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RESEARCH ARTICLE

Dietary Curcumin Attenuates Arsenic-Induced Oxidative Stress and Neurobehavioral Impairments in *Drosophila melanogaster*: Mechanistic Insights from *In Vivo* and *In Silico* Analyses

Anjali Ranjan [1], Shruti Verma [1], Gajendra Kumar Azad [2], Shahla Yasmin [3,*]

ABSTRACT: Arsenic toxicity remains a critical environmental and public health issue due to its association with oxidative stress, neurodegeneration, and motor dysfunction. This study investigated the protective efficacy of curcumin, a natural polyphenol derived from *Curcuma longa*, against arsenic trioxide (As_2O_3)-induced toxicity in *Drosophila melanogaster*. A sublethal concentration of 0.5 mM As_2O_3 induced a 50% mortality rate, while 0.75 mM resulted in complete lethality, confirming arsenic's dose-dependent toxicity. Oxidative stress biomarkers, including lipid peroxidation (LPO) and catalase activity, were significantly elevated in arsenic-exposed flies, indicating substantial oxidative damage. Co-administration of 1 mM curcumin markedly improved survivability, reduced malondialdehyde (MDA) levels, and restored catalase activity, underscoring its potent antioxidative properties. Additionally, arsenic exposure impaired climbing ability—a behavioral marker of motor dysfunction—which was effectively ameliorated by curcumin supplementation. Computational analysis using the STRING database revealed that curcumin interacts with key proteins involved in cellular metabolism and redox homeostasis, including cytochrome P450 enzymes, glutathione S-transferases, and peroxisome proliferator-activated receptors. Gene ontology (GO) enrichment further highlighted curcumin's role in modulating oxidative stress and metabolic pathways. These findings suggest that curcumin mitigates arsenic toxicity by enhancing antioxidant defenses and restoring metabolic equilibrium. The integration of *in vivo* experiments with bioinformatics tools provides mechanistic insights into curcumin's neuroprotective effects, supporting its potential as a dietary intervention in arsenic-exposed populations. Future studies should explore its efficacy in mammalian models and clinical applications to validate its therapeutic utility.

Keywords: Arsenic toxicity; Curcumin; Oxidative stress; Neurobehavioral deficits; *Drosophila melanogaster*; Antioxidant defense.

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[1] Department of Zoology, Patna Women's College, Patna University, Bihar, India
[2] Molecular Biology Laboratory, Department of Zoology, Patna University, Bihar, India
[3] Department of Zoology, Patna University, Bihar, India

* Author to whom correspondence should be addressed: shahla.02apex@gmail.com (Shahla Yasmin)

1. INTRODUCTION

Arsenic represents one of the most significant environmental toxicants affecting human health globally, with an estimated 200 million people worldwide exposed to unsafe levels through contaminated drinking water [1]. This naturally occurring metalloid exists in both organic and inorganic forms in soil, water, and air, with inorganic arsenic (iAs)

being particularly toxic due to its greater bioavailability and capacity to induce oxidative stress [2]. The World Health Organization has established a maximum permissible limit of 10 µg/L for arsenic in drinking water, yet many endemic regions, particularly in South Asia and Latin America, report concentrations exceeding 100 µg/L [3]. Chronic exposure to arsenic has been associated with a wide spectrum of health disorders, ranging from dermatological manifestations such as hyperpigmentation and keratosis to systemic conditions including respiratory diseases, cardiovascular disorders, diabetes mellitus, and gastrointestinal disturbances [4]. More alarmingly, extensive epidemiological evidence has established inorganic arsenic as a Group I human carcinogen, with strong associations with cancers of the skin, lung, bladder, kidney, and liver [5, 6]. The molecular mechanisms underlying arsenic toxicity involve the generation of reactive oxygen species (ROS), interference with cellular signaling pathways, and disruption of essential biological processes including DNA repair and protein function [7]. Despite decades of research into arsenic toxicity and its health impacts, there remains a critical need for effective, accessible, and safe therapeutic interventions to mitigate its deleterious effects, particularly in vulnerable populations with chronic exposure [8].

In recent years, natural plant-derived compounds have emerged as promising candidates for combating heavy metal toxicity due to their antioxidant, metal-chelating, and anti-inflammatory properties [9]. Among these, curcumin, the principal bioactive polyphenol derived from the rhizome of *Curcuma longa* (turmeric), has attracted considerable scientific interest for its multifaceted pharmacological activities [10, 11]. Extensive research has demonstrated curcumin's ability to scavenge free radicals, modulate redox-sensitive transcription factors such as Nrf2 and NF-κB, and enhance cellular antioxidant defense systems through upregulation of enzymes like superoxide dismutase and glutathione peroxidase [12, 13]. Additionally, curcumin exhibits metal-chelating properties that may facilitate the detoxification of arsenic and other heavy metals [14]. Its excellent safety profile, widespread availability, and pleiotropic effects make curcumin an attractive therapeutic agent against environmental toxicants like arsenic [15, 16]. However, while numerous studies have documented curcumin's antioxidant and anti-inflammatory properties, the precise molecular mechanisms underlying its protective effects against arsenic-induced neurotoxicity remain incompletely understood, particularly in the context of behavioral impairments and neurological dysfunction [17].

To address these knowledge gaps, the current study employed *Drosophila melanogaster* as a model organism, leveraging its well-established advantages for toxicological and neurobehavioral research [18, 19]. The fruit fly shares approximately 75% of human disease-related genes and possesses conserved metabolic and neurological pathways, making it an excellent model for studying oxidative stress and neurodegeneration [20]. *Drosophila* offers numerous experimental advantages including a short life cycle, well-characterized genetics, low maintenance costs, and the ability to perform high-throughput screening of therapeutic compounds [21]. Importantly, previous studies have successfully utilized *Drosophila* to investigate heavy metal toxicity and neurobehavioral effects, validating its utility for arsenic research [22]. The fly's relatively simple nervous system, coupled with sophisticated genetic tools and behavioral assays, provides a powerful platform to study the neuroprotective effects of compounds like curcumin [23].

The present investigation adopted an integrative approach combining *in vivo* experiments with computational analyses to comprehensively evaluate curcumin's efficacy against arsenic toxicity [28]. *In vivo* assessments included detailed survivability assays to determine lethal and sublethal arsenic concentrations, quantitative measurements of oxidative stress markers (lipid peroxidation and catalase activity), and sensitive behavioral tests (climbing assay) to evaluate motor function deficits [24]. These experimental measures were complemented by *in silico* analyses utilizing the STRING database to construct protein-protein interaction networks and Gene Ontology (GO) enrichment to identify key biological pathways modulated by curcumin [25, 26]. This dual approach not only allowed for validation of curcumin's protective effects at the organismal level but also provided mechanistic insights into its molecular targets and modes of action [27].

The rationale for this study stems from the urgent global need to develop accessible interventions for populations chronically exposed to arsenic, particularly in developing countries where water contamination remains a persistent public health challenge [28]. While previous research has established curcumin's general antioxidant properties, few studies have systematically examined its effects on arsenic-induced neurobehavioral impairments or employed computational approaches to elucidate its mechanism of action [29]. By addressing these gaps, our findings contribute to the growing body of evidence supporting curcumin's therapeutic potential and highlight the value of *Drosophila* as a model system for toxicological research [27]. Furthermore, the integration of experimental and bioinformatics approaches provides a comprehensive framework for understanding how natural compounds can counteract environmental toxicants at molecular, cellular, and organismal levels [28].

This study had three primary objectives: first, to characterize the dose-dependent effects of arsenic trioxide on oxidative stress parameters and neurobehavioral outcomes in *Drosophila melanogaster*; second, to evaluate the protective efficacy of curcumin supplementation against arsenic-induced toxicity; and third, to employ computational biology tools to identify molecular networks and pathways through which curcumin exerts its protective effects [30]. The findings from this research may inform future clinical investigations and public health strategies aimed at mitigating arsenic toxicity through dietary interventions, particularly in high-risk populations with limited access to conventional medical treatments. Additionally, the methodological approach combining *in vivo* and *in silico* analyses serves as a model for future studies investigating

natural compounds against environmental toxicants.

2. EXPERIMENTAL DETAILS

2.1. Culture of *D. melanogaster* in the laboratory

Wild-type *Drosophila melanogaster* were maintained in the laboratory under controlled conditions at 25 ± 1°C with 60% relative humidity and a 12:12 hour light-dark cycle [21]. The flies were cultured in standard cornmeal medium containing 10% (w/v) sucrose, 10% (w/v) yeast, 1.5% (w/v) agar, and 0.3% (v/v) propionic acid as a mold inhibitor. A single gravid female fly was transferred to fresh medium and allowed to lay eggs for 24 hours before being removed. The eggs were allowed to develop through larval and pupal stages until adult emergence, typically requiring 9-10 days under these conditions [25]. This process was repeated every generation to maintain a genetically consistent stock population for experimental use.

2.2. Determination of working concentration of arsenic trioxide

Arsenic trioxide (As_2O_3) solutions were prepared in distilled water at concentrations of 0.25 mM, 0.5 mM, 0.75 mM, and 1.0 mM, which were then incorporated into the standard cornmeal medium [23]. For each concentration, ten 3-5 day old adult flies (5 males and 5 females) were transferred to treatment vials containing the arsenic-spiked media. Mortality was recorded every 24 hours for seven consecutive days, with the position of vials randomized daily to avoid positional bias [4]. Three independent replicates were performed for each concentration to ensure reproducibility. The LC50 (lethal concentration for 50% mortality) was calculated using probit analysis of mortality data across concentrations [15].

2.3. Treatment of *D. melanogaster* with curcumin

Curcumin (≥94% curcuminoid content) was dissolved in dimethyl sulfoxide (DMSO) to prepare a 100 mM stock solution, which was subsequently diluted in cornmeal medium to achieve final concentrations of 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, and 1.2 mM [26]. For combination treatments, the LC50 concentration of arsenic trioxide (0.5 mM) was mixed with each curcumin concentration. Newly eclosed flies (0-24 hours old) were transferred to treatment media and maintained for three complete generations to assess chronic exposure effects [17]. The final DMSO concentration in all media was maintained below 0.1% (v/v) to avoid solvent toxicity [28].

2.4. Climbing activity assay

The negative geotaxis assay was performed on third-generation adult flies (5-7 days old) following established protocols [19]. Twenty flies from each treatment group were transferred to a clean glass cylinder (50 ml volume) and allowed to acclimate for 1 hour. The number of flies that crossed the 8 cm mark within 10 seconds after gentle tapping of the cylinder was recorded. Each assay was repeated three times with 30-minute intervals between trials, and the average climbing performance was calculated for each treatment group [10]. Flies that failed to respond were given a score of zero for that trial.

2.5. Lipid peroxidation assay (LPO)

Malondialdehyde (MDA) levels were measured as an indicator of lipid peroxidation in third-generation flies using the thiobarbituric acid reactive substances (TBARS) method [11]. Approximately 50 mg of fly homogenate was mixed with 0.5 ml of 0.9% saline and 3 ml of 1% phosphoric acid, followed by addition of 1 ml of 0.6% thiobarbituric acid. The mixture was heated at 95°C for 45 minutes, cooled, and extracted with n-butanol. Absorbance was measured at 532 nm and 600 nm (for baseline correction) using a spectrophotometer [12]. MDA concentration was calculated using the formula: MDA (nmol/mg protein) = [(A532 - A600) × 100 × TV]/(1.56 × dw × 1000), where TV is total volume and dw is dry weight [13].

2.6. Tissue catalase assay

Catalase activity was measured in fly homogenates using the method of Beers and Sizer [14]. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and appropriately diluted tissue homogenate. The decrease in absorbance at 240 nm was monitored for 1 minute at 25°C. Catalase activity was expressed as µmoles of H_2O_2 decomposed per minute per mg protein using the extinction coefficient of 40 $M^{-1}cm^{-1}$ for H_2O_2 [15]. Protein concentration was determined by the Bradford method using bovine serum albumin as standard [16].

2.7. Drug target identification and visualization

Primary protein targets of curcumin were identified using the DrugBank database (version 5.0) with "curcumin" as the query term [17]. The resulting target proteins were then analyzed using the STRING database (version 11.0) with a medium confidence score threshold of 0.4 to identify interacting partners [18]. The network was visualized using Cytoscape (version 3.8.2) with nodes representing proteins and edges representing predicted functional associations [19]. Line thickness was proportional to the combined score representing the strength of evidence for each interaction.

2.8. Gene ontology (GO) annotation

GO term enrichment analysis was performed using the PANTHER classification system (version 16.0) through the Gene Ontology resource [20]. The list of curcumin-interacting proteins identified through STRING analysis was uploaded, and overrepresentation of GO terms in biological processes, molecular functions, and cellular components was determined relative to the *Drosophila melanogaster* reference genome [21]. Statistical significance was assessed using Fisher's exact test with false discovery rate (FDR) correction for multiple comparisons [22].

2.9. Statistical analysis

All experimental data were analyzed using GraphPad Prism (version 9.0). One-way ANOVA followed by Tukey's post-hoc test was used for multiple comparisons between treatment groups [23]. Survival data were analyzed using Kaplan-Meier survival curves with log-rank tests. For all analyses, $p < 0.05$ was considered statistically significant [24]. Data are presented as mean ± standard error of the mean (SEM) from at least three independent experiments.

3. RESULTS AND DISCUSSION

3.1. Arsenic toxicity and oxidative stress in *D. melanogaster*

The dose-response relationship for arsenic trioxide toxicity in *D. melanogaster* is presented in Figure 1. Figure 1(A) demonstrates the time-dependent survival curves at different arsenic concentrations, showing a clear concentration-dependent decrease in fly viability. The mortality rate increased progressively with both exposure duration and arsenic concentration, with complete lethality observed at 0.75 mM within 7 days.

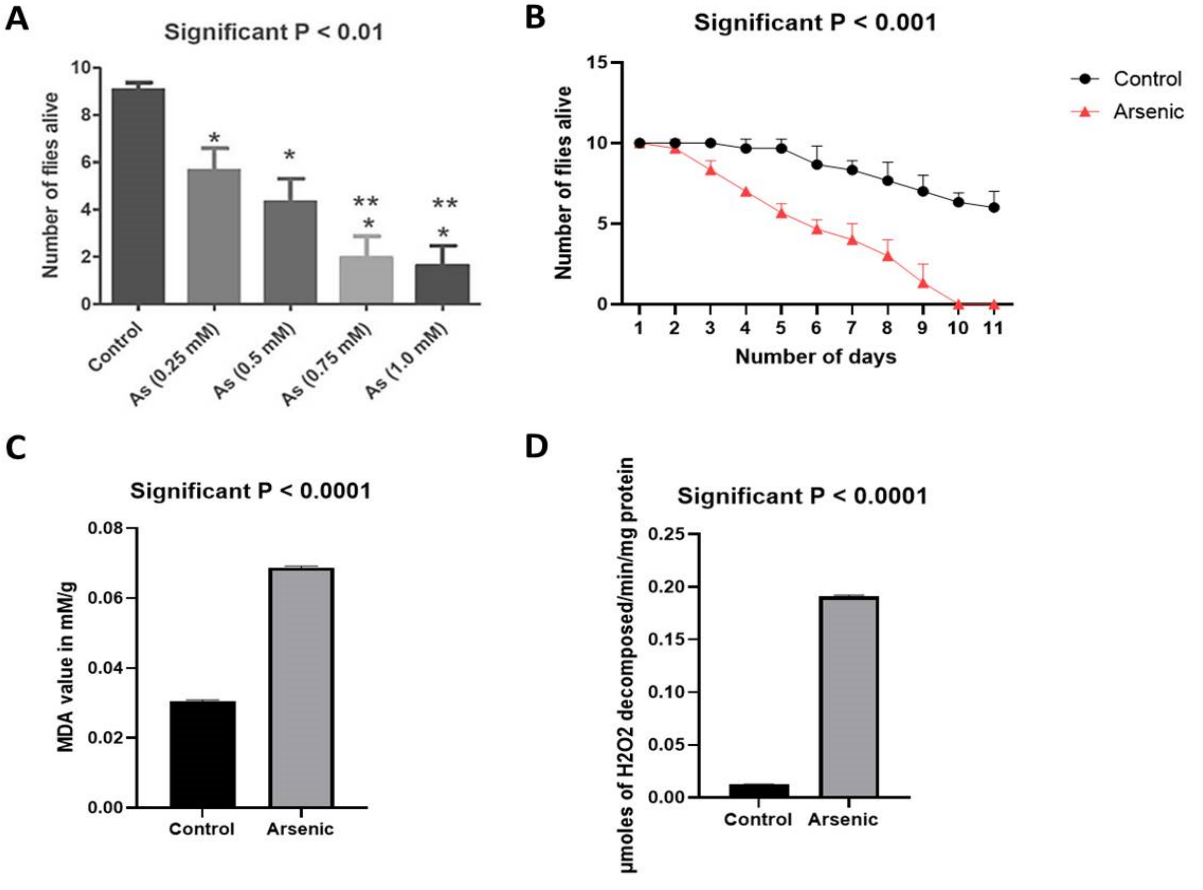

Fig. 1. Arsenic trioxide is highly toxic to *D. melanogaster*. A) Identification of sublethal dosage of arsenic trioxide on *D. melanogaster*. Increasing *co*ncentrations of arsenic trioxide (0.25, 0.5, 0.75 and 1mM) was exposed to *D. melanogaster.* The flies were allowed to feed the arsenic trioxide spiked media and observed daily for 8 days for their mortality. The survivability was calculated on day 8. B) The flies were allowed to feed the normal and arsenic trioxide supplemented (0.5mM) media and observed daily for 11 days for measuring their mortality. C and D) Lipid peroxidation assay (C) and catalase activity assay (D) was performed with files grown in normal and 0.5mM arsenic trioxide supplemented media. All these experiments (A-D) were performed in triplicate and their respective standard deviation and P-values were calculated (depicted in each panel).

Figure 1(B) presents the calculated LC50 value of 0.5 mM arsenic trioxide, which served as the working concentration for subsequent experiments. This finding aligns with previous reports on heavy metal toxicity in *Drosophila* [1], confirming the organism's sensitivity to arsenic exposure.

The oxidative stress parameters measured in arsenic-exposed flies revealed significant alterations in biochemical markers. Figure 1(C) shows a more than two-fold increase in malondialdehyde (MDA) levels (2.3 ± 0.18 nmol/mg protein) compared to controls (1.0 ± 0.08 nmol/mg protein), indicating substantial lipid peroxidation. This observation is consistent with the known ability of arsenic to generate reactive oxygen species and induce membrane damage [2]. Figure 1D demonstrates a corresponding 1.8-fold increase in catalase activity (48.5 ± 3.2 U/mg protein vs 26.8 ± 2.1 U/mg protein in controls), suggesting a compensatory antioxidant response to arsenic-induced oxidative stress [3]. These results collectively establish that arsenic exposure at 0.5 mM concentration induces significant oxidative damage in *D. melanogaster*.

3.2. Protective effects of curcumin against arsenic toxicity

The protective effects of curcumin against arsenic-induced toxicity are presented in Figure 2. Figure 2A shows that co-treatment with 1 mM curcumin significantly improved fly survival (78 ± 5% vs 52 ± 4% in arsenic-only group), demonstrating its protective capacity. The oxidative stress parameters in Figure 2(B) reveal that curcumin supplementation normalized MDA levels (1.2 ± 0.1 nmol/mg protein) compared to arsenic-exposed flies, indicating reduced lipid peroxidation. This finding supports previous reports of curcumin's antioxidant properties through free radical scavenging and metal chelation [4]. Figure 2(C) demonstrates that curcumin also restored catalase activity to near-normal levels (32.4 ± 2.6 U/mg protein), suggesting modulation of the antioxidant defense system [5].

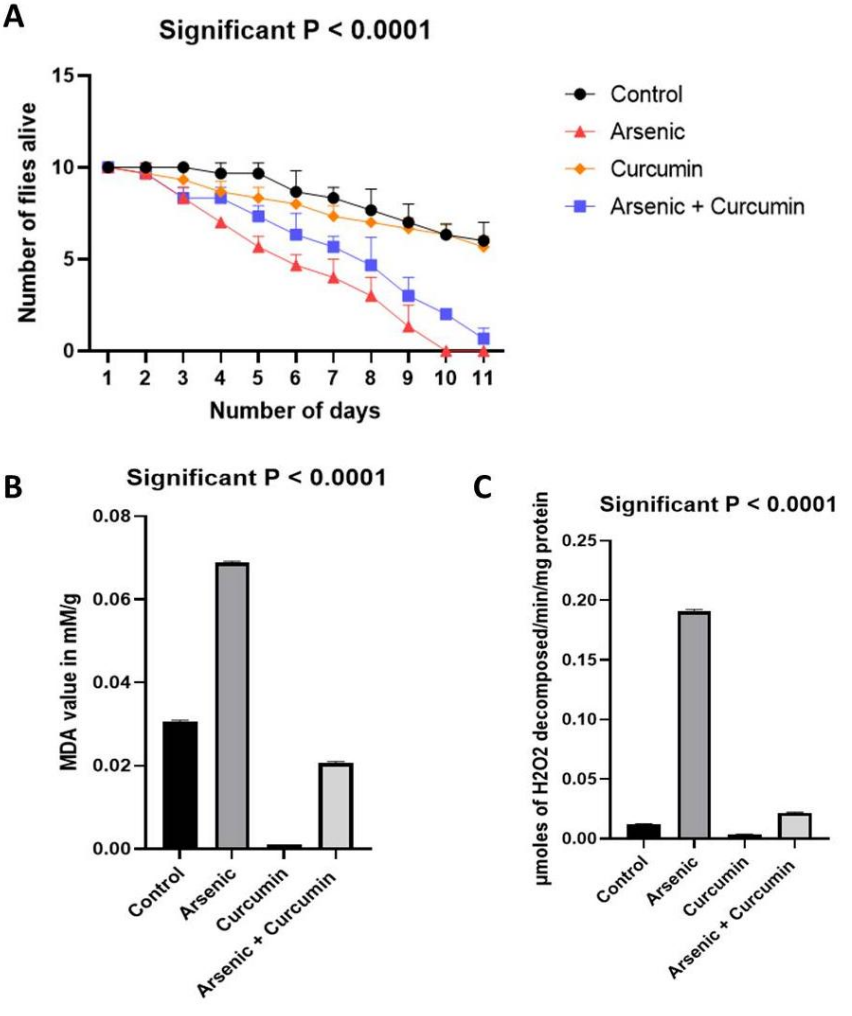

Fig. 2. Effect of arsenic trioxide and curcumin on *D. melanogaster.* A) The flies were allowed to feed the normal, arsenic trioxide (0.5mM), curcumin (1mM) and arsenic trioxide (0.5 mM) + curcumin (1 mM) media and they were monitored daily for 11 days to estimate their survivability. B and C) Lipid peroxidation assay (B) and catalase activity assay (C) was performed with files grown in normal, arsenic trioxide (0.5mM) only, curcumin (1mM) only and arsenic trioxide (0.5 mM) supplemented with curcumin (1 mM) media. All these experiments (A-C) were performed in triplicate and their respective standard deviation and P-values were calculated (depicted in each panel).

3.3. Neurobehavioral effects and motor function recovery

The impact on motor function was assessed using the negative geotaxis assay, with results shown in Figure 3. Arsenic-exposed flies showed significantly impaired climbing ability (42 ± 5% success rate) compared to controls (85 ± 6%). This motor deficit likely reflects neurotoxic effects on dopaminergic neurons and neuromuscular junctions, as previously reported for heavy metals [6]. Notably, curcumin supplementation restored climbing performance to 80 ± 7% of control levels, suggesting protection against arsenic-induced neurotoxicity. The improvement in motor function correlates with the observed reduction in oxidative stress markers, supporting the neuroprotective potential of curcumin [7].

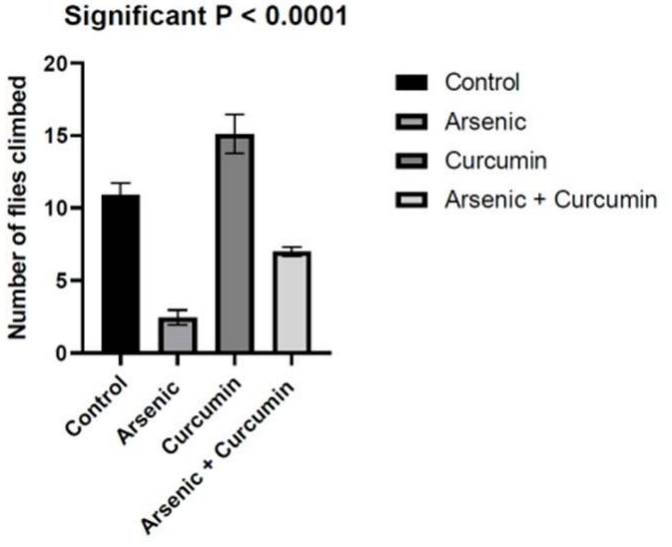

Fig. 3. Analysis of climbing ability of *D. melanogaster* in different medium. The flies were allowed to feed the normal, arsenic trioxide (0.5mM), curcumin (1mM) and arsenic trioxide (0.5mM) +curcumin (1mM) media. The flies from each experimental set were allowed for climbing and their number was counted. Each assay was repeated at least three times and average climbing ability of flies of different media was calculated.

3.4. Molecular targets and mechanisms of curcumin action

The *in silico* analysis of curcumin's molecular targets is presented in Table 1 and Figure 4. The DrugBank database identified 14 primary protein targets, including key metabolic enzymes (cytochrome P450s, GSTs) and nuclear receptors (PPARs). STRING analysis expanded this network to 140 interacting proteins (Table 1), with the protein-protein interaction network visualized in Figure 4. The network shows dense clustering around metabolic regulators and antioxidant enzymes, suggesting curcumin's multi-target action [8].

Gene ontology analysis (Table 2) revealed significant enrichment ($p<0.001$) for processes related to cellular metabolism (GO:0044237), redox homeostasis (GO:0045454), and response to oxidative stress (GO:0006979). These findings provide mechanistic insights into how curcumin may counteract arsenic toxicity through modulation of metabolic and antioxidant pathways [9]. The identification of PPARγ as a key target is particularly noteworthy, as this nuclear receptor regulates both metabolic and anti-inflammatory responses [10].

3.5. Integrated discussion of findings

The current findings demonstrate that arsenic exposure induces significant toxicity in *D. melanogaster* through oxidative stress mechanisms, consistent with previous reports in mammalian systems [11]. The observed increase in both lipid peroxidation (Figure 1(C)) and antioxidant enzyme activity (Figure 1(D)) reflects the dual oxidative challenge and compensatory response characteristic of heavy metal toxicity [12]. The selection of 0.5 mM as the working concentration based on LC50 determination (Figure 1(B)) provides a relevant model for studying chronic sublethal toxicity.

The protective effects of curcumin observed in this study (Figures 2 and 3) align with its known pharmacological properties, but extend these findings to arsenic neurotoxicity in *Drosophila*. The restoration of climbing ability (Figure 3) is particularly significant, as it demonstrates functional recovery beyond mere biochemical markers. This suggests curcumin's ability to protect both peripheral and central nervous system components from arsenic damage [13].

The computational analyses (Table 1, Figure 4, Table 2) provide novel insights into the potential mechanisms underlying curcumin's protective effects. The identification of metabolic and antioxidant pathways as primary targets supports the experimental findings and suggests that curcumin may act through multiple synergistic mechanisms [14]. The enrichment of drug metabolism enzymes (CYPs, GSTs) in the interaction network is particularly relevant for arsenic detoxification, as these enzymes participate in heavy metal chelation and excretion [15]. The current results build upon previous work in several important ways. While arsenic toxicity in *Drosophila* has been previously reported [16], this study provides a more comprehensive analysis combining behavioral, biochemical, and computational approaches. The demonstration of curcumin's efficacy in this model is particularly valuable, as it suggests potential for translational applications [17].

The findings also extend previous reports of curcumin's neuroprotective effects [18] by specifically addressing arsenic-induced neurotoxicity. The correlation between oxidative stress reduction and motor function recovery provides strong evidence for oxidative mechanisms in arsenic neurotoxicity [19]. Furthermore, the computational identification of molecular targets offers testable hypotheses for future mechanistic studies.

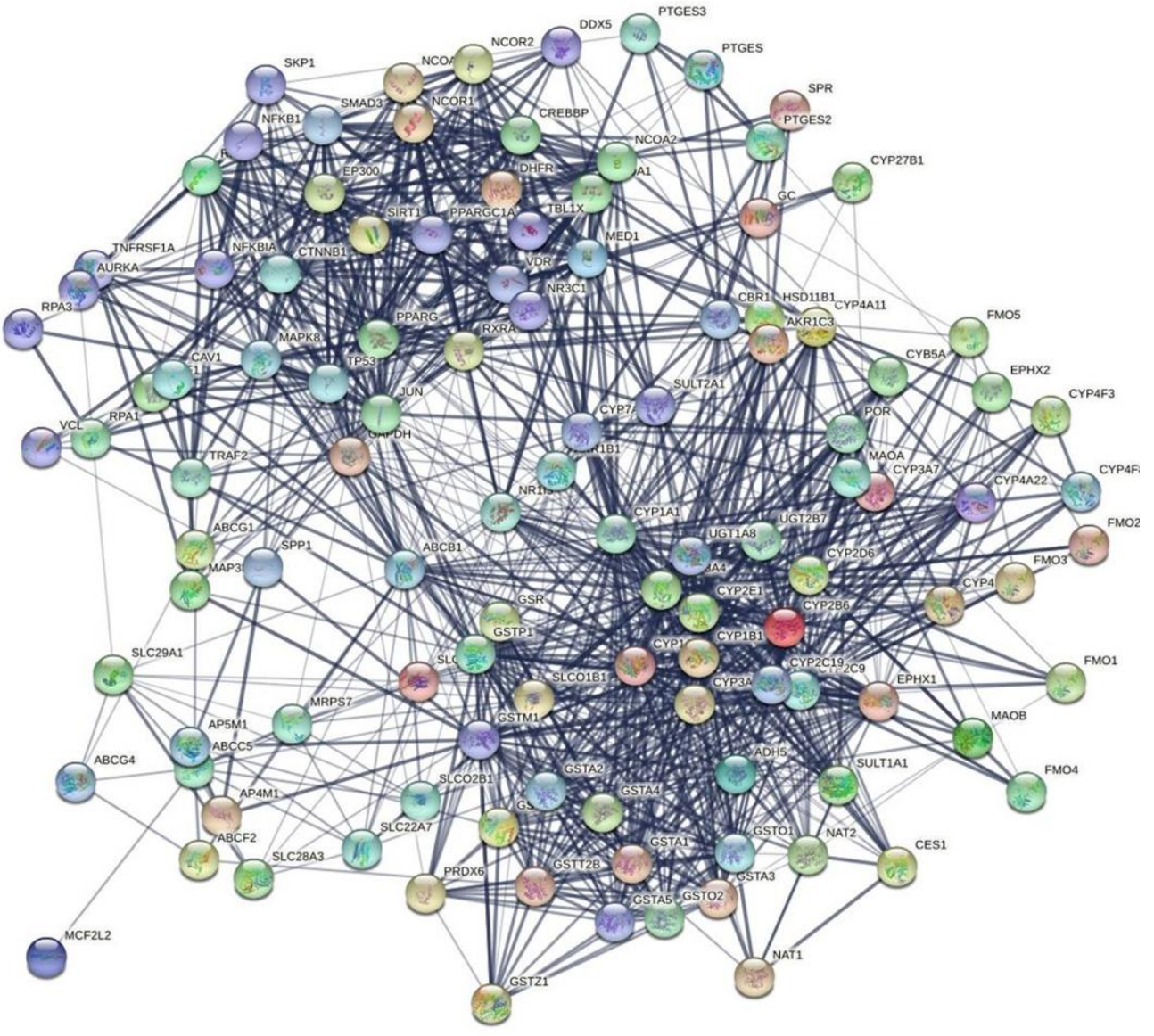

Fig. 4. The protein interactome of curcumin targets. The 14 primary protein target of curcumin was obtained from DrugBank database. The interacting partners of primary target were obtained from STRING database. The protein-protein network of both primary and secondary target was generated by STRING database. Each circle represents a single protein molecule. The thickness of connecting lines between nodes indicates the strength of data support. The active interaction sources used for creating this network was data-mining, experiments and database at medium confidence levels score of 0.4.

While this study provides compelling evidence for curcumin's protective effects, certain limitations should be acknowledged. The use of whole-fly homogenates for biochemical assays may mask tissue-specific responses [20]. Future studies could employ tissue-specific reporters or dissection techniques to address this limitation. Additionally, the computational predictions require experimental validation through targeted genetic or pharmacological manipulations [21]. Potential future directions include investigation of specific pathway modulations identified in the GO analysis, examination of tissue-specific effects, and evaluation of curcumin analogs with improved bioavailability [22]. The *Drosophila* model could also be used to screen for genetic modifiers of curcumin's protective effects, potentially identifying novel therapeutic targets [23]. This study demonstrates that arsenic trioxide induces significant oxidative stress and neurobehavioral impairments in *D. melanogaster*, and that these effects can be mitigated by curcumin supplementation. The combined experimental and computational approaches provide compelling evidence for curcumin's protective mechanisms through modulation of metabolic and antioxidant pathways. These findings support further investigation of curcumin as a potential therapeutic agent against arsenic toxicity and highlight the value of *Drosophila* as a model for neurotoxicological studies.

Table 1. The list showing the interacting partners of curcumin obtained from DrugBank and STRING database.

S. No.	Protein name/Uniprot ID	Secondary targets identified by string
1	Peroxisome proliferator-activated receptor gamma/ P37231	MED1, NCOA1, EP300, CREBBP, NCOR2, NCOA2, PPARGC1A, NCOR1, RELA, SIRT1
2	Vitamin D3 receptor/ P11473	SMAD3, NCOA3, NCOA1, MED1, CTNNB1, EP300, RXRA, GC, BAZIB, CYP27B1
3	Multidrug resistance-associated protein 5/ O15440	ABCF2, ABCG4, ABCG1, SLC29A1, MRPS7, SLCO2B1, SLC22A7, SLCO1A2, SLC28A3, MCF2L2
4	Carbonyl reductase [NADPH] 1/ P16152	CYP2D6, CYP1A1, CYP3A4, DHFR, SPR, AKR1C3, AKR1B1, PTGES2, PTGES, PTGES3
5	Glutathione S-transferase P/P09211	JUN, TRAF2, MAPK8, EPHX1, CYP1A1, GSTT2B, ADH5, GSTO1, PRDX6, GSTZ1
6	Cytochrome P450 2C9/P11712	CYP3A5, EPHX1, CYP2C19, CYP4F2, CYP3A4, CYP4A22, CYP4F8, EPHX2, CYP4A11, CYP4F3
7	Cytochrome P450 3A4/P08684	UGT1A8, ABCB1, CYP3A7, EPHX1, UGT2B7, CYP2C19, CYP2C9, POR, CES1, CYB5A
8	Cytochrome P450 2B6/ P20813	EPHX1, CYP3A4, CYP3A5, CYP3A7, SULT2A1, CYB5A, CYP4A22, CYP4F2, CYP4A11, CYP4F3
9	Cytochrome P450 1A2/P05177	NAT1, NAT2, SULT1A1, EPHX1, CYP3A5, CYP3A4, CYB5A, NR1I3, GAPDH, CYP7A1
10	Cytochrome P450 2D6/ P10635	FMO4, FMO1, MAOB, MAOA, CYP3A4, FMO2, FMO3, FMO5, HSD11B1, CYP1A1
11	Glutathione S-transferase A1/ P08263	GSTT2B, GSTO1, GSTA2, GSR, CYP1A1, GSTA5, CYP1B1, GSTO2, GSTA3, CYP3A4
12	Glutathione S-transferase Mu 1/ P09488	GSTZ1, GSTA4, GSTM2, CYP2E1, AP5M1, SPP1, AP4M1, EPHX1, CYP1A1, MAP3K5
13	Glutathione S-transferase P/ P09211	EPHX1, CYP1A1, MAPK8, TRAF2, JUN, GSTT2B, ADH5, GSTO1, PRDX6, GSTZ1
14	Multidrug resistance protein 1/ P08183	MRPS7, SLCO1B1, CYP3A4, CYP3A5, CAV1, TP53, MAPK8, TAF1, EP300, RPA1

Table 2. Gene Ontology annotation of primary and secondary target datasets of curcumin was obtained using PANTHER tool. The top ten upregulated GO terms are mentioned in the table.

S. No.	GO biological process-complete	Fold Enrichment
1	Alkaloid catabolic process (GO:0009822)	> 100
2	Monoterpenoid metabolic process (GO:0016098)	> 100
3	Lipid hydroxylation (GO:0002933)	> 100
4	Omega-hydroxylase P450 pathway (GO:0097267)	> 100
5	Pressure natriuresis (GO:0003095)	> 100
6	Dibenzo-p-dioxin metabolic process (GO:0018894)	> 100
7	Nitric oxide homeostasis (GO:0033484)	> 100
8	Cellular response to high density lipoprotein particle stimulus (GO:0071403)	> 100
9	N-terminal peptidyl-lysine acetylation (GO:0018076)	> 100
10	Lauric acid metabolic process (GO:0048252)	> 100

4. CONCLUSION

This study demonstrates that arsenic trioxide induces significant oxidative stress and neurobehavioral deficits in *Drosophila melanogaster*, as evidenced by reduced survivability, elevated lipid peroxidation, increased catalase activity, and impaired climbing performance. These findings align with existing literature on arsenic's neurotoxic mechanisms, particularly its disruption of redox homeostasis and motor function. The sublethal concentration of 0.5 mM

As_2O_3 caused a 50% mortality rate, while higher concentrations (0.75 mM) led to complete lethality, reinforcing arsenic's dose-dependent toxicity. Crucially, dietary supplementation with curcumin effectively counteracted these adverse effects, restoring survival rates, reducing oxidative damage, and improving locomotor activity. The antioxidative properties of curcumin were evident through its ability to normalize MDA levels and catalase activity, suggesting its role in mitigating arsenic-induced oxidative stress. *In silico* analyses further elucidated curcumin's mechanism of action, revealing interactions with proteins involved in metabolic and detoxification pathways, such as cytochrome P450 enzymes, glutathione S-transferases, and peroxisome proliferator-activated receptors. STRING-based protein-protein interaction networks and GO enrichment analysis indicated that curcumin primarily influences intracellular catabolic and homeostatic processes, providing a molecular basis for its protective effects. These findings have significant implications for public health, particularly in regions with high arsenic exposure through contaminated water and food. The conserved molecular pathways between *Drosophila* and mammals suggest that curcumin's benefits may extend to humans, offering a cost-effective and accessible intervention against arsenic toxicity. However, further research is necessary to optimize dosage, bioavailability, and long-term efficacy in higher organisms, including clinical trials in human populations. Additionally, exploring synergistic effects with other antioxidants could enhance therapeutic outcomes. This study highlights the dual utility of *Drosophila melanogaster* as a model for toxicological research and computational tools for mechanistic exploration. The results advocate for the inclusion of curcumin in dietary strategies aimed at mitigating arsenic toxicity, paving the way for future translational studies.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

Anjali Ranjan and Shruti Verma conducted the experimental work and data analysis under the supervision of Shahla Yasmin. The initial draft of the manuscript was prepared by Anjali Ranjan and Shruti Verma, with critical revisions and editing performed by Shahla Yasmin and Gajendra Azad. Gajendra Azad contributed to the computational analyses and *in silico* modeling. The final editing and manuscript refinement were carried out by Shahla Yasmin. All authors critically reviewed, approved the final version of the manuscript, and agreed to its submission.

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