Research

Albendazole-induced genotoxicity in the larvae of fall armyworm as a safe environmental tool

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Abstract
Anthelmintic drugs are considered a new alternative strategy to control insect pests. *Spodoptera frugiperda,* fall armyworm (FAW), is one of the most serious pests of maize and cultivated plants. Albendazole (ABZ) is one of the safest anthelmintic drugs and is used worldwide in human and veterinary medicine. ABZ has not previously been used for controlling FAW, so the present study aimed to investigate the insecticidal and genotoxic effects of ABZ on FAW. ABZ produced a significant effect on the mortality of FAW. Also, drug treatment significantly disrupted the larval, pupal, and adult durations associated with malformations. Because ABZ interferes with microtubule formation, it could induce mortality and affect all physiological processes in FAW. Additionally, it can disrupt the chromosomes' alignment and result in the formation of micronuclei (MN), DNA damage, and cell death, thus causing ABZ-induced genotoxic effects on FAW larval hemocytes. The present study shows that the ABZ drug has a strong insecticidal potency and is a promising environmental safety tool to control the fall armyworm pest.

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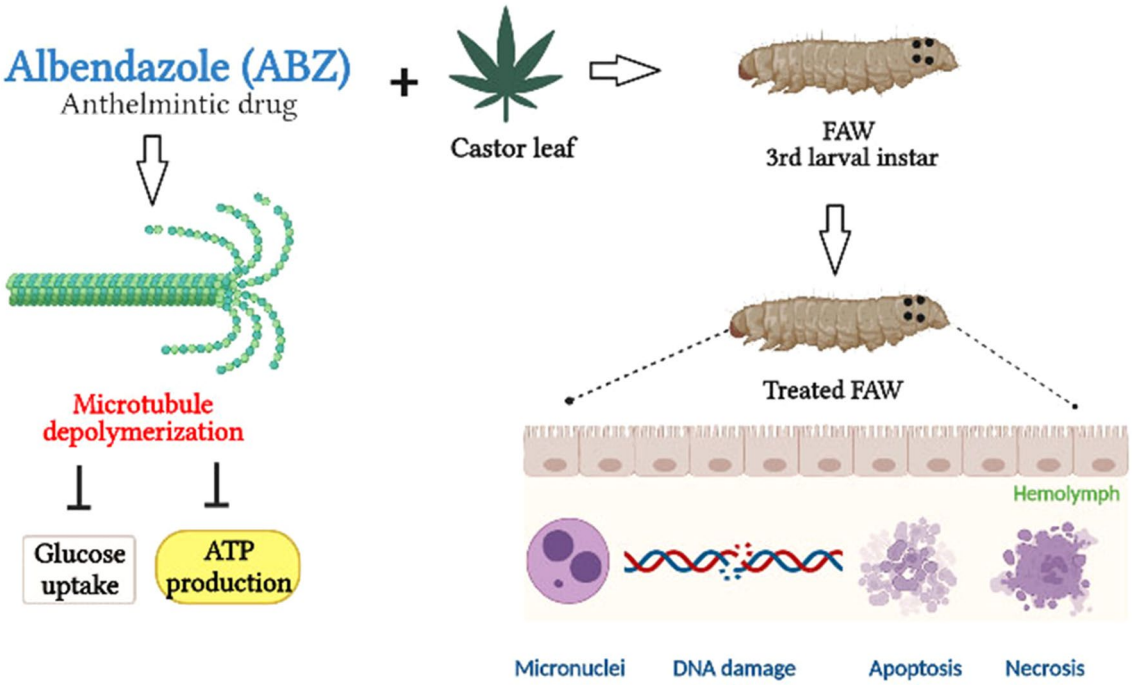
Graphical abstract



Article highlights

- ABZ interferes with the microtubule formation of organisms
- ABZ had significant insecticidal potency and induced genotoxicity in FAW larvae
- ABZ can be used to develop environmentally friendly insecticides.

Keywords *Spodoptera frugiperda* · Albendazole · Genotoxicity · Micronucleus · DNA damage · Apoptosis · Hemolymph

1 Introduction

Spodoptera frugiperda (Smith, J.E.) (Lepidoptera: Noctuidae) is a widespread destructive insect pest. It is also known as the fall armyworm (FAW). Rice, soybeans, beans, sugar cane, cotton, and maize are just a few of the crops that suffer significant economic losses as the FAW larvae's ability to consume at least 353 distinct plant species from 76 different botanical families [1]. Currently, FAW is the most significant maize pest in the world, and its global danger to agricultural production leads to a substantial negative influence on food security [2, 3]. The larvae primarily fed on the whorl of the plant, causing poor growth, development, and enormous losses, which led to vigorous economic damage [4]. Interestingly, due to its high fecundity rate, long-distance migratory behavior, polyphagous larvae with continual generations throughout the year, and ability to adapt to different climate conditions, FAW was recently recorded as an "A1 quarantine pest" [5, 6]. As FAW has been firstly invaded the African continent in 2016 and quickly expanded there [7]. According to the Agricultural Pesticide Committee (APC), Ministry of Agriculture in Egypt, *S. frugiperda* was found on maize plants in the Aswan governorate, Upper Egypt, in 2019 [8], and by 2021, it had quickly invaded and spread to nearly all of Egypt's governorates [9]. Polyphagous FAW could widen their infestation range and annual generation rate as a result of warmer temperatures and climate change worldwide [10].

As a result of this new pest's economic importance, researchers around the world have studied several methods to control FAW. The extensive use of chemical insecticides has unintended detrimental effects on the environment, wildlife, beneficial insects, plants, and human health [11–13]. Moreover, *S. frugiperda* has a high degree of adaptability and is widely known for developing a strong resistance to synthetic pesticides [14]. Several research investigations have

focused on the development of eco-friendly control strategies for managing *S. frugiperda*, as a crucial component of an integrated pest management approach [15].

One strategy that shows promise as a tool for controlling pests is the use of medications and drugs, particularly anthelmintic drugs. For instance, the anthelmintic abamectin has proved effective in controlling *Cydia pomonella* [16]. Rafoxanide and levamisole are environmentally safe drugs for controlling *Spodoptera littoralis* because of their potent larvicidal properties [17]. Oxyclozanide had significant effects on the survival, development, and adult longevity of *Galleria mellonella* [18]. In addition, benzimidazoles as broad-spectrum anthelmintic drugs are widely applied in human, veterinary medicine, and pesticides [19]. Mebendazole and triclabendazole, two anthelmintics in the benzimidazole group, have been shown to have negative effects on *G. mellonella*'s biology and physiology [20, 21].

Albendazole (ABZ), methyl(5-propylthio)-1H-benzimidazol-2-yl)-carbamate, was developed as an affordable drug and is one of the safest benzimidazole derivatives now utilized in clinics to treat a range of internal parasitic worms (helminths) [22]. Also, ABZ might be effective against head lice [23], and it is well known for its potency and low hazard in both humans and agricultural animals [24]. The eukaryotic cytoskeletal protein, β-tubulin appears to be ABZ's principal target, as the interaction of ABZ prevents tubulin from polymerizing into microtubules [25]. When ABZ inhibits the formation of microtubules, it can disrupt the chromosomes' alignment during mitosis and result in the formation of micronuclei (MN) and DNA damage [26]. Moreover, ABZ causes chromosomal aberrations and ultimately causes mitotic arrest, apoptosis, and necrosis [27]. However, albendazole's anthelmintic effectiveness for the control of fall armyworms has not yet been evaluated.

In order to find alternative novel methods as efficient integrated pest management strategies against FAW, the present study's objective is to evaluate the effect of the anthelmintic drug ABZ on the developmental biological parameters of the third larval instars. The genotoxicity induced by ABZ in *S. frugiperda* hemocytes was also investigated, with a focus on some parameters such as the micronuclei assay, single-cell gel electrophoresis, and annexin assay to estimate the extent of DNA damage and apoptosis in the hemolymph of FAW larvae following albendazole treatment.

2 Materials and methods

2.1 Insect rearing

Fall armyworm larvae were collected from maize fields near West Nubariyah, El Beheria governorate, 5,713,104, Egypt. The insect was identified and confirmed through morphological characters at Plant Protection Research Institute, Agricultural Research Center (ARC), Egypt. FAW is a polyphagous insect that feeds on a variety of plants, including maize and castor (*Ricinus communis*). Since maize plants are not available throughout the year, castor leaves, on the other hand, are readily available and are now widely utilized for FAW rearing, feeding, and oviposition preference [28, 29]. The larvae were reared in plastic containers (25 cm length, 15 cm width, and 12 cm height) with fine muslin and provided daily with fresh castor leaves as food at the Faculty of Education laboratory. Larvae were kept individually and separately from the 3rd instar onwards to overcome cannibalism until pupation [30]. Pupae were observed and collected daily. On emergence, adults were served with a 10% sugar solution and fresh castor oil leaves for egg deposition inside the mating jars (20 cm in height and 15 cm in diameter). Egg masses were collected daily and kept in separate containers. The fall armyworm culture was reared for five generations before being used in an experiment to obtain a strong culture without any chemical contamination. The insects were kept under controlled laboratory conditions to ensure progressive culture continuity (temperature of 27 ± 1 °C, relative humidity of 70 ± 5%, and photoperiod L12:D12).

2.2 Drug concentration and biological experiment

The anthelmintic drug albendazole was obtained from Pharma Swede Company (10th of Ramadan City Industrial Zone B3, Egypt). Each 1 mL contains 25 mg of ABZ. One concentration was prepared by diluting 1 mL of the drug with 1 mL of distilled water [31]. The leaf dip method was used to perform the experiment [32]. The fresh and equal-sized castor oil leaves were collected from plants grown in the university yard. First, the plant leaves were cleaned under running water and allowed to air dry. Then, they were immersed in the drug concentration for 60 s and were left to air dry for 1 h under laboratory conditions and transferred to separate jars. The 3rd larval instar was used in this experiment to study the biological parameters of FAW after ABZ treatment. Fifteen larvae were fed for 24 h on treated castor oil leaves, followed by fresh leaves until pupation. A control group of another fifteen 3rd larval instars was fed on clean castor leaves. Five replicates were performed for either the control or treated groups. The study evaluated the following biological parameters: percentage of larval mortality, pupation and adult emergence, and developmental period of larvae, pupae, and adults, in addition to resulted malformations. The observations were recorded at each 24-h interval.

2.3 Hemolymph sample preparation

Hemolymph was obtained after 24 and 48 h from control and treated third larval instars of FAW using scissors to carefully cut the prolegs of survival larvae at their base, avoiding the collection of any other organs [33]. Hemolymph was taken from 10 larvae fed on either treated castor leaves with ABZ or clean castor leaves as a control group for each genotoxicity parameter. Hemolymph was collected by calibrated capillaries and put directly in vials kept at − 20 °C for further assays. Three replicates were performed for each genotoxicity parameter.

2.4 Genotoxicity assay

2.4.1 Micronucleus assay

To determine the genotoxicity of ABZ on larval hemocytes, the micronucleus test (MN) analysis was performed. The MN test was carried out with minor modifications [34]. Micronuclei preparations were obtained from hemolymph specimens of control and treated FAW larvae. The tissue was removed into a saline solution and incubated in tap water as a hypotonic treatment. In order to get a large number of mitotic metaphases, tissue samples were immersed in a colchicine solution 1 h earlier than the hypotonic treatment. The MN was scored using a LEICA DM6 M LIBS microscope at × 100 magnification. The number of cells scored was determined depending on the level of change in the MN index. The micronuclei formation score was performed in 1000 cells. For each slide, at least 1000 binucleate (BN) cells were scored for the presence or absence of MN [35].

2.4.2 Alkaline single-cell gel electrophoresis

Isolated hemolymph from control and treated groups of insects was subjected to the modified single-cell gel electrophoresis or comet assay [36]. To obtain the cells, a small piece of tissue was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately 1 mm^3 pieces while immersed in HBSS with a pair of stainless-steel scissors. After several washings with cold phosphate-buffered saline (PBS) (sodium chloride NaCl 40.0 g, potassium chloride KCl 1.0 g, and potassium dihydrogen phosphate anhydrous Na_2HPO_4 4.6 g), then washed in distilled water to make up to 51 mL (4 °C). The minced tissues were dispersed into single cells using a pipette. In brief, the protocol for electrophoresis involved embedding the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 min to denature the DNA, and electrophoresis was made under alkaline conditions (30 min) at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a fluorescence LEICA DM6 M LIBS microscope with a green filter at × 40 magnification. For each larva, about 600 cells were examined to determine the percentage of cells with DNA damage that appear like comets in *S. frugiperda* hemocytes of control and treated larvae with ABZ. The nonoverlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with a length between 1 × and 2 × the nuclear diameter; and class 3 = tail longer than 2 × the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus [37].

2.4.3 Annexin V-FITC assay

The Annexin analysis was carried out using the Annexin V-FITC assay kit (Sigma-Aldrich, Germany) according to the protocols provided to determine whether ABZ affected necrosis and apoptosis in the hemocytes of *S. frugiperdas*. Briefly, treated and control insects' hemolymph was homogenized in PBS [38–40]. Then, Annexin V-FITC (5 µL) was added to 195 µL of cell suspension binding buffer (50 mL binding buffer and 150 mL distilled water), mixed, and incubated in the dark for approximately 10 min. A total of 100 cells in three replicates were counted per slide. The cells were then washed and resuspended in 190 µL binding buffer, and finally, 10 µL propidium iodide (PI) solution was added. The cells were kept on ice and then analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA). The obtained data were analyzed using CellQuest software (Becton Dickinson).

2.5 Data analysis

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp.). Categorical data were represented as numbers and percentages. Quantitative data were expressed as the mean and standard error. Student t-test was used to compare two groups for normally distributed quantitative variables, while one way ANOVA test was used for comparing the different studied groups, followed by a Post Hoc test (Tukey) to determine the significant differences. The significance of the obtained results was judged at the 5% level.

3 Results

3.1 Biological experiment

ABZ efficacy against the 3rd larval instar of *S. frugiperda* is presented in Fig. 1. The present study has demonstrated that the ABZ drug had more potency to achieve larval mortality of more than 80% (84 ± 3.40) during the experiment, while there were no mortalities recorded in the control group (Fig. 1a). Moreover, the tested ABZ concentration significantly reduced the percentage of pupae and adults' survival when compared to the ABZ-free control leaves. The percentage of reaching the pupal and adult stages in the control group was 100%, while the percentage of pupae and adults significantly decreased to 30% and 4% in the treated group, respectively ($p < 0.001$).

Furthermore, the mean longevity of larvae and adults was (9 ± 0.32) and (8 ± 0.37) days in the control group, while it significantly declined (4 ± 0.45) and (4 ± 0.37) days

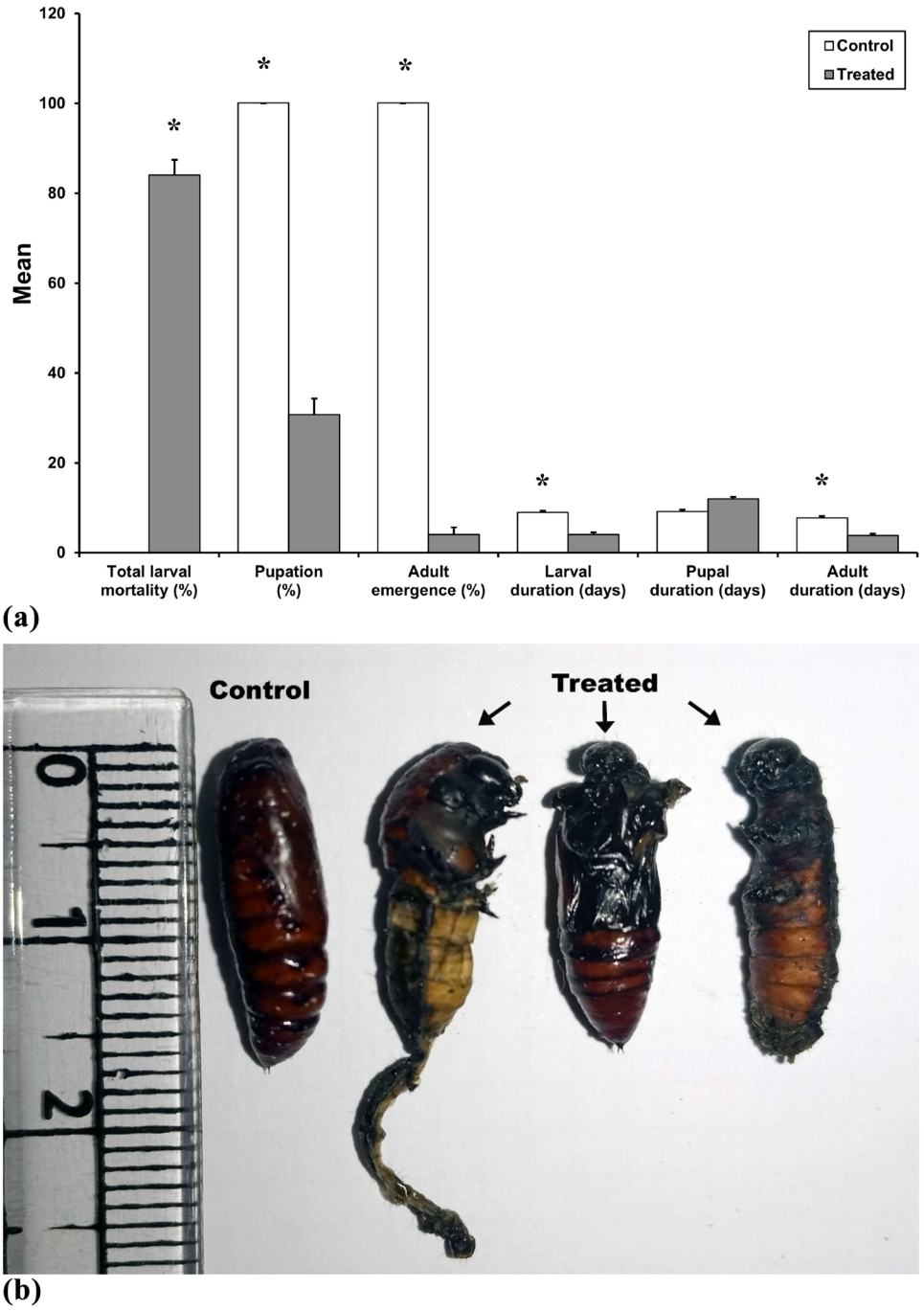
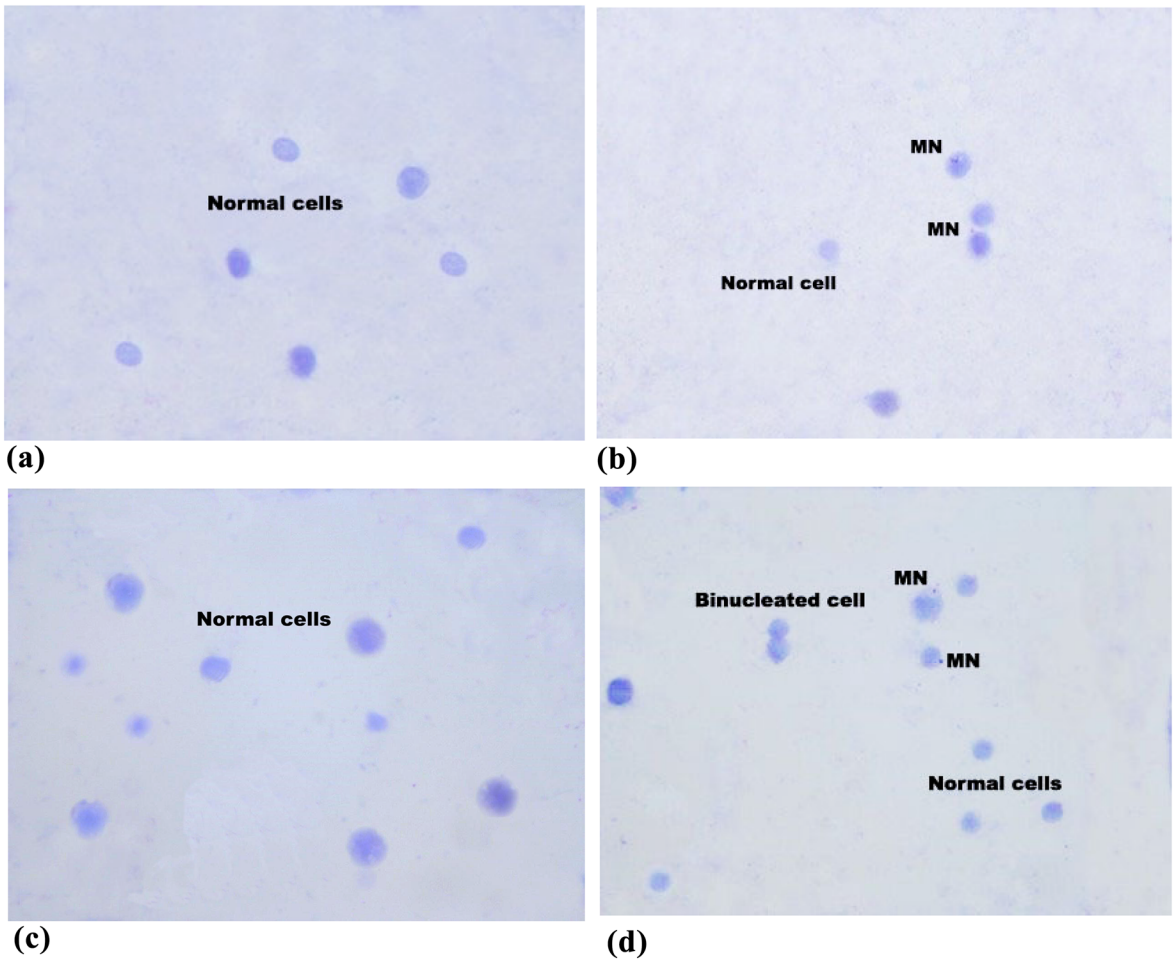
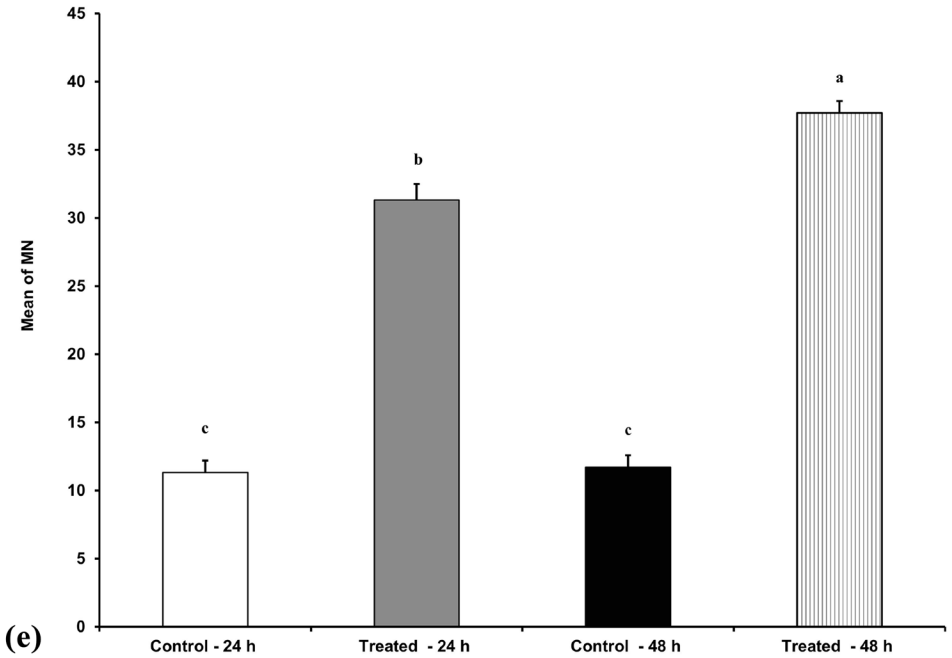


Fig. 1 The lethal effect of albendazole drug on 3rd larval instar of *Spodoptera frugiperda*. **a** Mean percentages (± SE) of larval mortality, pupation, adult emergence, duration of larvae, pupae, and adults after drug treatment. **b** Representative image of *S. frugiperda* showing control pupa versus different abnormalities. *SE* standard error of mean; *Statistically significant at $p \leq 0.001$ level


Normal cells
(a)
MN
MN
Normal cell
(b)
Normal cells
(c)
Binucleated cell
MN
MN
Normal cells
(d)


Mean of MN
45
40
35
30
25
20
15
10
5
0
c
b
c
a
Control - 24 h
Treated - 24 h
Control - 48 h
Treated - 48 h
(e)

◂**Fig. 2** Effect of albendazole drug on micronuclei formation (MN) in hemocytes of third larval instars of *S. frugiperda* at two-time intervals (24 and 48 h). **a** and **c** The normal hemocytes after 24 and 48 h, respectively. **b** and **d** The incidence of micronuclei and binucleated cells in FAW hemocytes fed castor leaves treated by albendazole drug after 24 and 48 h, respectively, Magnification: 100X. **e** Mean ± SE. of micronuclei formation in isolated hemocytes of FAW. Means followed by the different letters of alphabets are significantly different according to Tukey's test at $p \leq 0.001$ level

in the treated group, respectively. In contrast, prolongation of pupal duration was evident in the group fed on drug-treated leaves (12 ± 0.45) days when compared to (9 ± 0.37) days in the control group.

Noticeably, the ABZ drug is effective in causing several abnormalities in *S. frugiperda* bodies, as shown in Fig. 1b**,** compared with the control one. The incompleteness of pupae with part of the last larval skin remaining on their bodies was recorded, as well as abnormally blackened bodies.

3.2 Micronuclei assay

The incidence of micronuclei in hemocytes of *S. frugiperda* due to the ABZ effect is demonstrated in Fig. 2a–e. After 24 and 48 h, hemocytes that showed intact nuclei were of control FAW (Fig. 2a, c), whereas the treated group showed visible micronuclei (Fig. 2b, d) and binucleated cells (Fig. 2d). The mean values of micronucleated cells among the treated group were highly significant, being (31.3 ± 1.2) after 24 h compared with (11.3 ± 0.88) in the control group. The obtained data also revealed that at 48 h of ABZ treatment, the formation of micronuclei significantly increased (37.7 ± 0.88) when compared to the control group (11.7 ± 0.88) ($p < 0.001$) (Fig. 2e).

3.3 Alkaline single-cell gel electrophoresis

The images for the Comet assay also revealed a detrimental effect of ABZ and a crucial impairment of hemocyte nuclei (appeared as a comet) of *S. frugiperda* in comparison with the cells of control larvae at two-time intervals (24 and 48 h) **(**Fig. 3a–d). The DNA-damaged cells were classified into four classes from 0 to 3 according to the length of the DNA tails, where class 0 represents intact DNA. Classes 1 and 2 specify the different disruption levels of DNA in the investigated cells, and class 3 indicates the extensive damage of DNA in the nuclei of cells.

From the data in Fig. 3e, the mean value of nuclei without evidence of DNA damage (class 0) in FAW from the control group was (559 ± 2.1) and recorded (464.7 ± 2.6) in the treated group after 24 h and significantly declined to (444.3 ± 3.4) at 48 h. Additionally, the present study showed that in the control group, cells that belonged to class 1 were (34.7 ± 2.3) and (36 ± 1) after 24 and 48 h, respectively. In contrast to the control group, the hemocytes treated with the ABZ drug at 24 h showed nuclei with significant DNA damage (class 3) (54 ± 3.1), whereas the mean values of DNA damage (classes 1 and 2) were (39 ± 0.58) and (42.3 ± 1.3), respectively. Accordingly, the obtained data also revealed that at 48 h of ABZ treatment, the impairment of DNA significantly increased to (56.7 ± 1.3) in class 2 and (63 ± 2.1) in class 3 compared to (6.7 ± 1.2) and (0 ± 0) in cells of the control group, respectively.

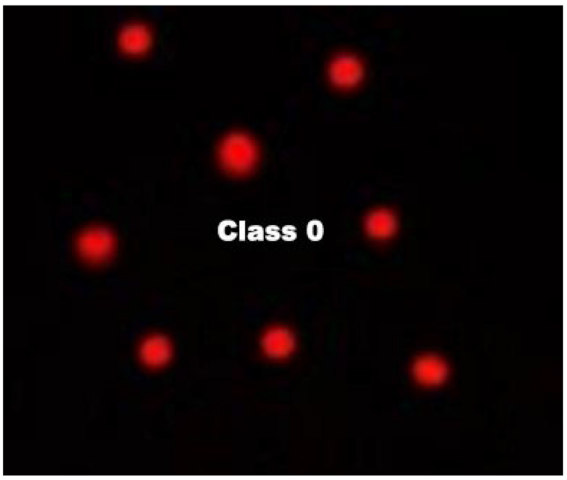
3.4 Annexin V-FITC assay

The FAW hemocytes were examined 24 and 48 h after anthelmintic drug treatment to observe whether ABZ was capable of inducing necrosis and apoptosis using flow cytometry. The lower left quadrant indicates normal cells, the upper left quadrant indicates necrotic cells, the upper right quadrant indicates early apoptotic cells, and the lower right quadrant indicates late apoptotic cells (Fig. 4a–d). Figure 4e showed that the mean value of necrotic cells significantly increased (6.8 ± 0.24), and the number of normal cells sharply decreased (64.6 ± 4) after ABZ treatment for 24 h when compared to the control group (2.1 ± 0.17) and (93.8 ± 0.52), respectively. Furthermore, at 48 h, the mean value of healthy cells significantly declined to a greater extent (58.7 ± 4.26) and no obvious change in necrotic cells was observed in the treated group (6.1 ± 1.2).

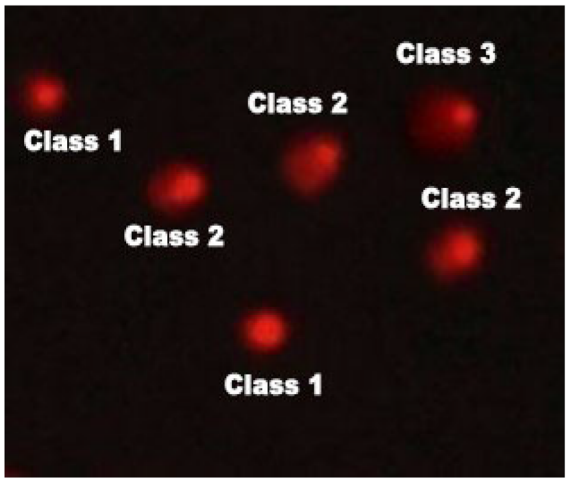
Compared with the control group, the treated group fed on the ABZ drug exhibited a significant increase in the mean value of cells gated in the upper right quadrant (early apoptotic cells) from (18.9 ± 3) after 24 h of treatment to (23.4 ± 2) after 48 h. Interestingly, ABZ caused a significant increase in the late apoptotic cells in the lower right quadrant from (9.7 ± 1.3) to (11.8 ± 1.5) after 24 and 48 h, respectively ($p < 0.001$).

4 Discussion

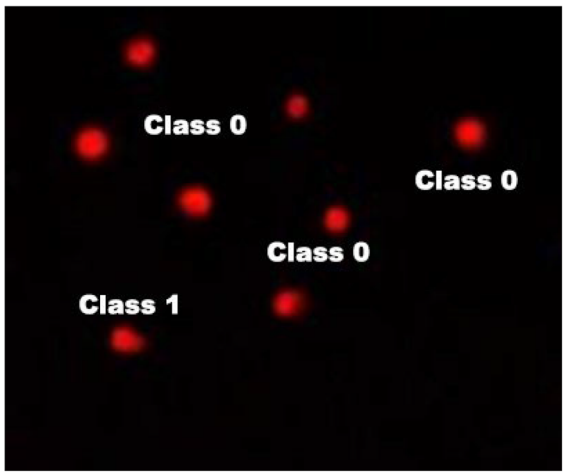
Albendazole (ABZ) is the anthelmintic drug that is most commonly used worldwide, but the current study found that the treatment also had a significant and very harmful effect on *S. frugiperda* third larval instars. A wide range of anthelminthic effects are displayed by ABZ, which interferes with microtubule formation and changes the dynamics of the protein [41]. The cytoskeleton of an organism consists primarily of microtubules. Critical molecular processes like intracellular transport, signal transduction, and morphological maintenance are regulated by their polymerization kinetics [42]. According to the results of


Class 0

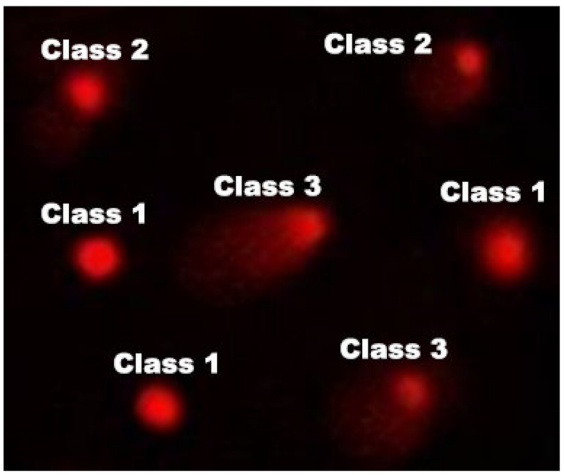
(a)


Class 3
Class 2
Class 1
Class 2
Class 2
Class 1

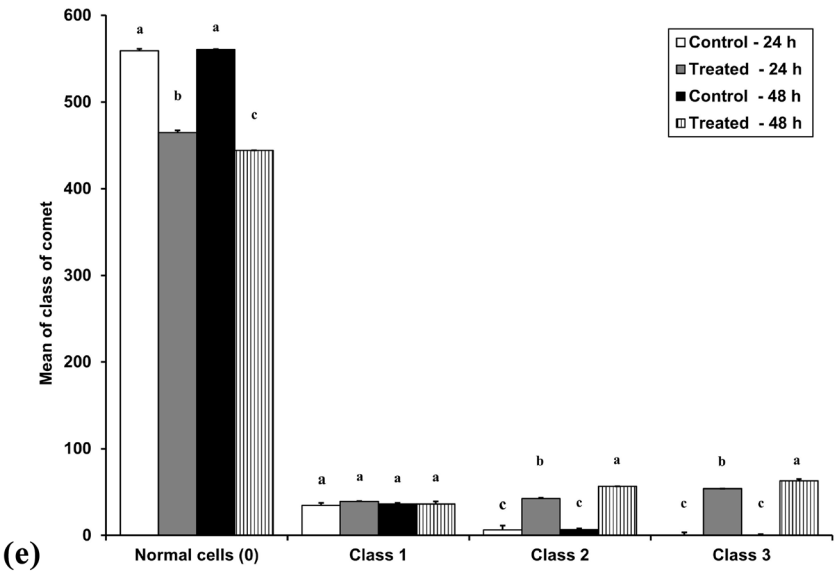
(b)


Class 0
Class 0
Class 0
Class 1

(c)


Class 2
Class 2
Class 3
Class 1
Class 1
Class 1
Class 3

(d)


Control - 24 h
Treated - 24 h
Control - 48 h
Treated - 48 h
Mean of class of comet
600
500
400
300
200
100
0
Normal cells (0)
Class 1
Class 2
Class 3

(e)

◂**Fig. 3** DNA damage induced by albendazole drug in hemocytes isolated from third larval instars of *S. frugiperda* at two-time intervals (24 and 48 h) using the alkaline Comet assay. Representative images of DNA damage from class 0 to 3 according to various degrees of DNA damage (**a–d**). Class 0 represents undamaged cells and class 3 represents the most heavily DNA damaged cells. Images **a, b** are shown control and treated larvae by albendazole for 24 h, respectively; **c**, **d** control and treated larvae for 48 h, respectively, Magnification: 40X. **e** Mean ± SE. of total DNA damage in isolated hemocytes of FAW. Means followed by the different letters of alphabets are significantly different according to Tukey's test at $p \leq 0.001$ level. Class 0 = no tail; Class 1 = tail length < diameter of nucleus; Class 2 = tail length between 1 and 2X the diameter of nucleus; and Class 3 = tail length > 2X the diameter of nucleus

this study, ABZ caused considerably greater mortality rates for FAW larvae, pupae, and adults when compared to the control group (Fig. 1a). The ability of ABZ to prevent insects from forming functional spindle cytoskeletons through microtubule polymerization has been proposed as the mechanism through which it raises death rates. The findings are consistent with the effect of cypermethrin that induced significant larval mortality percentages of the maize earworm, *Helicoverpa zea* (Boddie), (Lepidoptera: Noctuidae) [43]. Furthermore, larvae were unable to obtain the glucose and ATP required for energy to complete their healthy life cycle because ABZ inhibits the uptake of glucose into tissue and prevents the manufacture of ATP, resulting in the depletion of glycogen, inviability, and failure to develop and reproduce [44]. It is reasonable to expect that ABZ has detrimental effects on the biology of FAW larvae, pupae, and adults, which may also disrupt normal developmental periods. The addition of antibacterial compounds causes developmental retardation and short longevity in certain lepidopteran insects [45], that supporting our observation.

ABZ's capacity to destroy the organism's micro-cytoplasmic tubes decreases the production of ATP energy molecules through the uncoupling of oxidative phosphorylation [46]. This process may potentially be associated with the adverse side effects of ABZ that were seen in insects as morphological abnormalities (larval-pupal deformities) (Fig. 1b). Similar results were reported that broflanilide can cause *S. frugiperda* deformations [47].

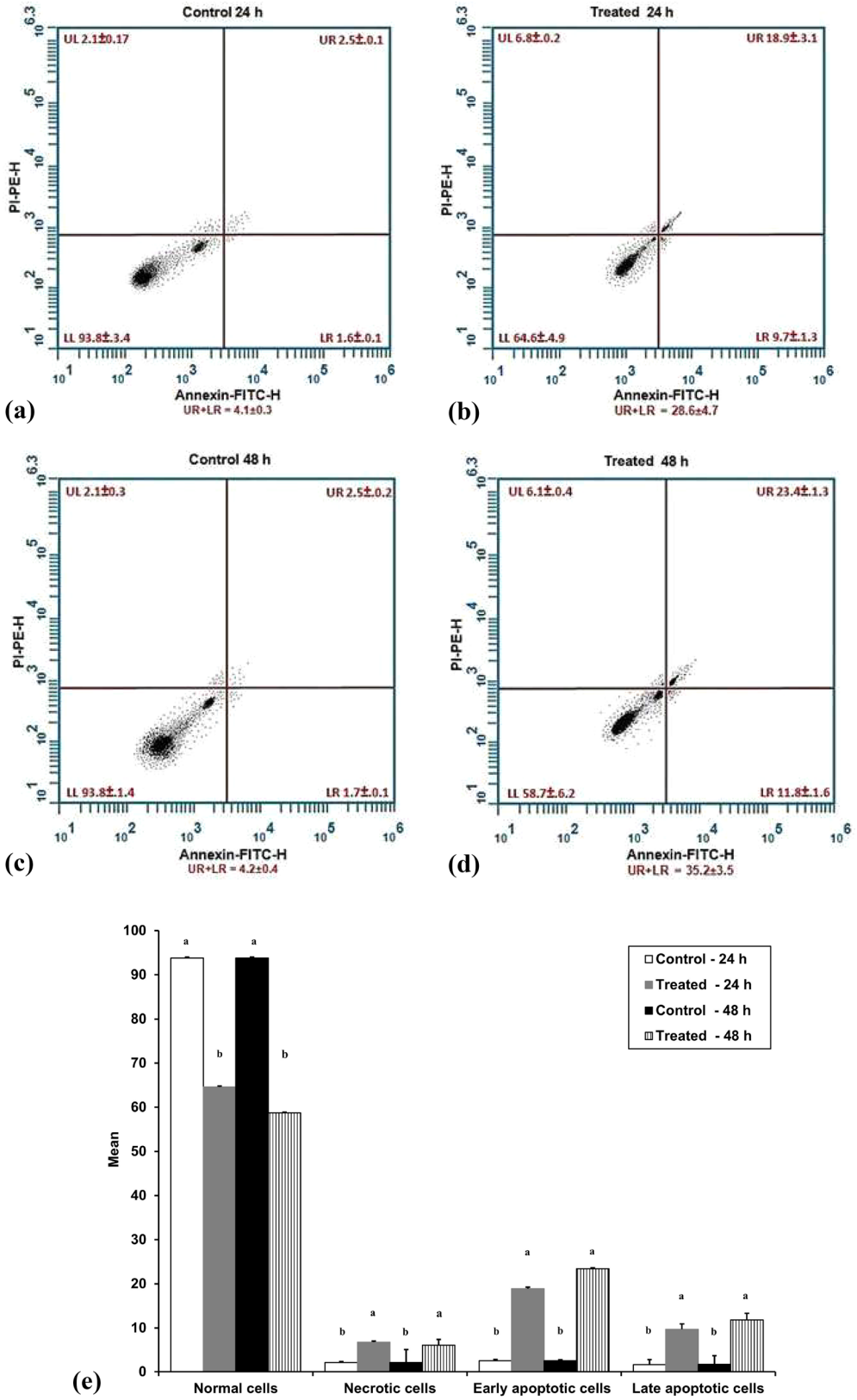
As demonstrated in the current study, feeding with ABZ also had a significant influence on cell proliferation and DNA. The insecticidal action of ABZ as mediated by genotoxic effects in *S. frugiperda* hemocytes is described here for the first time. Since hemocytes are known to be the primary source of energy for insects and are involved in the body's compensating mechanisms during the developmental stage, any genotoxic impairment of hemocytes could have a detrimental effect on *S. frugiperda*'s ability to grow and develop. It's interesting to note that ABZ functions as an agonist by binding to β-tubulin, a component of the cytoskeleton, which results in the absence or improper attachment of spindle microtubules [48], leading to the formation of micronuclei (MN) and the proper distribution of sister chromatids during cell division, as well as cell cycle arrest and ultimately cell death.

In this study, the difference in mean MN formation values between the control group and after albendazole treatment in 3rd larval instar hemocytes of *S. frugiperda* at two-time intervals (24 and 48 h) was statistically significant ($p < 0.001$) (Fig. 2). The MN assay is a sensitive biomarker for genotoxicity screening of applications that are promising in biology and medicine because MNs are tiny extranuclear bodies originating from acentric chromatid/chromosome fragments or whole chromatids/chromosomes that occur at the anaphase of dividing cells and are not present in the main nucleus during telophase [49]. The ABZ drug's potent induction of chromosomal missegregation and its detrimental effect on cell cycle progression may be the cause of the increase in MN formation [50]. Additionally, when ABZ binds to β-tubulin, it disrupts the polymerization of microtubules, impairing microtubular organization and resulting in the development of micronuclei [51]. Similar results were found that deltamethrin or boric acid, respectively, induced genotoxic damage by micronuclei formation in *G. mellonella* [52, 53]. An additional study revealed that chlorpyrifos had formed micronuclei in treated *Philosamia ricini* [54].

The formation of micronuclei is an additional sign of chromosome impairment and causes significant DNA damage that cannot be successfully repaired. Based on the fact that damaged DNA can migrate more readily in an electric field than intact DNA, the single-cell gel electrophoresis technique (comet assay) is used to evaluate DNA damage following ABZ treatment. In modern ecotoxicology, the comet assay has gained wide acceptance as a potent tool and an accurate technique for identifying even minute amounts of DNA damage [55].

In this experiment, comet appearance and classes (1–3) were significantly greater in ABZ-treated FAW hemocytes than in the control group at 24 and 48 h, suggesting that ABZ had a rapid and direct effect on DNA (Fig. 3). This might be a result of insufficient DNA repair accompanying the cellular damage caused by ABZ treatment. DNA damage has been reported [56] as the primary mechanism beyond the cytotoxic action of ABZ. Increased DNA fragmentation in the hemocytes of *G. mellonella* was also recorded when larvae were exposed to juglone [57]. A significant increase in DNA damage was documented following the treatment of *Helicoverpa armigera* larvae with a phytopesticide [58].

Significant DNA damage may be considered a trigger for necrotic and/or apoptotic cell death [59]. The findings obtained showed that the ABZ treatment group exhibited


Control 24 h
UL 2.1±0.17
UR 2.5±0.1
LL 93.8±3.4
LR 1.6±0.1
PI-PE-H
Annexin-FITC-H
UR+LR = 4.1±0.3
(a)
Treated 24 h
UL 6.8±0.2
UR 18.9±3.1
LL 64.6±4.9
LR 9.7±1.3
PI-PE-H
Annexin-FITC-H
UR+LR = 28.6±4.7
(b)
Control 48 h
UL 2.1±0.3
UR 2.5±0.2
LL 93.8±1.4
LR 1.7±0.1
PI-PE-H
Annexin-FITC-H
UR+LR = 4.2±0.4
(c)
Treated 48 h
UL 6.1±0.4
UR 23.4±1.3
LL 58.7±6.2
LR 11.8±1.6
PI-PE-H
Annexin-FITC-H
UR+LR = 35.2±3.5
(d)
Control - 24 h
Treated - 24 h
Control - 48 h
Treated - 48 h
Mean
Normal cells
Necrotic cells
Early apoptotic cells
Late apoptotic cells
(e)

◂**Fig. 4** Evaluation of cells viability obtained from hemocytes of third larval instars of *S. frugiperda* after albendazole drug treatment by flow cytometry analysis of annexin-V-FITC. **a**, **c** The cells from the control group at two-time intervals (24 and 48 h), respectively; **b**, **d** the cells from treated insects with albendazole for 24 and 48 h, respectively. **e** Mean ± SE. of normal, necrotic, and apoptotic cells (early and late) in hemocytes of FAW. Means followed by the different letters of alphabets are significantly different according to Tukey's test at $p \leq 0.001$ level. UL) The upper left quadrant corresponds to (PI + /Annexin V −) represents necrotic cells; (LL) the left lower quadrant corresponds to (PI − /Annexin V −) represents healthy cells; (UR) the upper right quadrant corresponds to (PI + /Annexin V +) represents early apoptotic cells and (LR) the lower right quadrant corresponds to (PI − /Annexin V +) represents late apoptotic cells

a significant decrease in viable cells and an increase in early and late apoptotic cells (Fig. 4). According to the findings of the present study, in addition to DNA damage in FAW larval hemocytes, ABZ also induced apoptosis and necrosis. It was found that ABZ interfered with microtubule development and activity, causing mitotic arrest and, eventually, cell death, as indicated by the positive staining of cells with annexin V PI. Annexin V can be considered an indication of apoptosis as it is a recombinant phosphatidyl serine-binding protein that binds completely and precisely with phosphatidyl serine residues of dead cells, which occurs in later stages of apoptosis or necrosis [60].

While ABZ induces DNA damage, it also prevents DNA damage from being repaired, which ultimately results in dysregulation of the cell cycle. In order to remove the damaged cell and maintain the body's health, the cell will therefore initiate the programmed cell death mechanism. Apoptosis and necrosis are the two main forms of cell death [61]. Furthermore, apoptosis and cytoskeletal component disintegration are associated [62]. The disruption and suppression of normal microtubule polymerization caused by ABZ is thought to be a major factor in the drug's ability to delay mitosis and induce apoptosis [63–65]. Enhanced apoptosis is a process that directly aims to preserve homeostasis [66] and indirectly shows that cells exposed to the ABZ drug exhibit greater abnormalities. Some natural products used against FAW can promote cell death by causing apoptosis and necrosis [67]. The pyrethrum extract can also cause DNA damage and induce apoptosis in the hemocytes of *G. mellonella* [68], that supporting our observation.

5 Conclusion

The present study provides further insight into using anthelmintic drugs as alternative strategies to control pests and replace chemical insecticides. To the best of our knowledge, ABZ has not previously been used for the control of fall armyworms. The data presented herein indicate that ABZ had insecticidal potential, as it drastically affected the survival of this pest. ABZ significantly had genotoxic effects on the hemocytes of *S. frugiperda*. The promising results in the laboratory encourage applying ABZ in the field to control FAW, though further studies aim to evaluate the potential effects of ABZ in natural environments.

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Data availability The datasets utilized and analyzed during this investigation are available upon reasonable request from the corresponding author.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval The research on insects was carried out according to the animal protection guidelines approved by the university authorities. All methods were performed in accordance with the relevant guidelines and regulations of Institutional Animal Care and Use Committee (IACUC), Faculty of Medicine, Alexandria University and Research Ethics Review Committee at Faculty of Education, Alexandria University, Alexandria, Egypt.

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