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Dioscorea alata L. Mitigates Oxidative Stress in *Drosophila* Larvae Induced by Sodium Dodecyl Sulfate

Apurva Jadhav [1], Archana Sharbidre [2], Suresh Jagtap [1,*]

[1] Herbal Medicine, Interactive Research School for Health Affairs, Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra, India; apurvajadhav201@gmail.com;
[2] Department of Zoology, Savitribai Phule Pune University, Pune, Maharashtra, India
* Correspondence: chiritatml@rediffmail.com;

Scopus Author ID 57213613663
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Abstract: The surfactant sodium dodecyl sulfate (SDS) causes severe environmental and ecological impacts. *D. melanogaster* is a cost-effective and genetically controllable model organism for studying toxicity and prevention. This study examined the protective effect of *Dioscorea alata* L. (purple yam) in restoring SDS-induced oxidative damage in the developmental stages of *Drosophila melanogaster*. Sub-lethal SDS dosage caused developmental impairments in *Drosophila* larvae. Increased toxicity caused pupation delays, reduced survival rates, and intestinal damage. The side effects were decreased by simultaneous *D. alata* (DA) tuber powder administration. The larvae treated with DA had reduced pupation times and eclosion rates similar to those of the control group. DA significantly restored SDS-induced intestinal damage in Smurf larvae, leading to the maintenance of epithelial integrity. Moreover, DA administration reduced oxidative stress indicators and modulated enzyme activity, strengthening larvae's defense mechanism against SDA oxidative damage. The protective effects are attributed to the antioxidant and polysaccharide properties of DA. Furthermore, DA powder reduced SDS-induced intestinal damage and accelerated developmental toxicity recovery, corroborating restored DA-associated impairments in larvae. Therefore, the study reported the potential of DA to alleviate SDS-induced oxidative damage and intestinal dysfunction. However, further investigations are required to understand the possible molecular underpinnings responsible for mitigating SDS-associated developmental defects and mitigation upon DA treatment.

Keywords: SDS; ROS; *Drosophila*; antioxidant; gut damage.

1. Introduction

The SDS is utilized as an ingredient in household products, including toothpaste, shampoos, shaving foams, bubble baths, floor cleaners, car wash soaps, creams and lotions as dispersing agents, cosmetics as cleansing agents, dried egg products as whipping aids, and food additive, etc. [1]. Although SDS has various applications, SDS inclusion in consumer products has sparked concerns regarding the potential health and environmental hazards [2]. It is crucial to consider the possible adverse effects of extensive SDS utilization on human health and the environment. The intestinal epithelium is regularly subjected to a wide range of harmful substances. Hence, a highly effective and robust immune system is necessary [3]. As per the report, vertebrates and insects have different physiological characteristics, and it is possible to simulate human intestinal diseases in *Drosophila melanogaster* (hereinafter *Drosophila)* [4]. Casali et al. documented that *Drosophila* exhibits notable resemblances to mammals regarding

the signaling networks that govern intestine development, regeneration, and diseases. Moreover, the gastrointestinal tract of *Drosophila* demonstrates both anatomical and functional resemblances to those of mammals [5]. *Drosophila* is a powerful model used to study signaling systems for maintaining intestinal balance. It helps balance the immune system by suppressing harmful bacteria while preventing harm to native flora and promoting a strong response to invaders [6]. The host employs the formation of reactive oxygen species (ROS) by NADPH oxidase and dual oxidase 1 as an additional strategy to guard against infections [7]. Maintaining a proper equilibrium between generating and eliminating reactive oxygen species (ROS) is crucial for the well-being of the host. ROS can interfere with foreign pathogens' genetic material, RNA, and proteins and degrade lipids. Moreover, excessive ROS can lead to cytotoxicity, cancer, age-related illnesses, and damage to the intestinal epithelium [8]. Impaired turnover of intestinal cells can result in weakened tissue integrity or the development of cancer [9]. Additionally, the stress reaction caused by hazardous chemicals like SDS, DSS, and paraquat may also impair intestinal homeostasis by causing vascular leakage and malfunction. Loss of intestinal paracellular barrier integrity causes luminal contents to flow into adjacent tissues, inflammation, and tissue damage [10]. Studies have reported SDS as a toxicant in adult *Drosophila*, but the use of a colitis model in *Drosophila* larvae is rare. In 2020, Zanchi et al. adapted a non-invasive "Smurf" assay from *Drosophila* to two species of tenebrionid beetle larvae to study gut disintegration [11]. Therefore, in this study, SDS was used as a toxicant and *Drosophila* larvae and gut disintegration was seen through a smurf assay.

Yam is a term used to describe plants belonging to the *Dioscorea* genus in the *Dioscoreaceae* family of the cultivated root and tuber crop globally [12]. *Dioscorea* species are essential dietary supplement components in the cosmetics and pharmaceutical industries, and locals engaged in the medical plant trade also use them. They are used to treat diseases and ailments, including coughs, colds, stomach aches, leprosy, burns, fungal infections, dysentery, skin diseases, and birth control [13]. Bioactive substances abundant in *Dioscorea* species include diosgenin, carotenoids, water-soluble polysaccharides, phenols, flavonoids, saponins, anthocyanins, and allantoins [14]. Yam is a crucial staple food and energy source for millions of people in South America and Africa because of its high starch content. As per the study, in China, they are used for both culinary and medicinal purposes [15]. This study used Dioscorea alata L. (DA), commonly known as purple yam, because of its high antioxidants and polysaccharides, which have healthy gut benefits. Therefore, the study evaluated the potential ameliorative effects *of* DA on SDS-induced developmental toxicity in *Drosophila* larvae. This study seeks to bridge the gap in understanding the impacts of SDS on *Drosophila* larvae development and explore the therapeutic potential of *D. alata* in mitigating SDS-associated effects, particularly focusing on gut health and developmental outcomes. Furthermore, using *Drosophila* larvae as a model organism provides a cost-effective and time-efficient method for evaluating the safety and efficacy of herbal drugs in the context of gastrointestinal health.

2. Materials and Methods

2.1. Materials.

SDS, Bradford reagent, TAG, Griess reagent, glacial acetic acid, glycerol, propionic acid, Trypan blue, nitro blue tetrazolium, glucose, methylparaben, blue dye, agarose, Dimethyl Sulfoxide (DMSO), HCl, and phosphate-buffered saline (PBS) were obtained from Himedia.

2.2. Collection and processing of plant materials.

The native *Dioscorea alata* tubers were collected from the Dist. Raigad, Maharashtra State, India. Required prior approval of the biodiversity board was obtained (MSBB/Research/ 630/2023-24). Fresh tubers were collected. Initially, the tubers were washed, peeled, sliced, and dried. Then, samples were ground in powder form.

2.3. Fourier transform infrared spectroscopy (FTIR).

FTIR spectroscopy is considered to be a reliable and sensitive method for the detection of bio-molecular composition. FTIR was conducted on *a D. alata (tuber)* powder sample. The dried sample was loaded in an FTIR spectroscope (SHIMANZU UV-2600-FTIR affinity), with a scanning range of 4000–400 cm^{-1} [16].

2.4. Fly stock and culture.

The Oregon R *Drosophila* stock was obtained from ISSER in Pune, India. A standard diet of yeast, sugar, maize meal, and type I agar was prepared to raise the flies. Propionic acid and methylparaben were added as antimicrobial substances to stop the growth of microbes.

2.5. Treatment.

The study used third-instar larvae. They were split into five groups: a control group, a model group treated with 0.25% and 0.5% SDS, and two medication administration co-treatment groups given 10 and 15 mg/ml of DA. We dispersed the powdered SDS in distilled water, thoroughly mixed it, and then transferred the larvae to the vials. Larvae were reared at 22°C constant temperature, 70% humidity, and 12h of light/dark conditions. Each experiment was replicated thrice.

2.6. Effect on larval development.

We analyzed the development cycles of treated and control larvae to check if they altered. The larvae's development was monitored at six-hour intervals across several phases of development. The percentages of larval pupation and adult eclosion were also recorded throughout time to assess any potential developmental delay [17].

2.7. SDS-induced gut damage.

2.7.1. Trypan blue exclusion assay.

The trypan blue assay is a simplistic test that distinguishes between viable and non-viable cells. It is utilized to identify cellular demise in the larval gastrointestinal tract. The experiment was conducted following the methodology of Krebs and Feder [18] with a minor modification. Five third instar larvae were extracted from each vial and rinsed with 1X PBS to eliminate food particles adhering to the larvae. Subsequently, the larvae were placed into a solution containing 0.02% trypan blue and left undisturbed for 30 minutes under conditions of darkness. Following incubation, any surplus stain was removed by rinsing with PBS (1X). The larvae were examined using a stereomicroscope, and photographs were captured to assess any anomalies in the digestive tract.

2.7.2. Smurf assay.

The smurf assay was performed as previously described with minor modifications [19]. Briefly, larvae fed different diets were starved for 3 hours, cultured on the dyed medium for 48 hours, and then detected. The dyed medium comprised 2% bromophenol blue sodium, 5% sucrose, and 1% agarose. A fly was referred to as a "smurf" when the dye coloration could be observed outside the digestive tract. All experiments were carried out with three biological replicates.

2.8. *Neurobehavioral assays.*

2.8.1. Crawling.

Larval crawling is a straightforward approach to exploring larval rhythmic behavior and neurological disorders [20, 21]. Five third-instar larvae were placed on a 2% agarose Petri dish. On the surface, larvae were free to move. Cameras recorded the larva's movement. Each larva's trajectory was tracked on graph paper.

2.8.2. nociception.

A chemical nociception assay was conducted on mid-third instar *Drosophila* larvae [22]. Larvae were exposed to a 9% HCl solution, and their behavior was assessed. A complete roll of 360° along the body axis within 10 seconds of HCl exposure was scored as aversive behavior. The frequency distribution of responses was plotted, allowing comparison of total larvae, non-respondents, and responses at different latencies. From each diet, larvae were taken for the experiment (n=10).

2.9. Detection of ROS and antioxidant enzyme activity.

2.9.1. Preparation of larvae homogenates.

About 10 larvae were homogenized in lysis buffer (50 mM potassium phosphate buffer, pH 7.0, 1 mM phenylmethylsulphonyl fluoride, and 0.5 mM EDTA) at a 1:10 ratio. After centrifugation (10 min, 4°C) in an Eppendorf 10 rpm centrifuge, the supernatants were collected and used for biochemical assays with either a microplate reader or a spectrophotometer.

2.9.2. Total reactive oxygen species using nitroblue tetrazolium (NBT).

To measure the level of ROS in the larval hemolymph, the NBT test was conducted using the protocol recommended by [23]. In this experiment, materials containing ROS were treated with the yellow dye Nitroblue Tetrazolium (Y-NBT) and allowed to incubate. Following incubation, the dye underwent reduction into water-insoluble formazan particles (NBT) due to the presence of superoxide molecules, resulting in blue coloration. The absorbance measurement was conducted at a wavelength of 595 nm, which was directly related to the quantity of ROS molecules.

2.9.3. Nitrite production (NO).

In an aqueous solution at physiological pH, sodium nitroprusside spontaneously creates NO, which reacts with oxygen to yield nitrite ions that may be measured using a Griess reagent. NO scavengers compete with oxygen, reducing nitrite ion formation. Absorbance was taken at 550 nm [24].

2.9.4. Superoxide dismutase (SOD).

Using NBT, SOD activity was assessed [25]. To a 3 ml reaction mixture, 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 75 µM NBT, and 50 µL enzyme extract were added. All tubes were exposed to 400 W bulbs for 15 minutes before 560 nm absorbance was measured.

2.9.5. Catalase (CAT).

CAT activity was assessed using the usual procedure [26]. One milliliter of the reaction mixture contains the enzyme sample (homogenate), substrate (H_2O_2), and 0.1 M sodium phosphate buffer (pH 7). The activity was assessed by measuring the breakdown of H_2O_2 at 240 nm.

2.10. Statistical analysis.

Analysis was done with Graph Pad Prism 8.0. To compare between groups and one-way analysis of Variance (ANOVA) for multiple groups. Asterisks indicate the critical levels of significance (* p<0.05, **p<0.01**,** *** p<0.005, and ****p<0.0001).

3. Results and Discussion

3.1. FTIR spectroscopy analysis.

FTIR spectra for raw *D. alata* tuber powder are shown in Figure 1. The FTIR spectra curve was interpreted in three stages: below 800 cm^{-1}, 800–1500 cm^{-1}, and 1500–3500 cm^{-1}. In the spectra, a band is attributed to the presence of OH at 3253 cm^{-1}. These bands are considered to be characteristic of polysaccharide absorption.

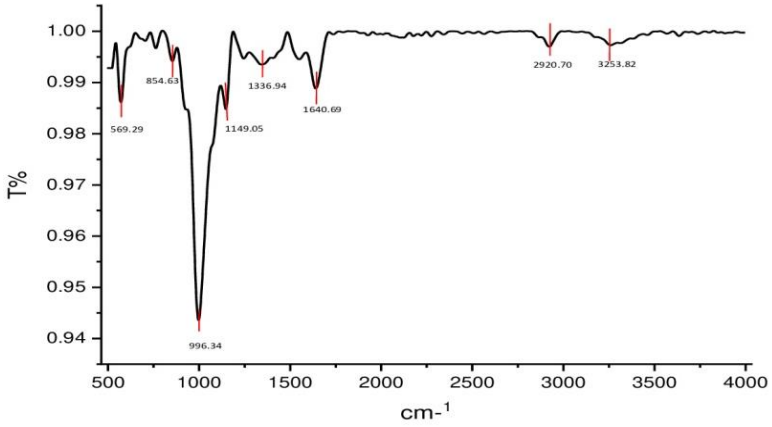


Figure 1. FTIR spectra of powder of *Dioscorea alata* L. tuber powder.

The absorbance bands at 856, 996, 1077, 1149, and 1242 cm^{-1} were linked to the C-O stretching vibrations, the C-H rocking vibrations, and the C-O-C asymmetric valence vibrations. Studies with other *Dioscorea* spp. are similar to those in this work. The 'fingerprint'

region for carbohydrates is FTIR spectra between 950 and 1200 cm^{-1}, which can identify important chemical groups in polysaccharides because the position and strength of the bands are unique to each polysaccharide [27]. Previous studies have demonstrated the potential anti-inflammatory properties of polysaccharides obtained from *Dioscorea* species [28]. The carbohydrate fractions from D. *alata* tuber are high in inulin-like fructooligosaccharides (FOS) with strong antioxidant and prebiotic activity [28].

3.2. Effects of SDS on developmental time course.

Developmental phases of *Drosophila* comprise the egg, larva, pupae, and adult stages. Each stage is fixed at a specific time point and remains unchanged unless there is an internal or external stressor [29]. In this study, larvae were fed with 0.25% and 0.5% SDS and SDS and DA-mixed diet (10 and 15mg/ml) to examine the effect of DA supplementation. An experimental control group of larvae was fed a normal diet. The right shift of the time to pupation curves and pupation time (PT) demonstrated dose-dependent delays in development in larvae raised on different SDS meal concentrations (Figure 2a). As the dose increased, delay became apparent. At 0.5% SDS, larval lethality increased, and total larvae attaining pupation decreased (Figure 2b). Compared to larvae fed normal food, PT rose by more than 7 days at this dose (Figure 2), whereas the 0.25% SDS group dropped 54%. Co-treatment with DA 10mg/ml and SDS 0.25% reversed this delay. Pupation was 97%, and PT was the same as the control group. The delay in PT affects eclosion time and percentage. Figure 2c-d shows adult eclosion from larvae at varied SDS concentrations. The eclosion rate reduced as SDS concentration increased, indicating progressive death. At 0.25% SDS concentration, mortality was estimated to be over 50%.

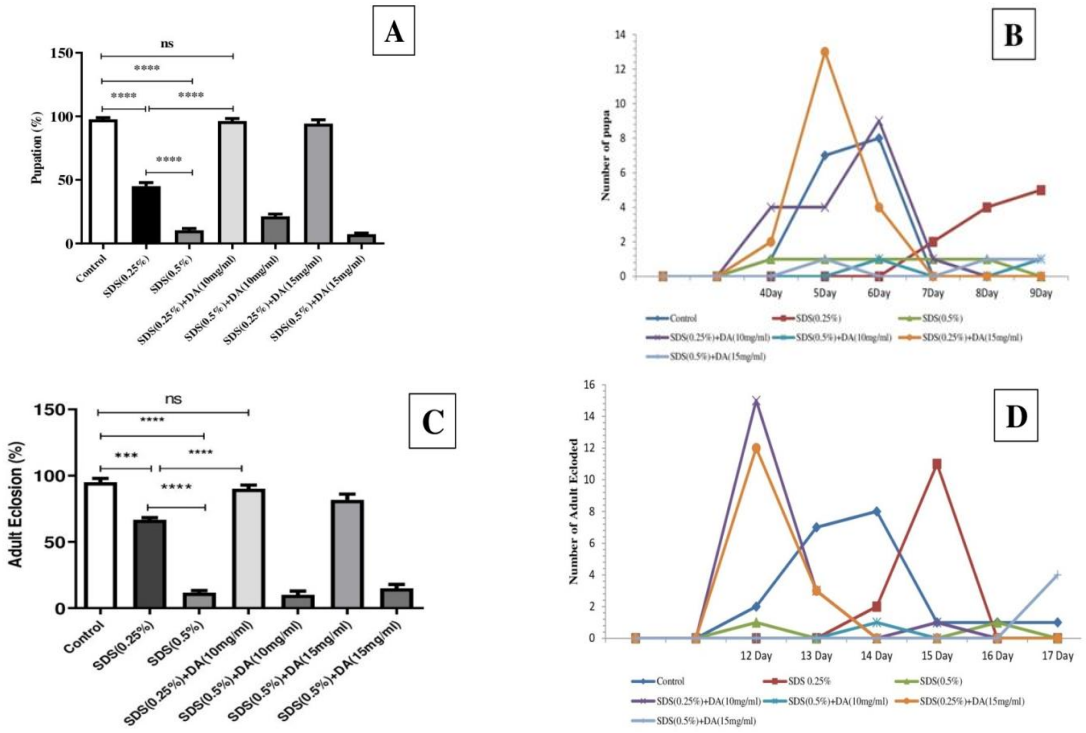
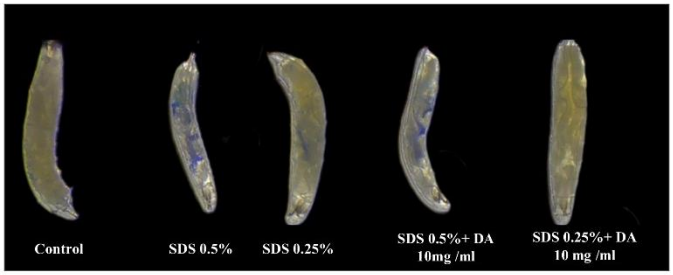


Figure 2. Evaluation of the development of *Drosophila melanogaster* with different treatments (Control, SDS 0.25, 0.5 % and SDS+ DA 10 mg/ml, SDS+15 mg/ml) during the developmental cycle. SDS exposures prolong the developmental time of *Drosophila*. SDS exposures prolong the developmental time of *Drosophila*. **(A-B)** Pupation time and percentage; **(C-D)** Adult eclosion time and percentage. Data are expressed as mean ± SEM. (**P < 0.05, ***p < 0.001, ****p< 0.0001, ns = not significant).

3.3. Protective Effect of DA against intestinal damage with SDS.

3.3.1. Midgut cell damage.

Trypan blue stains the damaged part of the gut. Gut damage was observed mainly in the midgut region of SDS-treated larvae, and gut damage was minimal in the case of co-treated larvae with DA 10 mg/ml and 15 mg/ml (Figure 3a).



A

Figure 3. (A) Trypan blue assay: DA alleviated SDS-induced cell damage in *Drosophila* larvae. Representive photos of *Drosophila* third instar larvae with trypan blue staining. Trypan blue staining signal level indicated the degree of damage to midgut cells.

3.3.2. Gut leakage.

The Smurf assay measured larvae intestinal integrity. The larvae had more blue dispersion after SDS exposure than the control group (Figure 3b).

B

Figure 3. (B) Smurf assay: DA alleviated SDS-induced impairment of intestinal morphology and barrier integrity.

The larvae's intestines turned blue after 48 hours of exposure to 0.5% and 0.25% SDS solutions. These results are in agreement with the study done on adult flies when treated with SDS [30]. The control larvae's digestive tract had little blue dye. However, the color spread throughout the body in SDS-treated larvae. We administer (DA 10mg/ml) with 0.25% and 0.5% SDS to prevent this. These findings show that SDS exposure compromises the epithelial barrier by increasing intestinal permeability. The loss of intestinal barrier function in the larvae resulted in the appearance of the Smurf phenotype, characterized by the presence of blue color throughout the entire body. This phenomenon can be measured using Smurfs. To examine the defensive impact of DA on larvae treated with SDS, Collectively, our results indicate that DA shields against the impairment of the intestinal barrier induced by SDS in larvae.

3.4. Neuro behavior of larvae.

Crawling is a coordinated motion that involves the simultaneous activation of numerous muscles and neurons [31]. Crawling of the larvae depicts that control larvae can cover more distance than the SDS-treated ones. Larvae become sluggish with the SDS treatment. In another investigation, a delay in crawling speed was noted when the larvae were exposed to cadmium toxic exposure [19]. Among the different treatments, the crawling distance increases with co-treatment with DA concentration up to a certain extent, i.e., 10mg/ml concentrations (Figure 4a).

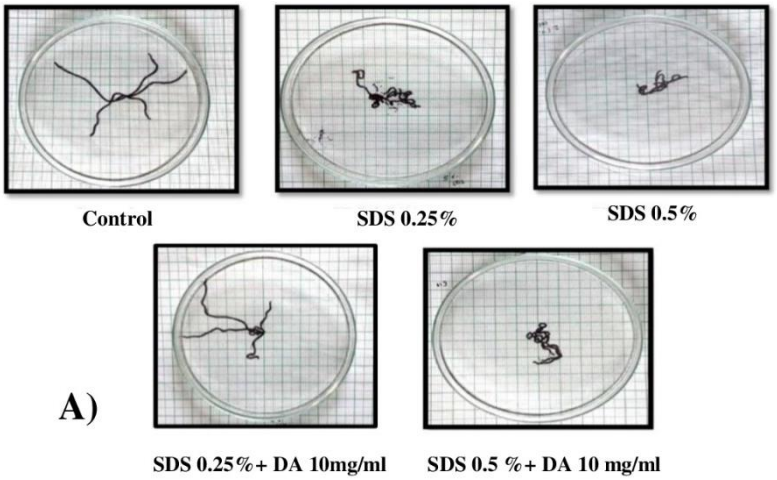


Figure 4. Impact of SDS on Neurobehavioral of *Drosophila* larvae **(A)** larval crawling assay showing larval trailing path.

The findings revealed that high amounts of SDS caused aberrant behavior in *Drosophila* larvae, such as irregular turns and twists. The crawling pattern of third-instar larvae was evaluated to determine any deficiency in their locomotor function and subsequent behavioral alterations. At varying doses of co-treatment of DA with SDS exposure, the crawling pattern of the larvae showed a smooth movement.

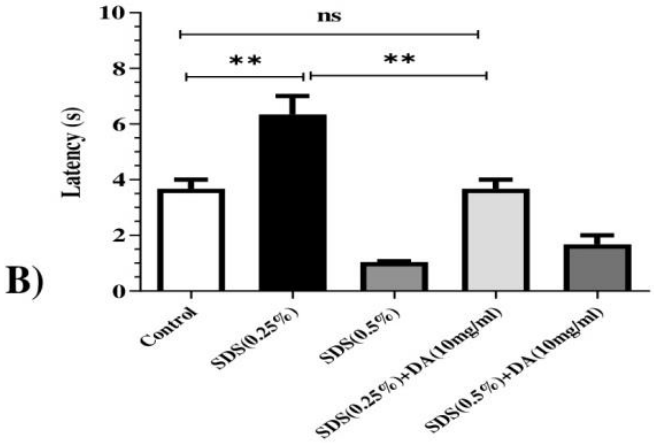


Figure 4. (B) Nociception activity in larvae where SDS has increased the latency period and DA significantly restored the latency time. Data are expressed as mean ± SEM (**P < 0.05, ***p < 0.001, ****p< 0.0001, ns = not significant).

Drosophila larvae display a wide range of nociceptive reactions, varying based on the specific stimulation they experience. Nociception is a fundamental sensory capacity found in

all animal species, enabling the detection and avoidance of potentially dangerous stimuli. Exposure to SDS led to a prolongation of the latency time of nociception. As depicted in Figure 4b, the SDS model group 0.25% exhibited a considerable latency increase compared to the control group ($p < 0.0001$). Co-treatment with DA reduced latency, indicating the beneficial impact of DA on nociceptive behavior (Figure 4b).

Dioscorea spp. Diosgenin (DG) has immunomodulatory, anti-inflammatory, anti-oxidative, anti-thrombotic, anti-apoptotic, anti-depressant, and anti-nociceptive properties, according to studies [32]. In our previous study, results obtained from HRMS analysis confirmed the presence of DG in the sample [33].

3.5. DA mitigates SDS-induced Drosophila larval toxicity.

The gastrointestinal tract is particularly susceptible to oxidative stress as a result of prolonged exposure to oxidants in the lumen, external stimuli, or an imbalanced microbiota [34,35]. Exposure to SDS significantly increased ROS levels relative to the control group ($p<0.0001$) (Figure 5a). The co-treatment of 10mg/ml DA dramatically reduces the antioxidant activity by up to 90%. The utilization of SDS has led to a substantial increase in nitric oxide levels. The DA scavenged 26% nitric oxide. Antioxidant enzyme functions were studied. Figure 5d-e shows that SDS lowered SOD and CAT enzyme activity by 36% and 14%, respectively, compared to the control. However, adding DA restored these enzymes' activities (54% increase in SOD activity and 9% increase in CAT activity compared to the SDS group, Figure 5c-d). The findings demonstrated that elevated SDS stress led to a notable elevation in ROS levels in the larvae, and DA successfully mitigated the level of ROS by showing antioxidant properties.

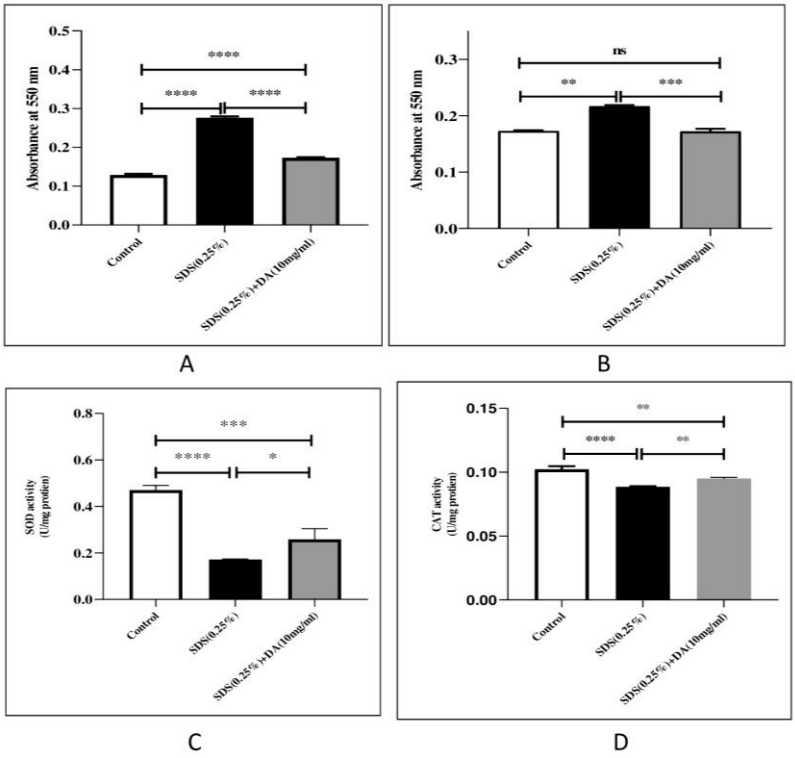


Figure 5. ROS in *Drosophila* larvae increased due to SDS stress. **(A)** Nitroblue Tetrazolium reduction assay for the estimation of reactive oxygen species (ROS) in *Drosophila* larvae exposed to SDS; **(B)** Nitric oxide scavenging activity of DA in larvae when exposed to SDS; **(C-D)** DA supplementation alleviated the activities of superoxide dismutase (SOD) and Catalase (CAT). (Mean ± SD, n = 3, 10 larvae per sample, ***P* < 0.05, ***p < 0.001, ****p< 0.0001, ns = not significant.

4. Conclusion

We administered SDS treatment for the first time during the larval stage when the organisms experience rapid growth and develop into adults. The toxicity of SDS in *D. melanogaster* is caused by an imbalance between reactive oxygen species (ROS) and the antioxidant defense system. This leads to a decrease in various biological and biochemical parameters. *Dioscorea alata* tuber has shown potential as a treatment for SDS-induced toxicity in *Drosophila melanogaster*, likely due to its antioxidant and anti-inflammatory properties. These findings suggest that DA could be a cost-effective and safe therapeutic agent for preventing SDS toxicity. The safety of DA in larvae also indicates its potential for treating gut leakage. However, further studies are needed to fully understand the mechanisms behind the protective effects of *Dioscorea alata* L. against SDS toxicity and to assess its safety and effectiveness as a therapeutic agent for SDS toxicity. While adult flies have been extensively researched in this model, this study represents the first investigation of gastrointestinal toxicity and behavioral alterations in *Drosophila* larvae using SDS.

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Conflicts of Interest

The author declared no conflict of interest.

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