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Research Article

Genotoxic Effects of *Aristolochia albida* Extract Using *Drosophila melanogaster*

***Ibrahim Malami[1,2], Adamu Zubairu Adamu[1], Aisha Shehu Tambuwal[1] and Zakiyyah Yakubu Yahaya Ibrahim[1]**

[1]Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria
[2]Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University Sokoto, Nigeria
****Corresponding Author's email*:** ibrahim.malami@udusok.edu.ng; **Phone:** +2348166923199

ABSTRACT

This study investigated *Aristolochia albida* extracts/toxicological effects using antioxidant defense assays and the Drosophila Somatic Mutation and Recombination Test (SMART) assay. For acute toxicity, adult *Drosophila melanogaster* was exposed to *A. albida* extracts at concentrations of 25, 50, 100, and 200 µg/mL, with mortality recorded over 7 days. In the 28-day treatment, flies were fed extracts at 12.5, 25, and 50 µg/mL, and antioxidant enzyme activities (catalase, superoxide dismutase, and glutathione) were measured. In the SMART assay, Drosophila larvae were exposed to *A. albida* extracts at 12.5, 25, and 50 µg/mL concentrations. The adult flies were analysed to evaluate genotoxicity through wing spot formation. The LC_{50} was determined to be 128.5 µg/mL. Antioxidant enzyme activities (catalase, superoxide dismutase, and glutathione) significantly increased, indicating an oxidative stress response. The SMART assay revealed genotoxic effects with a higher frequency of wing spots in treated groups than controls. These results suggest that *A. albida* exposure exerts toxic effects on Drosophila flies by inducing oxidative stress and mutations.

Keywords: *Aristolochia albida*; Aristolochic acid; Drosophila; Genotoxicity; Oxidative stress; Mutagenicity

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INTRODUCTION

The genus *Aristolochia* has been traditionally used in herbal medicine for various illnesses in many parts of the world. However, it's important to note that the use of *Aristolochia* plants in herbal medicine is highly debated and potentially dangerous. Certain species of *Aristolochia* contain toxic compounds known as aristolochic acid (AAs) and these compounds can cause severe kidney damage (Chan *et al*., 2019) and have been associated with the development of urinary tract cancer (NIEHS, 2021). These compounds are well known to form DNA adducts, especially in the kidney, causing mutagenicity and nephrotoxicity in patients with a history of using the herbal material (Han *et al*., 2019). When these compounds are ingested, they can cause severe damage to the kidneys due to their DNA adducts formation in the kidney, thereby causing renal failure associated with urothelial carcinoma (Michael *et al*., 2009; Han *et al*., 2019). The genotoxic effects of AAs have been extensively studied, particularly in the context of *Aristolochia fangchi* and *Aristolochia clematitis*, which are known to contain high levels of these compounds (Sgamma *et al*., 2018; Chan *et al*., 2019). Interestingly, *Aristolochia albida* Duch is one of the *Aristolochia* species commonly used as herbal medications in many parts of Nigeria including Sokoto State.

Aristolochia albida Dutch is native to various parts of Africa commonly known as Dutchman's pipe and it is widely found in the Northern region of Nigeria as

'Duman Dutse'. The herbal material is locally used in many parts of Sokoto for various illnesses and has been prescribed for cancer treatment (Malami *et al*., 2020). The use of *A. albida* in herbal medicine for different illnesses raises concerns regarding potential health risks. While the plant has been traditionally used for its perceived medicinal properties, several scientific research has identified AAs as the toxic compounds present in the species of Aristolochia. The US FDA and other countries have banned or restricted the sale and use of AAs-containing products (Han *et al*., 2019). However, the frequent use of herbal medications containing AAS still occurs in Nigeria. Therefore, continued use of *A. albida* in herbal remedies without understanding the potential health risks associated with the herbal medicine and the lack of knowledge of the toxicity of AAs may expose individuals to unnecessary harm and undermine public health efforts. There is a need to investigate herbal medicine, which may pose health risks to consumers. This research aims to investigate the genotoxic effect of the extract of *A. albida* using the Drosophila model by employing genotoxicity assessment assays.

MATERIALS AND METHODS

Preparation of plant material

The plant material was obtained from a local herbal market and authenticated at the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The sample was assigned a voucher number (PCG/UDUS/Aris/0001) corresponding to the existing voucher specimen in the Department. The stem bark of the plant material was peeled, shade-dried, and pulverised into a fine powder. An aqueous extraction was performed on the powdered material using the decoction method, whereby the sample was boiled in water for two hours. The resulting solution was cooled to room temperature and filtered using filter paper. The filtrate was then evaporated to dryness at 40°C until a completely dry extract was obtained.

Drosophila Stocks and Culture

Wild-type *Drosophila melanogaster* stocks were obtained from the Centre for Advanced Medical Research and Training (CAMRET). All flies were maintained in an incubator at a controlled temperature of 20–22°C with a 12-hour light/12-hour dark photoperiod cycle.

Food Preparation

The diet for the flies consisted of 1000g of prepared food media, which included 100g of corn flour, 20g of yeast, 10g of agar-agar, and 1g of methylparaben with water added to achieve the appropriate consistency. To prepare this media, agar-agar was gradually added to boiling water and stirred continuously for 10 minutes. Moist corn flour was then incorporated and cooked for an additional 10 minutes. Yeast was subsequently added, followed by methylparaben, with stirring maintained for another 10 minutes. The media was then allowed to cool briefly before being transferred into culture bottles.

Acute toxicity study

The food media were supplemented with *A. albida* extract at concentrations of 25, 50, 100, and 200 µg/mL. Groups of 20 adult flies were placed in separate food vials containing each concentration of *A. albida* and mortality was recorded daily over a 7 day period following initial exposure. The median lethal concentration (LC_{50}) was determined using Probit analysis to assess the toxicity of the *A. albida* extract across concentrations.

Chronic toxicity study

The *A. albida* extract was supplemented into a standard diet medium at concentrations of 12.5, 25, and 50 µg/mL. A total of 40 flies were introduced into separate vials, each containing a different concentration of the extract. Each treatment group was prepared in triplicate. Mortality among adult flies was monitored daily over 28 days, with observations recorded every 24 hours following initial exposure.

Haemolymph extraction

At the end of the 28-day treatment period, flies were homogenised in phosphate-buffered saline (PBS) to collect haemolymph for biochemical analysis.

Determination of antioxidant defences Catalase

The catalase (CAT) activity assay was quantified by measuring the decomposition of H_2O_2 using the catalase activity assay kit (Elabsicence, USA) according to the manufacturer's protocol. Briefly, 1 mL of buffer solution was added to each control tube and a mixture of sample 0.1 mL of haemolymph and 1 mL of buffer solution was added to each sample tube. All tubes were incubated at 37°C for 5 minutes. Next, 0.1 mL of substrate was added to each tube, mixed thoroughly, and incubated at 37°C for precisely 1 minute. The reaction was stopped by adding 1 mL of chromogenic solution and 0.1 mL of clarificant. After standing for 10 minutes at room temperature, absorbance was measured at 405 nm using a microplate reader (Infitek, China). The CAT activity (U/mL) was calculated as:

$$\text{CAT activity}(\text{U/mL}) = \frac{\Delta A \times 32.5}{1 \times V} \times f$$

Where, 32 5: reciprocal of the slope; 1: Reaction time; ΔA: Absolute OD ($OD_{Control} - OD_{Sample}$); V: Volume of the sample; and f: Dilution factor of the sample before the test.

GSH

The Reduced Glutathione (GSH) assay was performed using the Reduced Glutathione colorimetric assay kit (Elabsicence, USA) according to the manufacturer's protocol. Briefly, the GSH assay was by mixing each 0.7 mL sample with 0.7 mL of acid reagent and centrifuging at 4500×g for 10 minutes, after which the supernatant was collected for analysis. Separate 5 mL tubes were prepared for the assay, with 1 mL of acid reagent in the blank tube, 1 mL of 20 µmol/L GSH standard solution in the standard tube, and 1 mL of the supernatant in each sample tube. Each tube was then supplemented with 1.25 mL of phosphate application solution, 0.25 mL of DTNB solution, and 0.05 mL of salt reagent, mixed thoroughly, and incubated at room temperature for 15 minutes and measured at 420 nm using a microplate reader (Infitek, China). The GSH content in the samples was subsequently calculated based on the optical density (OD) values obtained from the blank, standard, and sample tubes, following the established quantification formula.

$$\text{GSH content (mgGSH/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times M \times 2 \times f$$

Where, ΔA_1: $OD_{sample} - OD_{blank}$); ΔA_2: $OD_{standard} - OD_{blank}$); c: Concentration of standard, 20 $\times 10^{-3}$ mmol/L; M: Molecular weight of GSH, 307; 2: Dilution factor of sample pretreatment, 2 times; f: Dilution factor of the sample before the test.

SOD

The Total Superoxide Dismutase (T-SOD) assay was performed using the Total Superoxide Dismutase (T-SOD) activity assay kit (Elabsicence, USA) according to the manufacturer's protocol. The T-SOD assay was conducted by first preparing sample and control tubes, each containing 1 mL of buffer working solution with either the sample or double distilled water. Then, 0.1 mL of nitrosogenic agent, 0.1 mL of substrate solution, and 0.1 mL of enzyme stock working solution were sequentially added into each tube. The tubes were then vortexed thoroughly and incubated at 37°C for 40 minutes. Following incubation, 2 mL of chromogenic agent was added to each tube, mixed thoroughly, and left to stand at room temperature for 10 minutes. Absorbance was subsequently measured at 550 nm using a microplate reader (Infitek, China). SOD activity was calculated from the optical density values of the sample and control tubes, based on the inhibition percentage formula:

$$\text{T} - \text{SOD activity (U/mL)} = i \times 50\% \times \frac{V_1}{V_2} \times f$$

Where, i: inhibition ratio, Inhibition ratio = ($OD_{Control} - OD_{Sample}/OD_{Control}$)×100%; V_1: the total volume of the reaction solution; V_2: the volume of sample added; f: Dilution factor of the sample before the test.

Somatic Mutation and Recombination Test (SMART) Assay

The SMART assay was used to evaluate the genotoxicity in Drosophila flies by detecting somatic mutations and recombination events in wing cells. Drosophila larvae were exposed to different concentrations (0, 12.5, 25, and 50 mg/mL) of the *A. albida* extract supplemented into a standard diet medium. Subsequently, adult flies were collected, and wing cells were examined microscopically for single and multiple spot mutations. Mutation frequency was observed for each concentration and results were compared to controls to assess potential genotoxic effects.

Data Analysis

Data analysis was conducted using GraphPad Prism Version 9.0. Data were expressed as mean ± standard deviation (SD). One-way ANOVA assessed differences between groups, followed by Dunnett's post hoc test for pairwise comparisons. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The LC_{50} of *A. albida* extract was determined to be 128.5 mg/mL. This indicates that this concentration caused mortality in 50% of the exposed *D. melanogaster* population. Mortality rates were observed to increase progressively with higher concentrations of the extract (Figure 1A) in a dose-dependent manner. The chronic toxicity study results demonstrated a dose-dependent increase in mortality among flies exposed to *A. albida* stem extract. A substantial number of mortality was recorded while surviving flies exhibited observable toxicity symptoms such as reduced mobility and impaired flight ability. Among the treatment groups (Figure 1B), average mortality rates increased with the higher extract concentrations, with 20% mortality observed at 12.5 µg/mL, 21% at 25 µg/mL, and 30% at 50 µg/mL compared to the control group (14%).

The results of the antioxidant defense levels, as represented in Figure 2, demonstrate significant changes in CAT, GSH, and T-SOD activities in response to treatment with varying concentrations (12.5, 25, and 50 µg/mL) of *A. albida* extract. The antioxidant defense levels measured in the study revealed a dose-dependent response in CAT, GSH, and T-SOD activities across the tested concentrations of the extract. Specifically, CAT activity was significantly ($p < 0.05$) elevated at both 12.5 and 25 µg/mL concentrations, with a further substantial increase at 50 µg/mL ($p < 0.01$) compared to the control group. GSH content exhibited a significant ($p< 0.05$) increase at the 25 µg/mL concentration, while no statistically significant changes were observed at 12.5 and 50 µg/mL when

compared with the control. T-SOD activity significantly increased at 25 and 50 µg/mL, whereas no significant difference was observed at the 12.5 µg/mL concentration.

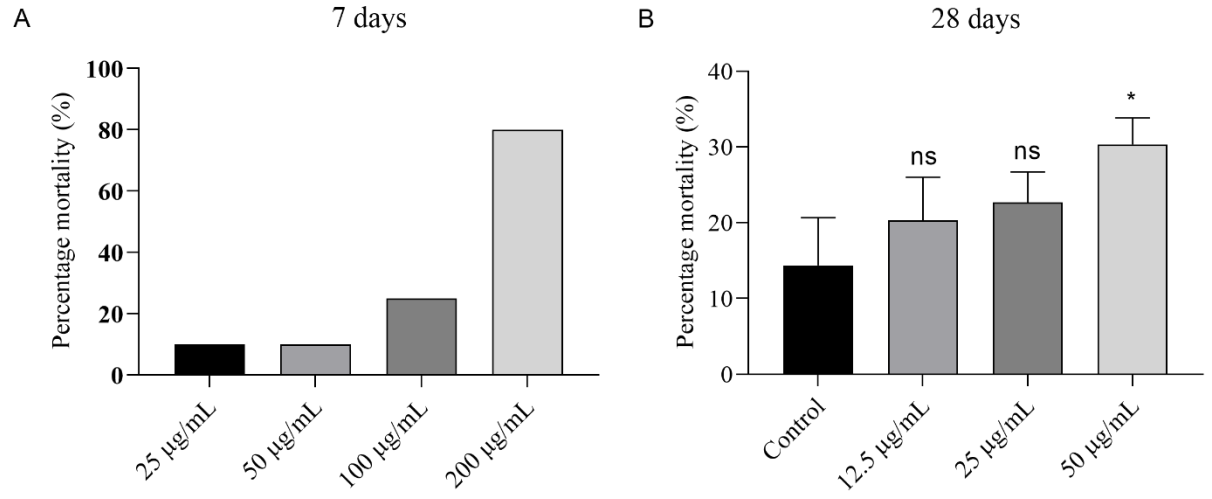

Figure 1. Toxicity effects of *A. albida* stem extract on *D. melanogaster*. (A) Dose-dependent mortality observed during the acute toxicity study over a 7 day exposure period. (B) Chronic toxicity effects showing mortality rates at over a 28 day exposure period. Data are presented as mean ± standard deviation (SD). Statistical significance relative to the control is indicated as: *p < 0.05 and ns (not significant).

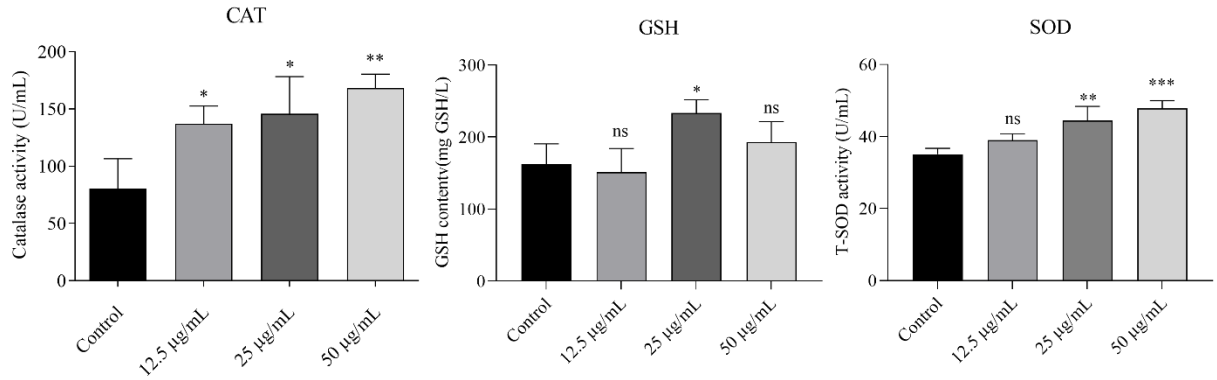

Figure 2. Effects of different concentrations of *A. albida* extract (12.5, 25, and 50 µg/mL) on antioxidant defence levels in *D. melanogaster*. (A) Catalase (CAT) activity, (B) Glutathione (GSH) content, and (C) Total Superoxide Dismutase (T-SOD) activity. Data are presented as mean ± standard deviation (SD). Statistical significance relative to the control is indicated as: *p < 0.05, **p < 0.01, ***p < 0.001, and ns (not significant).

The results of this study indicate that exposure to *A. albida* extract leads to a dose-dependent increase in the activities of antioxidant enzymes, specifically CAT and SOD. This increase demonstrates a protective response against oxidative stress, a typical manifestation of toxicity associated with aristolochic acid-containing plants. These plant constituents are known to generate reactive oxygen species (ROS), which can cause significant cellular damage, including lipid peroxidation, protein oxidation, and DNA mutations (Yu *et al*., 2011; Niemitz, 2013; Wang *et al*., 2021; Li *et al*., 2022; Liu *et al*., 2024). The observed elevation in these antioxidant enzyme activities suggests an adaptive cellular response aimed at neutralising the ROS generated by exposure to *A. albida*. The minimal increase in GSH activity may imply that the oxidative stress was not severe enough to activate a strong antioxidant response. This response is consistent with findings from previous studies, which show that exposure to aristolochic acid triggers oxidative stress pathways through GSH depletion, resulting in oxidative DNA damage (Yu *et al*., 2011).

The results of the SMART assay, as shown in Figure 3, reveal the effects of *A. albida* extract on Drosophila wing spot formation. Wing spots are reliable

indicators of genotoxicity, as they reflect genetic alterations caused by agents that induce DNA damage, mitotic recombination, or chromosomal aberrations (Pitchakarn *et al*., 2021). In Figure 2C through E, red-circled areas highlight the spot formation regions. The increased spot formation in treated groups compared to control suggests a mutagenic effect of *A. albida* constituents, as the wing spots result from somatic mutation and recombination events. The data imply that *A. albida* extract exposure elevates mutation frequency in developing Drosophila larvae, which provides new evidence of genotoxic potential from *A. albida* in Drosophila. The presence of wing spots in multiple regions of the wing in exposed groups indicates that the constituents in the plant exert mutagenic effects across different developmental stages of the flies, which requires further investigation.

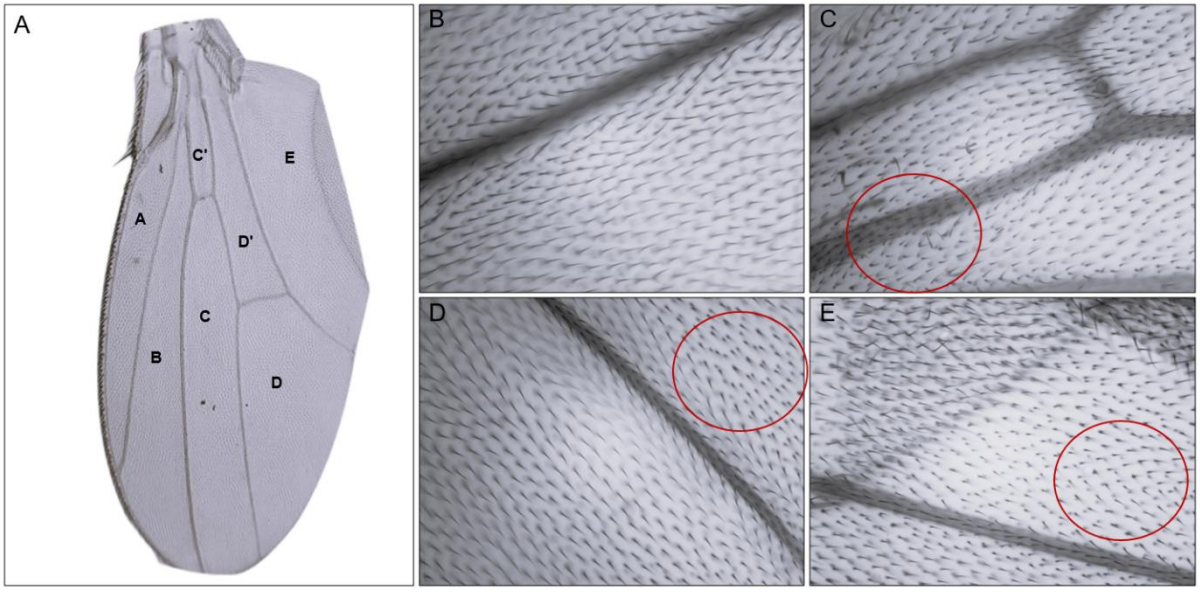

Figure 3. Representative images of Drosophila wing regions from the SMART assay showing the effects of *A. albida* extract on somatic mutation and recombination. (A) Drosophila wing with labelled regions analysed ((magnification ×40), (B–E) Magnified views of wing sections highlighting spot formations (circled in red) in different treatment groups (magnification ×400).

CONCLUSION

This study revealed the toxic effects of *A. albida* extracts on *D. melanogaster*, demonstrated by increased antioxidant enzyme activities (CAT, SOD, and GSH) and genotoxic effects observed through the SMART assay. These findings suggest oxidative stress and genotoxic effects *A. albida* extracts.

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