Department of Life and Consumer Sciences, School of Agriculture and Life Sciences, University of South Africa, Johannesburg 1710, South Africa. ✉email: adewaleob@abuad.edu.ng; solaustine200357@gmail.com

mimicking how pharmaceuticals or therapeutic substances attach to protein targets in the body. This technique was particularly influential during the COVID-19 pandemic, where it helped identify potential treatment candidates like remdesivir and nirmatrelvir[3]. Molecular docking cuts down the time and expense associated with conventional medication development and allows researchers to filter through vast libraries of possible candidates.

Natural antioxidants, present in various parts of plants, have shown to exhibit different health-promoting effects, by providing affordable management of free radical-causing diseases and avoiding possible toxicities associated with conventional medication[4]. Several medicinal plants, possessing antioxidant properties, have shown promising therapeutic potential against several diseases, including diabetes mellitus. *Carica papaya* Linn. is commonly called pawpaw, and it belongs to the family *Caricaceae*. The ripe pawpaw fruit is juicy and sweet when eaten raw. The plant has been reported to possess antimicrobial, antioxidant, anti-inflammatory, anti-hyperlipidemic, anticancer, anti-obesity, and anti-diabetic properties, and these have been attributed to the presence of several phytochemicals in various parts of the plant[5].

Although, the anti-diabetic potential of *Carica papaya* extracts has been reported, most especially with the fruits. However, there is less information on the anti-hyperglycemic and anti-diabetic potential of the leaf extracts on diet-induced hyperglycemia/diabetes using non-mammalian models. This study therefore, centers on the use of in silico methods to study the interaction of some compounds present in *C. papaya* leaf with protein targets that are relevant to hyperglycemia and diabetes, highlighting the possible compounds that could be responsible for the pharmacological properties of the plant. In addition, this study supports the use of *Drosophila melanogaster* in biomedical research and the protective effect of the leaf extract on high sucrose diet-induced hyperglycemia and oxidative stress were also investigated.

Materials and methods

Chemicals

Ellman's reagent (5'5-dithiobis(2-nitrobezoic acid) (DTNB)), 1-chloro-2,4,-dinitrobenzene (CDNB), trichloroacetic acid (EDTA), hydrogen peroxide, and Greiss Reagents were products of Sigma Aldrich Chemical Co. (St. Loius, MO, USA). Glucose kit was sourced from Randox laboratories limited (Crumin country Antrim, UK). Agar was a product of Readymed (Chaitanya group of industries, India). Every chemical used in this study is of analytical grade.

Plant material

Carica papaya leaves were obtained in July, 2023 from Iworoko-Ekiti in Ekiti state, Nigeria, and the plant was authenticated at the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria, and a voucher number (2024029) was assigned to it. The leaves were separated, air-dried for three weeks, and pulverized using an electric blender. The blended leaves were sieved to obtain a fine powder.

Preparation of aqueous extract of *Carica Papaya*

Ten grams of the powdered leaf of *Carica papaya* were soaked in distilled water for 48 h at room temperature and then filtered. The filtrate was evaporated to dryness using a water bath at 45 °C, with a yield of 15%. The resulting extract was stored in an airtight container at −4 °C until further use.

In silico study

Selection of compounds present in Carica papaya Leaves

The compounds within the *Carica papaya* leaves were examined through searches on different platforms like PubMed, Google Scholar, Science Direct, and ResearchGate. The goal was to compile data on these bioactive compounds, employing diverse range of scholarly sources to ensure a comprehensive and thorough investigation.

Collection and preparation of bioactive compounds in Carica Papaya leaves

The 3D files of bioactive compounds present in *C. papaya* leaves were retrieved from the PubChem database. The 3D files underwent a comprehensive preparation procedure, such as the removal of water molecule and heteroatoms using Discovery Studio Visualizer software.

Source of diabetes protein targets

The 3D structure of the five diabetes protein targets (alpha amylase, beta glucosidase, dipeptidyl peptidase 4 (DDP-4), peroxisome proliferator-activated receptors (PPARG), sodium-dependent glucose cotransporter (SGLT-2), and sulfonylurea receptor 1 (SUR-1) were retrieved from RCSB protein data bank server. The resolution, dependability, and relevance of the chosen structure to the target enzyme served as guiding principles in the selection process.

Virtual screening workflow

Preparation: *Carica papaya* leaf's bioactive compounds were retrieved from the PubChem database and downloaded as 3D structures and then prepared for docking using Discovery Studio software.

Docking: Each compound was docked against the five diabetes protein targets using Python Prescription (PyRx) software with Autodock Vina embedded for binding interactions. Docked poses were ranked based on their predicted binding affinity scores.

Docking and scoring methods

Molecular docking simulations were performed utilizing PyRx with AutoDock vina, during the virtual screening procedure. The scoring functions used in the docking simulations were selected based on their capacity to precisely forecast the binding affinities and interactions between the *Carica papaya* leaf compounds and the multiple diabetes protein targets.

Absorption, distribution, metabolism, and excretion (ADME) screening and toxicity (T) prediction

ADME prediction

The pharmacokinetic profiles of the selected bioactive compounds were evaluated using the SwissADME web tool (http://www.swissadme.ch/), provided by the Swiss Institute of Bioinformatics. SMILES (Simplified Molecular Input Line Entry System) notations of each compound were submitted to predict key descriptors relevant to ADME. The parameters analyzed included gastrointestinal (GI) absorption, P-glycoprotein (P-gp) substrate identification, lipophilicity (LogKp), water solubility (ESOL model), topological polar surface area (TPSA), blood-brain barrier (BBB) permeability, cytochrome P450 (CYP450) enzyme inhibition, and bioavailability score. These descriptors were used to infer the drug-likeness, oral bioavailability, and pharmacokinetic behavior of the compounds, providing early insights into their suitability for therapeutic application.

In silico toxicity evaluation

An in silico toxicity evaluation was performed for the fourteen compounds using the Greenstone Bio ADMET Prediction Platform (https://admet.ai.greenstonebio.com/), a web-based tool powered by machine learning. The platform predicts a wide range of ADMET properties based on quantitative structure–activity relationship (QSAR) models developed from curated experimental datasets. For each compound, the simplified molecular-input line-entry system (SMILES) notation was input into the platform's interface. The tool returned predictions for several toxicity-related endpoints, including hERG channel inhibition, clinical toxicity, drug-induced liver injury (DILI), carcinogenicity, acute toxicity (LD_{50}), and skin reaction potential. The output data were compiled and tabulated to facilitate a comparative toxicity analysis across all fourteen compounds. This computational approach provided preliminary insight into the safety profiles of the compounds, offering a rapid, cost-effective alternative to early-stage experimental screening.

Predictions of endocrine disruptors properties

The metabolic activity of 15 nuclear receptors was predicted using the Endocrine Disruptome web server (http://endocrinedisruptome.ki.si/, accessed on 09 October 2024). This server simulates the docking of each metabolite with crystal structures of various nuclear receptors, including androgen receptors (AR), oestrogen receptors α and β (ER α/β), glucocorticoid receptor (GR), liver X receptors α and β (LXR α/β), mineralocorticoid receptor (MR), peroxisome proliferator-activated receptors α, β, and γ (PPAR α, PPAR β, and PPAR γ), progesterone receptor (PR), retinoid X receptor α (RXR α), and thyroid receptors α and β (TR α and TR β). The results from the web server are categorized into three levels: red indicates a high binding potential, orange and yellow suggest a moderate binding probability, and green signifies a low likelihood of binding to the receptors.

In vivo study

Drosophila melanogaster stock and culture

Drosophila melanogaster of both genders (Harwich strain, 1–3 days old) was cultured on a cornmeal medium under controlled conditions at the Drosophila Laboratory, Biochemistry Program, College of Sciences, Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria. The flies were fed on basal diet containing a mixture of cornmeal, brewer's yeast (1% w/v), agar-agar (1% w/v), and methyl paraben (as preservative, 0.08% v/w), and were maintained at temperature of 23 ± 2 ∘C, under 12 h dark/light cycle.

Determination of survival rate

Experimental flies were cultivated on basal diets supplemented with different concentrations (0, 50, 100, 200, and 400 mg/kg diet) of *Carica papaya* leaf extract (AECP). Survival rates were recorded for 7 days as described by Oboh, et al.[6].

Treatments of D. melanogaster with sucrose and AECP

Based on the survival studies, doses of 50 and 100 mg/kg AECP were chosen for further studies. Flies were fed with sucrose and AECP for 5 days, divided into groups as follows: Control (basal diet only), sucrose (30% w/v) diet only, sucrose (30% w/v) + AECP (50 mg/kg diet), sucrose (30% w/v) + AECP (100 mg/kg diet), AECP (100 mg/kg diet) only.

Preparation of flies homogenate for biochemical assays

Flies were anesthetized, weighed, homogenized in 0.1 M potassium phosphate buffer, and centrifuged. The supernatants were used for biochemical assays.

Parameters investigated

Parameters such as glucose level[7], total protein level[8], level of total thiols[9], glutathione-s-transferase (GST) and catalase activities[10], and nitric oxide (NO) level[11], were carried out in whole flies' homogenate.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by intergroup multiple comparison using the Tukey post hoc test on the GraphPad Prism (version 8.1). Results were expressed as mean ± standard deviation (SD), $n = 5$, and values of $p < 0.05$ were considered statistically significant.

Results and discussion

In this study, the in silico model examined 14 bioactive compounds from *Carica papaya* leaves (Table 1) alongside two reference drugs against five protein targets. Results indicate that carpaine, myricetin 3-rhamnoside, orientin 7-O-rhamnoside, and quercetin demonstrated the strongest binding scores. These compounds, therefore, hold

S/N	Constituents	Canonical Smile String
1	Myricetin 3-rhamnoside	CC1C(C(C(C(O1)OC2 = C(OC3 = CC(= CC(= C3C2 = O)O)O)C4 = CC(= C(C(= C4)O)O)O)O)O)O
2	Quercetin	C1 = CC(= C(C = C1C2 = C(C(= O)C3 = C(C = C(C = C3O2) O)O)O)O)O
3	Protocatechuic acid	C1 = CC(= C(C = C1C(= O)O)O)O
4	Caffeic acid	C1 = CC(= C(C = C1C = CC(= O)O)O)O
5	Carpaine	CC1C2CCC(N1)CCCCCCCC(= O)OC3CCC(CCCCCCCC(= O)O2)NC3C
6	2-methoxy-4-vinylphenol	COC1 = C(C = CC(= C1)C = C)O
7	Equisetin	CC = CC1C = CC2CC(CCC2C1(C)C(= C3C(= O)C(N(C3 = O)C)CO)O)C
8	Tenuazonic acid	CCC(C)C1C(= C(C(= O)N1)C(= O)C)O
9	9-Octadecenamide	CCCCCCCCC = CCCCCCCCC(= O)N
10	Prunasin	C1 = CC = C(C = C1)C(C#N)OC2C(C(C(C(O2)CO)O)O)O
11	Orientin 7-O-rhamnoside	CC1C(C(C(C(O1)OC2 = C(C3 = C(C(= C2)O)C(= O)C = C(O3)C4 = CC(= C(C = C4)O)O) C5C(C(C(C(O5)CO)O)O)O)O)O)O
12	11-Hydroperoxy-12,13-epoxy-9-octadecenoic acid	CCCCCC1C(O1)C(C = CCCCCCCCC(= O)O)OO
13	Hexadecanamide	CCCCCCCCCCCCCCCC(= O)N
14	Ferulic acid	COC1 = C(C = CC(= C1)C = CC(= O)O)O

Table 1. Natural product library generated from plant constituents caricaceae (*Carica Papaya* leaf) as well as their various phytochemical constituent as well as the canonical smile generated for each plant constituent.

S/N	Docked ligand	Alpha amylase	Beta glucosidase	DDP 4	PPARG	SGLT – 2	SUR – 1
0	Reference (Metformin)	− 5.1	− 5.2	− 5.4	− 4.8	− 5.1	− 6.5
1	Reference 2 (Acarbose)	− 8.2	− 9.1	− 9.2	− 6.9	− 7.5	− 4.7
2	Myricetin 3-rhamnoside	− 9.6	− 9.8	− 9.6	− 8	− 8.2	− 7.5
3	Quercetin	− 8.8	− 8.9	− 8.3	− 7.3	− 9.7	− 7.1
4	Caffeic acid	− 6.5	− 7	− 7.1	− 5.9	− 7.7	− 5.8
5	protocatechuic acid	− 5.8	− 6.4	− 6.4	− 5.7	− 6.8	− 5.3
6	Carpaine	− 7.8	− 9.9	− 10	− 8.6	− 8.6	−7.9
7	2-methoxy-4-vinylphenol	− 5.6	− 6.4	− 6	− 5.8	−6.8	− 5.1
8	Equisetin	− 8.7	− 7.7	− 8.2	− 7.1	−7.6	− 6.5
9	Tenuazonic acid	−7	− 6.3	− 6.5	− 5.6	− 6.8	− 5.3
10	9-Octadecenamide	− 5.4	− 6.1	− 5.5	− 6	− 6.8	− 4.9
11	Prunasin	− 7.2	− 7	7.2	− 6.9	− 8.7	− 5.9
12	Orientin 7-O-rhamnoside	− 9.1	− 9.2	− 9.7	− 8	− 8.5	− 7.7
13	11-Hydroperoxy-12,13-epoxy-9-octadecenoic acid	− 6.2	− 6.4	− 6.5	− 6.1	− 6.1	− 5.3
14	Hexadecanamide	− 5.1	− 5.6	− 5.1	− 5.4	− 6.6	− 5.6
15	Ferulic acid	− 6.5	− 6.8	− 6.8	− 6	− 7.4	− 6

Table 2. Binding affinity and binding energy (kcal/mol) of plant derived natural compounds in *Carica Papaya* leaves against multiple macromolecular targets in diabetes mellitus.

potential as inhibitors of alpha-amylase, beta-glucosidase, DPP-4, PPARG, SGLT-2, and SUR-1 (Table 2), offering diverse pathways for managing diabetes. This aligns with findings by Kong, et al.[12] and suggests the potential of *Carica papaya*-based diabetes treatments. In another research, bioactive compounds in natural compounds, such as quercetin, have been found to inhibit key enzymes involved in carbohydrate digestion, including alpha-amylase and beta-glucosidase[13]. By inhibiting these enzymes, AECP reduces the rate of carbohydrate breakdown and glucose absorption, thereby lowering blood glucose levels. These findings suggest that developing *Carica papaya*-based treatments could help manage diabetes.

Molecular investigations were undertaken on the inhibitory activities of the relative compounds in the *Carica papaya* leaves (Table 2). Two reference medicines (acarbose and metformin) were used to compare the docking score with the compounds in *Carica papaya* leaves. Four comparable compounds exhibited a good docking score compared to the two reference medicines utilized against various macromolecular targets. The four compounds with good docking scores were carpaine, myricetin 3-rhamnoside, orientin 7-O-rhamnoside and quercetin. These results revealed the following components might be the bioactive compounds contained in *Carica papaya* leaves that can potentially regulate OS complications like diabetes.

The data in Table 3 reveal the pharmacokinetic properties and bioavailability of various metabolites, examining key characteristics including GI absorption potential, ability to cross the BBB, P-gp substrate status, bioavailability score, and skin permeation (Log Kp). Differences in GI absorption values are observed among the metabolites, with myricetin 3-rhamnoside and orientin 7-O-rhamnoside showing low absorption potential, while quercetin, caffeic acid, carpaine, and protocatechuic acid exhibit high GI absorption values, indicating more effective absorption after

Compound	Mol. Wt. (g/mol)	GI Absorption	*P*-gp Substrate	LogKp (Skin permeation)	Water Solubility (ESOL)	TPSA (Å²)	BBB Permeability	CYP Inhibitors	Bioavailability Score
Myricetin 3-rhamnoside	464.38	Low	No	−0.23	Soluble	210.51	No	None	0.17
Quercetin	302.24	High	No	1.23	Soluble	131.36	No	1A2, 2D6, 3A4	0.55
Protocatechuic acid	154.12	High	No	0.65	Very soluble	77.76	No	3A4	0.56
Caffeic acid	180.16	High	No	0.93	Very soluble	77.76	No	None	0.56
Carpaine	478.71	High	No	4.64	Poorly soluble	76.66	No	None	0.55
2-methoxy-4-vinylphenol	150.17	High	No	2.14	Soluble	29.46	Yes	1A2	0.55
Equisetin	373.49	High	Yes	2.93	Moderately soluble	77.84	Yes	2C9, 3A4	0.56
Tenuazonic acid	197.23	High	No	0.93	Very soluble	66.4	No	None	0.85
9-Octadecenamide	281.48	High	No	5.32	Moderately soluble	43.09	Yes	1A2, 2C9	0.55
Prunasin	295.29	High	No	−0.59	Very soluble	123.17	No	None	0.55
Orientin 7-O-rhamnoside	594.52	Low	Yes	−1.4	Soluble	260.2	No	None	0.17
11-Hydroperoxy-12,13-epoxy-9-octadecenoic acid	328.44	High	No	3.89	Soluble	79.29	No	2C9, 2D6	0.56
Hexadecanamide	255.44	High	No	4.83	Moderately soluble	43.09	Yes	1A2	0.55
Ferulic acid	194.18	High	No	1.36	Soluble	66.76	Yes	None	0.85

Table 3. Pharmacokinetic properties and bioavailability analysis of various metabolites (ADME).

oral intake. Five of the metabolites, including equisetin and ferulic acid could penetrate the BBB, implying potential activity on the central nervous system. Some metabolites are identified as P-gp substrates, such as equisetin and orientin 7-O-rhamnoside, influencing absorption processes in the body. Skin permeation values suggest that myricetin 3-rhamnoside, prunasin, and orientin 7-O-rhamnoside have poor skin penetration, while carpaine, Hexadecanamide and 9-octadecenamide show better absorption through the skin. Water solubility varies, with compounds like caffeic acid and protocatechuic acid being very soluble, while carpaine is poorly soluble. High TPSA in some compounds may limit permeability. Several compounds inhibit CYP enzymes, notably quercetin, equisetin, and 9-octadecenamide, indicating potential for drug–drug interactions. The bioavailability score indicates that most metabolites possess moderate scores, with compounds including ferulic acid, equisetin, quercetin, caffeic acid, and tenuazonic acid displaying high scores, suggesting their more effective use within the body. Overall, these findings reveal significant differences in the pharmacokinetic profiles of the metabolites, which may have important implications for their potential therapeutic effects, particularly for those with high GI absorption and bioavailability scores that warrant further investigation.

The toxicity prediction result for the fourteen compounds is presented in Table 4. Most compounds demonstrated low potential for hERG channel blocking, with values generally below 0.7, indicating a relatively low risk of cardiac arrhythmia. However, 9-octadecenamide (0.68) and carpaine (0.57) approached higher values, suggesting that their cardiotoxic potential should be monitored. In contrast, compounds like ferulic acid, caffeic acid, tenuazonic acid, and protocatechuic acid had minimal hERG blocking predictions (0.01), indicating a safer cardiac profile. Regarding clinical toxicity, most of the compounds showed low predictions, particularly 2-methoxy-4-vinylphenol (0.0021), and tenuazonic acid and hexadecanamide (0.01), suggesting a favorable safety profile in clinical settings. However, 11-hydroperoxy-12,13-epoxy-9-octadecenoic acid (0.35) was notably higher, implying a need for caution. Predictions for DILI were particularly high for myricetin 3-rhamnoside,

Compound	hERG (Pred)	Clinical Tox (Pred)	DILI (Pred)	Carcinogenicity (Pred)	LD50 (log(1/mol/kg))	Skin Reaction (Pred)
Myricetin 3-rhamnoside	0.47	0.28	0.93	0.11	3.38	0.37
Quercetin	0.45	0.03	0.93	0.03	2.51	0.89
Protocatechuic acid	0.01	0.05	0.59	0.11	1.72	0.6
Caffeic acid	0.01	0.1	0.63	0.07	1.86	0.61
Carpaine	0.57	0.2	0.26	0.08	2.15	0.71
2-methoxy-4-vinylphenol	0.11	0.0021	0.36	0.03	1.99	0.77
Equisetin	0.1	0.03	0.42	0.13	3.81	0.3
Tenuazonic acid	0.01	0.01	0.56	0.43	2.44	0.57
9-Octadecenamide	0.68	0.03	0.11	0.7	1.66	0.92
Prunasin	0.08	0.06	0.27	0.18	2.67	0.25
Orientin 7-O-rhamnoside	0.54	0.15	0.88	0.08	3.14	0.17
11-Hydroperoxy-12,13-epoxy-9-octadecenoic acid	0.24	0.35	0.43	0.2	2.13	0.84
Hexadecanamide	0.6	0.01	0.12	0.64	1.73	0.82
Ferulic acid	0.01	0.08	0.68	0.03	1.65	0.32

Table 4. Toxicity prediction of the compounds.

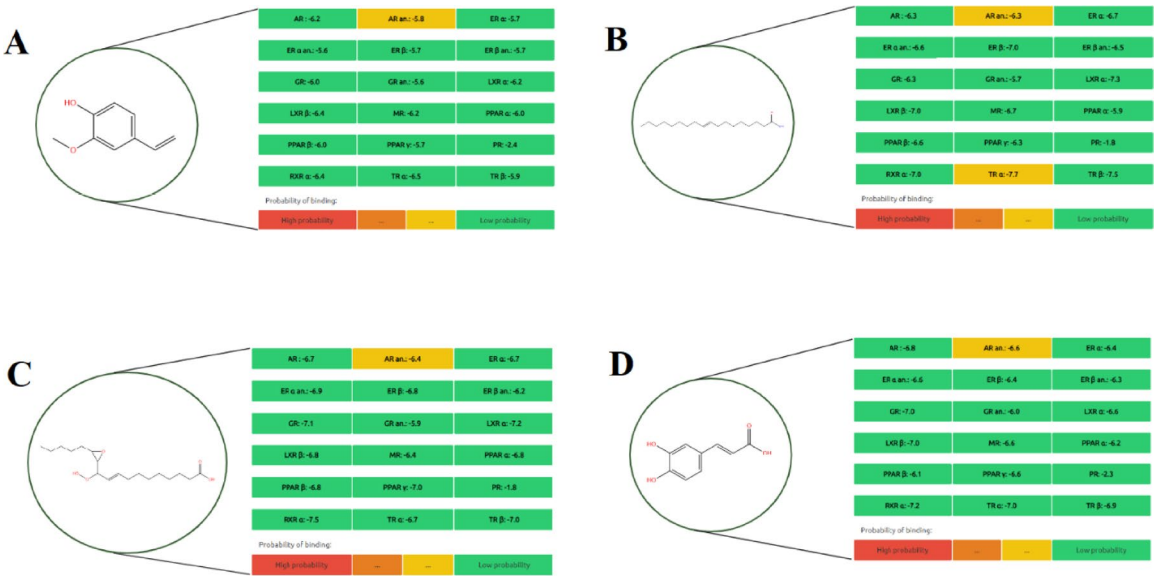


Fig. 1. The predicted endocrine disruption potential of four key metabolites: (**A**) 2-Methoxy-4-vinylphenol, (**B**) 9-Octadecenamide, (**C**) 11-Hydroperoxy-12,13-epoxy-9-octadecenoic acid, and (**D**) Caffeic Acid.

quercetin (both 0.93), and orientin 7-O-rhamnoside (0.88), highlighting a potentially significant hepatotoxic risk. This suggests their potential toxicity at high doses. On the other hand, hexadecanamide (0.12) and 9-octadecenamide (0.11) had lower DILI predictions, indicating better liver safety. Most compounds exhibited low carcinogenicity predictions, with quercetin, ferulic acid, and 2-methoxy-4-vinylphenol all at or below 0.03. However, 9-octadecenamide (0.70), hexadecanamide (0.64), and tenuazonic acid (0.43) had elevated values, suggesting a possible carcinogenic concern. In acute toxicity, measured as LD50 in log(1/mol/kg), higher values reflect lower toxicity. Equisetin (3.81), myricetin 3-rhamnoside (3.38), and orientin 7-O-rhamnoside (3.14) exhibited the highest LD50 values, suggesting lower acute toxicity. Lastly, for skin reaction, compounds like 9-octadecenamide (0.92), quercetin (0.89), and 11-hydroperoxy-12,13-epoxy-9-octadecenoic acid (0.84) had high predictions, implying potential for dermal irritation or allergic response.

In this study, the endocrine-disrupting potential of fourteen selected metabolites was evaluated using the **Endocrine Disruptome** platform, and the results are presented in Figs. 1, 2, 3 and 4. Each compound was assessed for its binding probability to 15 nuclear receptors, including AR, ER α/β, GR, MR, PR, PPAR α/β/γ, TR α/β, RXR α, and LXR α/β. The predicted binding scores were classified based on interaction likelihood as high (red), moderate (orange), or low (green).

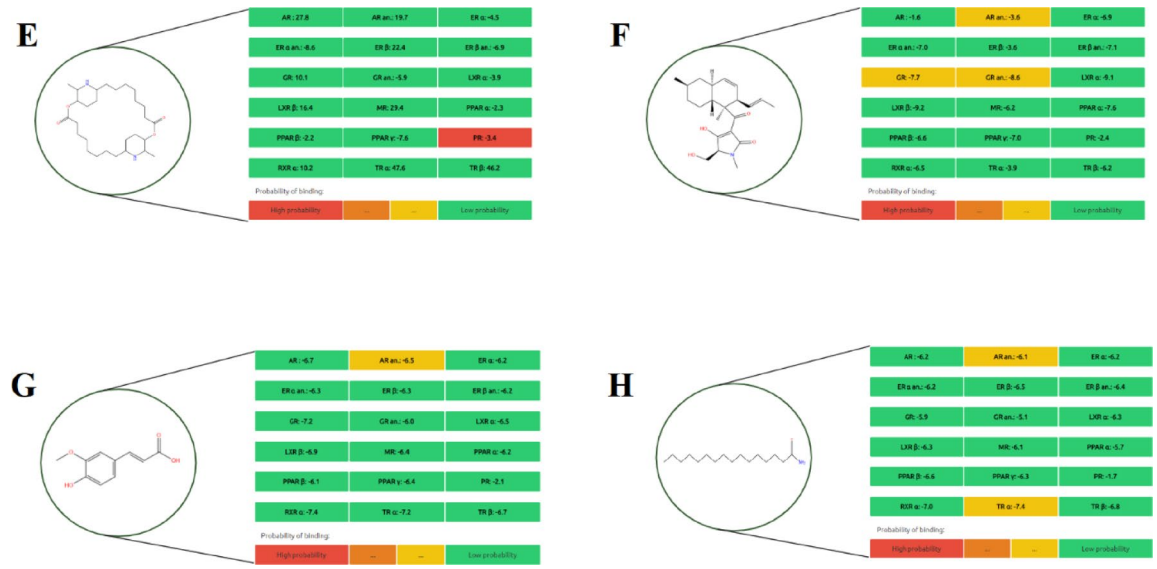


Fig. 2. The predicted endocrine disruption potential of four key metabolites: (**E**) Carpaine, (**F**) Equisetin, (**G**) Ferulic acid, and (**H**) Hexadecanamide.

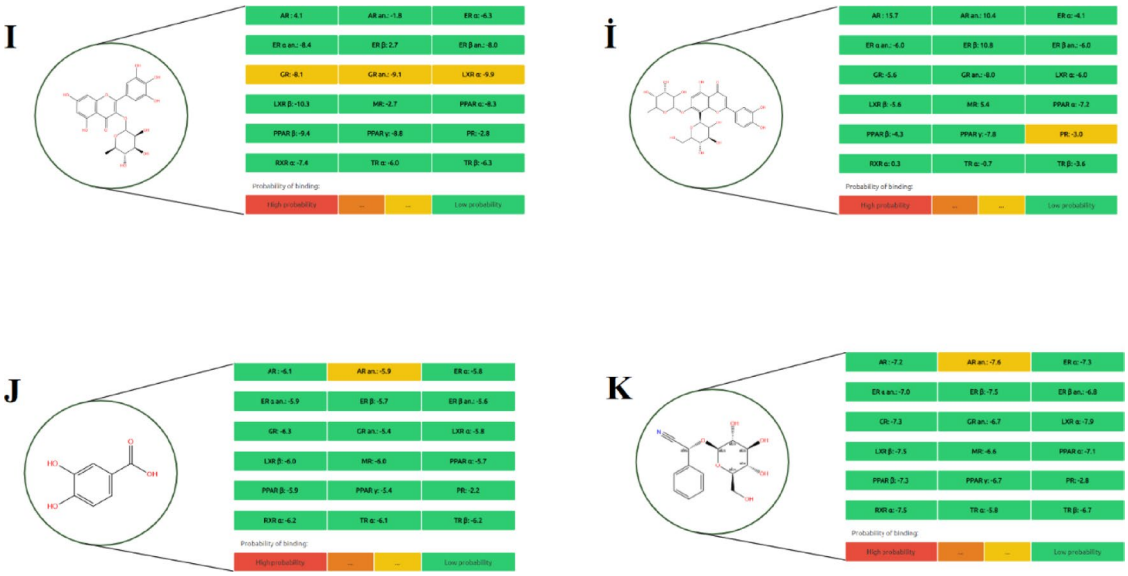
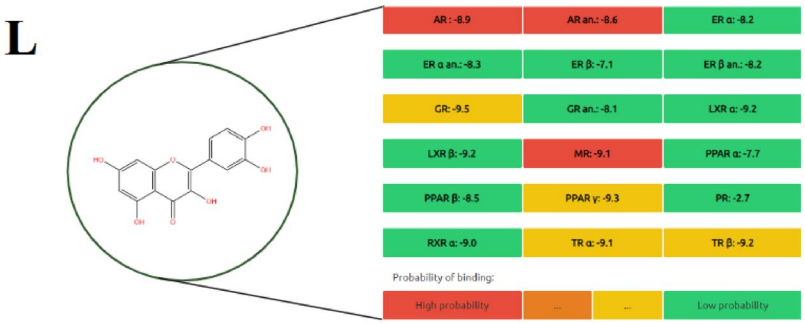


Fig. 3. The predicted endocrine disruption potential of four key metabolites: (**I**) Myricetin 3'-rhamnoside, (**İ**) Orientin 7-O-rhamnoside, (**J**) Protocatechuic acid, and (**K**) Prunasin.

Among all tested compounds, **quercetin** (Fig. 4L) demonstrated the highest binding potential across multiple receptors, particularly AR, GR, MR, PPAR γ, and TR α, indicating a broad spectrum of endocrine-disrupting capacity. Similarly, **carpaine** (Fig. 2E) exhibited high-affinity binding specifically to the PR receptor, warranting consideration as a potential endocrine-active compound. **Orientin 7-O-rhamnoside** (Fig. 3İ) showed a moderate binding probability to the PR receptor, suggesting selective interaction potential.

Other metabolites such as **ferulic acid** (Fig. 2G), **protocatechuic acid** (Fig. 3J), **prunasin** (Fig. 3K), and **tenuazonic acid** (Fig. 4M) demonstrated moderate binding only to the AR α receptor, while exhibiting low



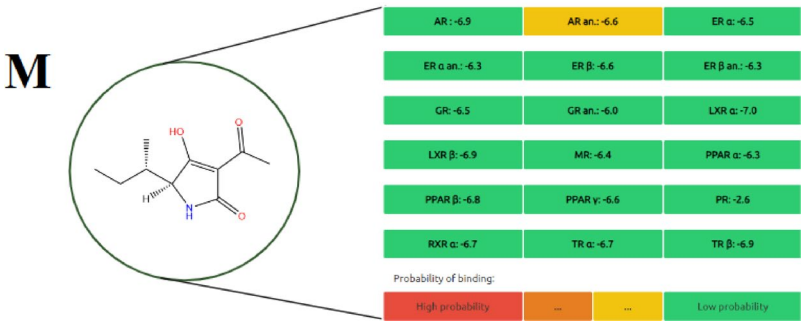


Fig. 4. The predicted endocrine disruption potential of four key metabolites: (**L**) Quercetin, (**M**) Tenuazonic acid.

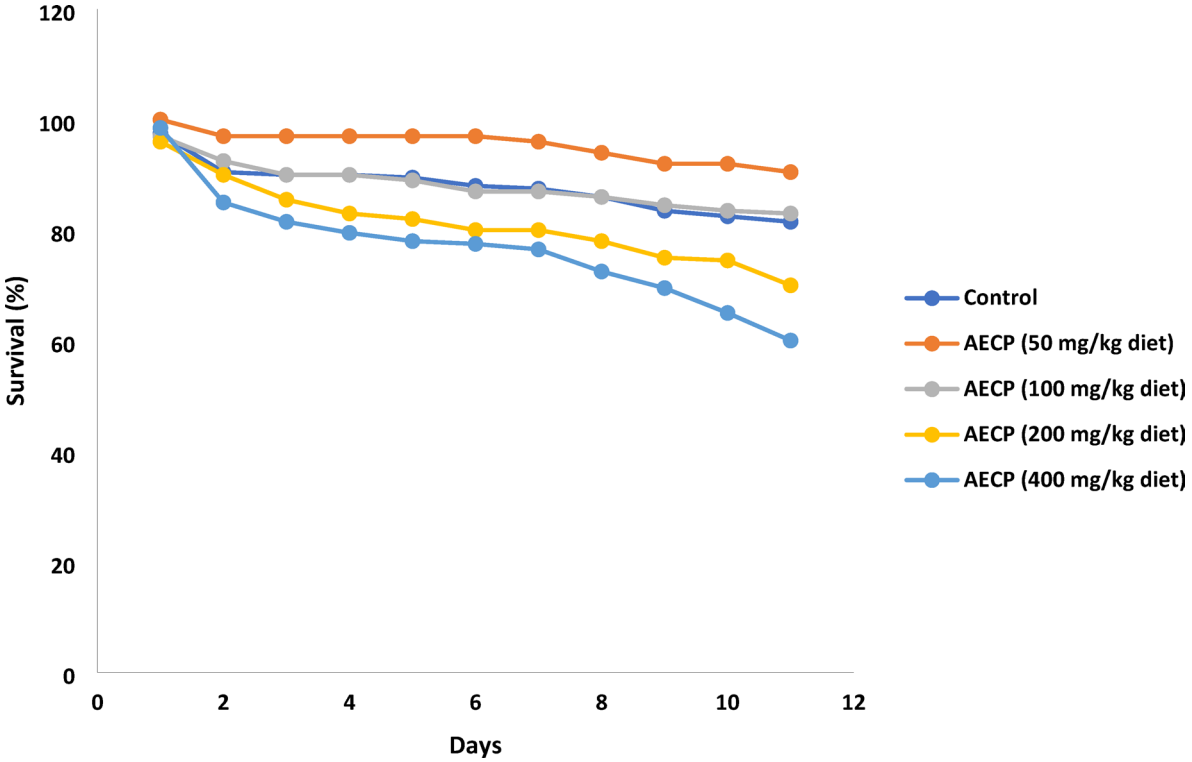


Fig. 5. Effect of aqueous extract of *Carica papaya* leaf on 12-day survival rate in adult *D. melanogaster*.

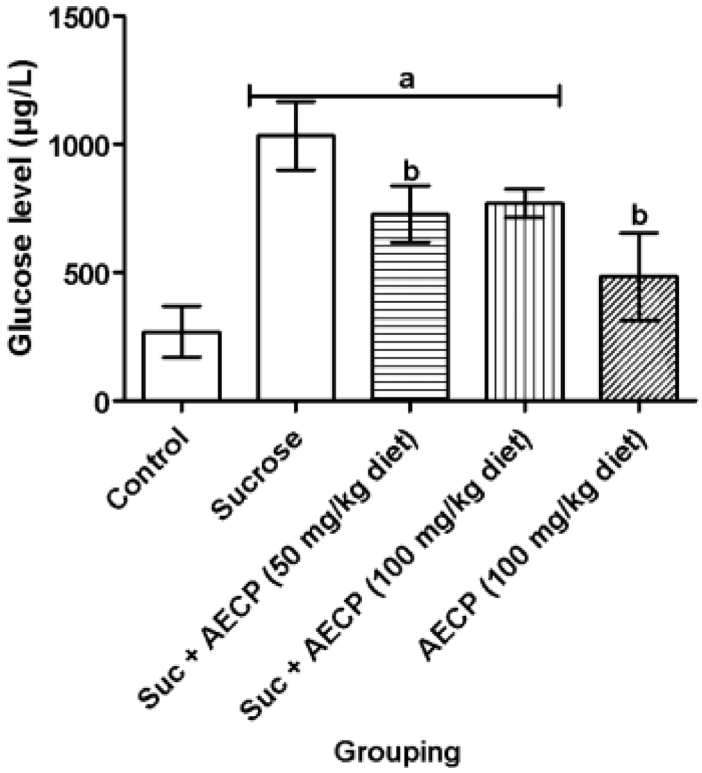


Fig. 6. Effect of aqueous extract of *Carica papaya* leaf on glucose level in sucrose-induced oxidative stress in flies. Data are presented as Mean ± SD of 40 flies/vial ($n = 5$). $^{a}p < 0.05$ when compared with the control, $^{b}p < 0.05$ when compared with sucrose only. AECP: Aqueous extract of *Carica papaya*; Suc: Sucrose.

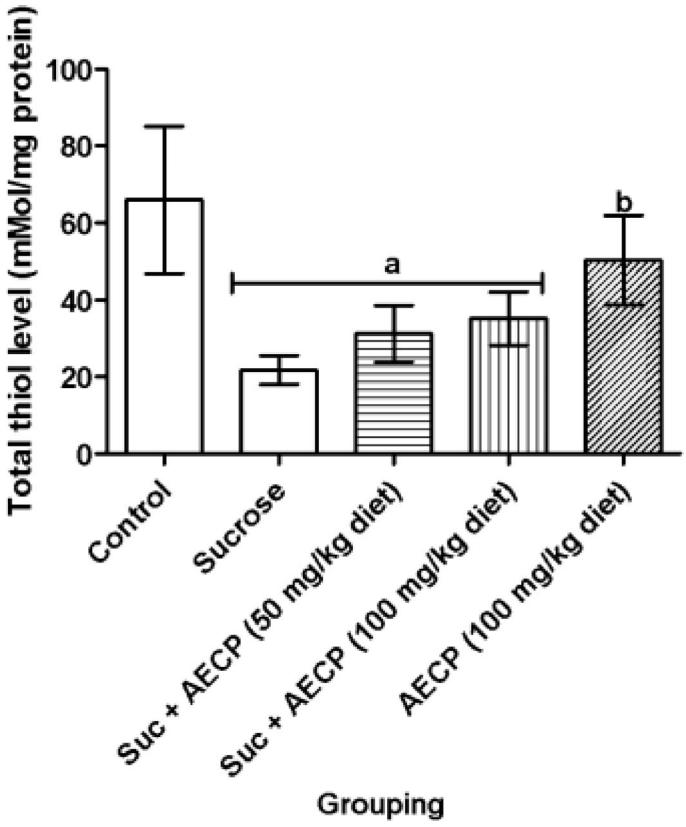


Fig. 7. Effect of aqueous extract of *Carica papaya* leaf on the level of total thiol in sucrose-induced oxidative stress in flies. Data are presented as Mean ± SD of 40 flies/vial ($n = 5$). [a]$p < 0.05$ when compared with the control, [b]$p < 0.05$ when compared with sucrose only. AECP: Aqueous Extract of *Carica papaya*; Suc: Sucrose.

affinity to other targets, indicating limited endocrine-disrupting potential. In contrast, compounds including **2-methoxy-4-vinylphenol** (Fig. 1A), **9-octadecenamide** (Fig. 1B), **11-hydroperoxy-12,13-epoxy-9-octadecenoic acid** (Fig. 1C), **hexadecanamide** (Fig. 2H), **equisetin** (Fig. 2F), and **myricetin 3'-rhamnoside** (Fig. 3I) displayed low binding across all nuclear receptors, suggesting minimal endocrine interference.

Overall, **AR α and PR** emerged as the most frequently affected receptors, with several metabolites showing moderate to high affinity toward them. These findings highlight receptor-specific binding tendencies of phytochemical metabolites and suggest that a subset of these compounds may exhibit endocrine-disrupting behavior, especially through interaction with androgenic and progestogenic pathways.

Oxidative stress results from an excess of ROS, thereby, causing harmful oxidation and inflammation. These processes have been linked to various health conditions, including diabetes, Alzheimer's disease, rheumatoid arthritis, cancers, cardiovascular diseases, cataracts, and even cosmetic issues like wrinkles[12].

The fruit fly, *Drosophila melanogaster*, is a valuable model for investigating human health due to the significant homology between its genome and that of humans. Its short lifespan, ease of maintenance, and well-understood genetics make it a powerful tool for studying diseases like cancer and developmental studies[14]. One of the major parameters used to assess the effect of plants and plant products in *D. melanogaster* is the survival rate[15]. It was noted that low doses of the AECP (50 and 100 mg/kg diet) improved the survival rate of the flies, which suggests that caution should be taken with the consumption of AECP at high doses (Fig. 5). The observed safe doses (50 and 100 mg/kg diet) were, therefore, used to investigate the protective effect against high-sucrose-induced hyperglycemia and oxidative stress in fruit flies.

Previous studies indicate that sucrose consumption elevates glucose levels, and excess sucrose consumption can trigger OS and its complications[16,17]. The present study explores the potential of AECP as a countermeasure. A significant ($p < 0.05$) increase in the level of glucose was noted in flies fed with sucrose alone and in sucrose-fed flies treated with 50 and 100 mg/kg AECP when compared with the control (Fig. 6). Furthermore, a significant ($p < 0.05$) reduction in glucose level was observed in the sucrose-fed diet fortified with 50 mg/kg AECP compared to the sucrose-only treated group. Results indicate that AECP reversed the glucose elevation caused by a high sucrose diet in the flies. This finding supports the result of the in silico analysis which suggest that the hit compounds (bioactive compounds) in the *Carica papaya* may interact with the diabetic protein targets to reduce the excess glucose level.

The antioxidant potential of *Carica papaya* has been documented in various studies. The high antioxidant activity of *Carica papaya* extracts can be attributed to bioactive compounds such as alkaloids, flavonoids, saponins, glycosides, phytosterol, flavonoids, tannins terpenoids, and vitamins[5]. These compounds neutralize free radicals and boost the body's antioxidant defense system. The role of catalase and GST in maintaining

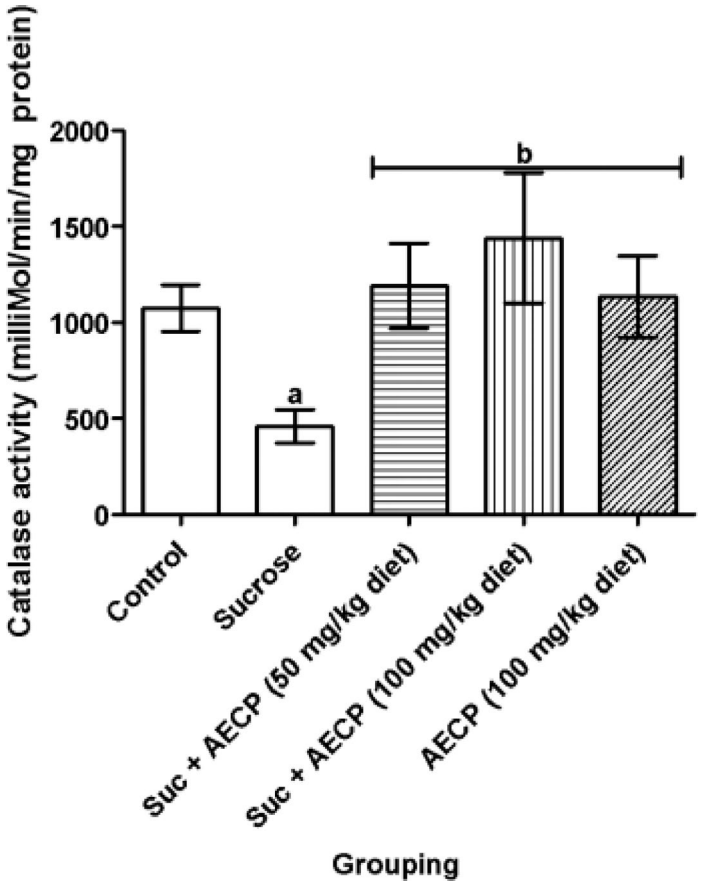


Fig. 8. Effect of aqueous extract of *Carica papaya* leaf on the activity of catalase in sucrose-induced oxidative stress in flies. Data are presented as Mean ± SD of 40 flies/vial ($n = 5$). $^{a}p < 0.05$ when compared with the control, $^{b}p < 0.05$ when compared with sucrose only. AECP: Aqueous extract of *Carica papaya*; Suc: Sucrose.

cellular health shows the importance of these enzymes in mitigating the effects of OS. Catalase, by breaking down hydrogen peroxide, prevents the formation of hydroxyl radicals, one of the most reactive and damaging types of free radicals[18]. Glutathione-s-transferase, through its detoxification role, helps eliminate reactive intermediates that could otherwise contribute to cellular damage and dysfunction as seen in this study.

In this study, the level of total thiols was significantly ($p < 0.05$) reduced in flies fed with a high-sucrose diet only and groups treated with a sucrose diet fortified with 50 and 100 mg/kg AECP when compared to the control group (Fig. 7). However, a significant ($p < 0.05$) increase in the level of total thiols in flies treated with 100 mg/kg AECP-only was observed when compared with sucrose-only fed flies. Also, a significant ($p < 0.05$) decrease in catalase activity was observed in sucrose-only treated flies when compared to the control (Fig. 8). The catalase activity was significantly ($p < 0.05$) increased in flies treated with both doses of AECP when compared to the group treated with sucrose only. The GST activity was significantly ($p < 0.05$) decreased in the group treated with sucrose only when compared to the control group and the group treated with 50 mg/kg diet AECP (Fig. 9). The GST activity was also significantly increased ($p < 0.05$) in the groups treated with AECP (100 mg/kg) when compared to the group treated with sucrose only. This could be associated with the antioxidant activity of the AECP. In addition, the binding score of carpaine as a potential compound, could enhance catalase activity as observed in the in silico analysis.

It has been reported that fruit extract of papaya increased the activities of antioxidant enzymes in biological systems, and ameliorated lipid peroxidation[5]supporting the notion that *Carica papaya* can enhance the body's ability to manage OS. This aligns with the findings of this study, where the antioxidant effects of AECP restored enzyme activities and potentially improved overall cellular health and resilience against OS. This study adds to the growing body of evidence supporting the health benefits of *Carica papaya*, particularly its role in enhancing antioxidant defenses. The restoration of total thiols level, catalase and GST activities in the treatment groups showed the potential of *Carica papaya* as a natural remedy for managing OS and its associated complications. These findings are particularly relevant in dietary management and the potential use of *Carica papaya* in functional foods and nutraceuticals to reduce OS and improve overall health.

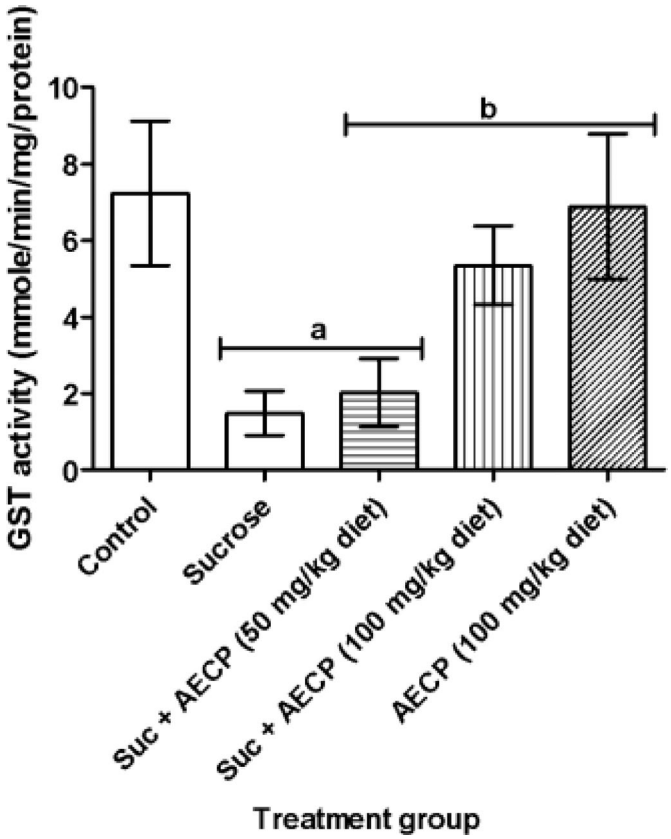


Fig. 9. Effect of aqueous extract of *Carica papaya* on the glutathione-s-transferase activity in sucrose-induced oxidative stress in flies. Data are presented as Mean ± SD of 40 flies/vial ($n = 5$). [a]$p < 0.05$ when compared with the control, [b]$p < 0.05$ when compared with sucrose only. AECP: Aqueous Extract of *Carica papaya*; Suc: Sucrose.

Elevated level of glucose (hyperglycemia) is associated with elevated production of NO through elevated expression of inducible NO synthase (iNOS) and endothelial NO synthase (eNOS) gene and protein levels[19]. In this study, a significant ($p < 0.05$) increase in the level of NO was noted in flies exposed to the sucrose diet only when compared to the control (Fig. 10). However, a significant ($p < 0.05$) reduction was noted in the sucrose diet-fed flies treated with both doses of AECP compared to the sucrose diet only. This suggests the ability of AECP to inhibit the production of NO.

Conclusion

Carica papaya exhibits promising potential in preventing or delaying the onset of oxidative stress (OS)-related diabetes and its associated complications. The anti-hyperglycemic and antioxidant activities of *Carica papaya* extracts (AECP) leaves (most especially at low doses) highlight its therapeutic relevance in the management of metabolic disorders. Molecular docking studies further support this potential by demonstrating strong interactions between key metabolites—particularly carpaine, quercetin and caffeic acid—and several diabetes-related targets and nuclear receptors, suggesting possible molecular mechanisms for their bioactivity. The BBB permeability of some compounds, including equisetin and ferulic acid, could suggest the action of the leaf extract of *C. papaya* against the complications of diabetes, most especially neuropathy. Further studies are recommended to confirm this. Taken together, these integrated in silico evaluations provide a strong rationale for further experimental and clinical investigations into the therapeutic applications of *Carica papaya* and its bioactive compounds, particularly those with high GI absorption and docking affinity. Based on this findings, it could be suggested that AECP has the ability to improve insulin secretion and sensitivity, as well as glucose metabolism. Although, more studies are warranted to elucidate the underlying molecular mechanisms and optimize their potential use in managing OS-associated diseases such as diabetes.

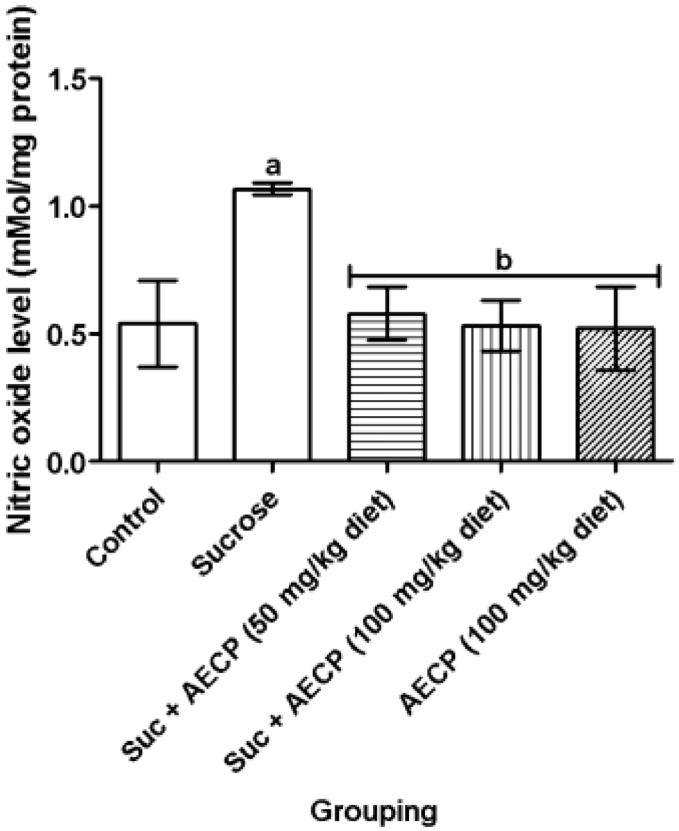


Fig. 10. Effect of aqueous extract of *Carica papaya* leaf on the level of nitric oxide in sucrose-induced oxidative stress in flies. Data are presented as Mean ± SD of 40 flies/vial ($n = 5$). $^{a}p < 0.05$ when compared with the control, $^{b}p < 0.05$ when compared with sucrose only. AECP: Aqueous extract of *Carica papaya*; Suc: Sucrose.

Data availability

All data generated or analyzed during this study are included in this article.

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Author contributions

O.I.O. and O.B.Ad. conceived and conceptualized the study. O.I.O. S.O.A., O.T.O. and O.B.Ad. supervised the work. S.J.I., O.S.O. and E.C.A. performed formal analysis and wrote the original draft. I.A.A. and O.B.Ak. performed formal analysis, data processing, and contributed to writing, review, and editing. All authors contributed to reviewing and editing of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to O.B.A.

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