Toxic effects of sodium dodecyl sulfate on planarian *Dugesia japonica*

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Sodium dodecyl sulfate (SDS) is an anionic surfactant, which is widely used in various fields in human life. However, SDS discharged into the water environment has a certain impact on aquatic organisms. In this study, planarian *Dugesia japonica* (*D. japonica*) was used to identify the toxic effects of SDS. A series of SDS solutions with different concentrations were used to treat planarians for the acute toxicity test , and the results showed that the semi-lethal concentration (LC_{50}) of SDS to *D. japonica* at 24 h, 48 h, 72 h, and 96 h were 4.29 mg/L, 3.76 mg/L, 3.45 mg/L, and 3.20 mg/L respectively. After the planarians were exposed to 0.5 mg/L and 1.0 mg/L SDS solutions for 1, 3, and 5 days, the activities of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) content were measured to detect the oxidative stress and lipid peroxidation in planarians. Random amplified polymorphic DNA (RAPD) analysis was performed to detect the genotoxicity caused by SDS to planarians. The results showed that the activities of SOD, CAT, and MDA content increased after the treatment, indicating that SDS induced oxidative stress in planarians. RAPD analysis showed that the genomic template stability (GTS) values of planarians treated by 0.5 mg/L and 1.0 mg/L SDS for 1, 3, and 5 days were 67.86%, 64.29%, 58.93%, and 64.29%, 60.71%, 48.21%, respectively. GTS values decreased with the increasing of SDS concentration and exposure time, indicating that SDS had genotoxicity to planarians in a time and dose-related manner. Fluorescent quantitative PCR (qPCR) was used to investigate the effects of SDS on gene expression of planarians. After the planarians were exposed to 1.0 mg/L SDS solution for 1, 3, and 5 days, the expression of *caspase3* was upregulated, and that of *piwiA*, *piwiB*, *PCNA*, *cyclinB*, and *RAD51* were downregulated. These results suggested that SDS might induce apoptosis, affect cell proliferation, differentiation, and DNA repair ability of planarian cells and cause toxic effects on planarian *D. japonica*.

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9   **Abstract**

10   Sodium dodecyl sulfate (SDS) is an anionic surfactant, which is widely used in various

11   fields in human life. However, SDS discharged into the water environment has a certain impact

12   on aquatic organisms. In this study, planarian *Dugesia japonica* (*D. japonica*) was used to

13   identify the toxic effects of SDS. A series of SDS solutions with different concentrations were

14   used to treat planarians for the acute toxicity test, and the results showed that the semi-lethal

15   concentration (LC_{50}) of SDS to *D. japonica* at 24 h, 48 h, 72 h, and 96 h were 4.29 mg/L, 3.76

16   mg/L, 3.45 mg/L, and 3.20 mg/L respectively. After the planarians were exposed to 0.5 mg/L

17   and 1.0 mg/L SDS solutions for 1, 3, and 5 days, the activities of superoxide dismutase (SOD),

18   catalase (CAT), and malondialdehyde (MDA) content were measured to detect the oxidative

19   stress and lipid peroxidation in planarians. Random amplified polymorphic DNA (RAPD)

20   analysis was performed to detect the genotoxicity caused by SDS to planarians. The results

21   showed that the activities of SOD, CAT, and MDA content increased after the treatment,

22   indicating that SDS induced oxidative stress in planarians. RAPD analysis showed that the

23   genomic template stability (GTS) values of planarians treated by 0.5 mg/L and 1.0 mg/L SDS for

24   1, 3, and 5 days were 67.86%, 64.29%, 58.93%, and 64.29%, 60.71%, 48.21%, respectively.

25   GTS values decreased with the increasing of SDS concentration and exposure time, indicating

26   that SDS had genotoxicity to planarians in a time and dose-related manner. Fluorescent

27   quantitative PCR (qPCR) was used to investigate the effects of SDS on gene expression of

28   planarians. After the planarians were exposed to 1.0 mg/L SDS solution for 1, 3, and 5 days, the

29   expression of *caspase3* was upregulated, and that of *piwiA*, *piwiB*, *PCNA*, *cyclinB*, and *RAD51*

30   were downregulated. These results suggested that SDS might induce apoptosis, affect cell

31   proliferation, differentiation, and DNA repair ability of planarian cells and cause toxic effects on

32   planarian *D. japonica*.

33   **Introduction**

34   Surfactants are a kind of amphipathic compounds, which are widely used in our everyday

35   life. Their global use is increasing every year, with consumption expected to reach \$28.8 billion

36   by 2023 (Brycki et al. 2017; Kaczerewska et al. 2020). Sodium dodecyl sulfate (SDS) is a

37   common anionic surfactant with the chemical formula $CH_3(CH_2)_{11}OSO_3Na$, which is easily

38   soluble in water and has the ability of decontamination, emulsification, and foaming (Cao et al.

39   2020). SDS is widely used in the production of cosmetics and cleaning products such as soaps,

40   shampoos, shower gels and toothpastes (Bondi et al. 2015; Cao et al. 2020). The content of SDS

41   varies depending on the product type and manufacturer, typically ranges from 0.01% to 50% in

42   cosmetic products and 1% to 30% in cleaning products (Bondi et al. 2015). Consumers expose to

43   SDS through the use of products containing this ingredient, and abuse of products may cause

44   skin inhalation contact and skin inflammation (Bondi et al. 2015; Cao et al. 2020). SDS is also

45   used in pharmaceutical and food products, it can be used as a food or drug additive to solubilize

46   hydrophobic aromas or some types of preservatives (Cid et al. 2019). In addition, as a tissue

47   lysate and protein denaturant, SDS can form complex with protein through hydrophobic

48   interaction, so it is often used in Western blot, Chromatin Immunoprecipitation, SDS-PAGE, and

49   other experiments in the field of biological research (Al-Tubuly 2000; Brunelle & Green 2014;

50   Lai et al. 2017).

51   Although most surfactants are degradable, their continuous use and excessive emissions

52   have caused pollution to the water environment(Bhattacharya et al. 2022; Lechuga et al. 2016;

53   Mustapha & Bawa-Allah 2020; Rosety-Rodríguez et al. 2002). The discharge of cleaning

54   products containing SDS into the water environment through domestic wastewater had toxic

55   effects on aquatic organisms (Bondi et al. 2015; Cruz de Carvalho et al. 2022; Jönander et al.

56   2022; Messina et al. 2014). SDS in the water entered the fish body through gills, skin, or

57   intestinal epithelial cells, circulated to various parts of the body, interrupted the normal

58   steroidogenesis process, and reduced the production of sex hormone (Moniruzzaman & Saha

59   2021; Rosety-Rodríguez et al. 2002). SDS was toxic to fish and sea urchin, the 96 hours LC_{50} of

60   SDS to *Tigriopus fulvus*, *Dicentrarchus labrax*, *Dunaliella tertiolecta*, and *Paracentrotus lividus*

61   were 7.42 mg/L, 7.34 mg/L, 4.80 mg/L, and 3.20 mg/L, respectively (Bondi et al. 2015; Mariani

62   et al. 2006).

63   The antioxidant defense system can protect organisms from oxidative damage caused by

64   external pollutants. Superoxide dismutase (SOD) and catalase (CAT) are important components

65   of the antioxidant defense system of organisms, and their activities can reflect the antioxidant

66   level of organisms (Liu et al. 2021; Zhang et al. 2022). Malondialdehyde (MDA) is one of the

67   degradation products of lipid peroxidation, it can be used as a biomarker of oxidative stress to

68   detect the degree of oxidative stress of organisms (Tsikas 2017). SDS could significantly

69   increase the activities of SOD, CAT, and MDA content in *Cirrhinus cirrhosus*, *Heteropneustes*

70   *fossilis*, and *Tubifex tubifex,* causing lipid peroxidation and oxidative stress in the organisms

71   (Bhattacharya et al. 2021; Moniruzzaman & Saha 2021). The toxic effects of SDS might be

72   related to the alteration of cellular ionic balance caused by the changes of cellular membrane

73   permeability and the induction of oxidative stress (Messina et al. 2014).

74   Planarian is a representative animal of the phylum Platyhelminthes, it is widely distributed

75   in clean waters around the world (Zhang et al. 2016a). Due to its high chemical sensitivity, many

76   chemical pollutants could cause toxic effects on planarians, resulting in the changes of

77   locomotion, regeneration, neurotransmission, and even chromosome (Lau et al. 2007; Ofoegbu et

78   al. 2016; Prá et al. 2005; Rink 2013; Simão et al. 2020; Yuan et al. 2018; Zhang et al. 2016a).

79   Planarian has become one of the indicator organisms for assessing the toxicity of environmental

80   pollutants in the field of neuropharmacology and ecotoxicology (Buttarelli et al. 2008; Hagstrom

81   et al. 2015; Prá et al. 2005; Wu & Li 2018). Therefore, we used planarian as the test animal to

82   study the toxic effects of SDS on aquatic organisms.

83   Randomly amplified polymorphic DNA (RAPD) is a technique for polymorphism analysis

84   of genomic DNA, which is often used for the detection of genetic diversity and genotoxicity

85   analysis (Pandey et al. 2018; Zare et al. 2019; Zhou et al. 2011). It is an important method for

86   detecting the genotoxicity of drugs and pollutants to planarians (Yin et al. 2022; Zhang et al.

87   2016b). However, the application of RAPD technology for detecting the genotoxicity of SDS to

88   planarians has not been reported. In this study, we analyzed the acute toxicity, oxidative stress

89   reaction and genotoxicity of SDS to planarians. The effects of SDS on the expression of genes

90   related to cell apoptosis, proliferation, differentiation, and DNA repair ability were further

91   detected by fluorescent quantitative PCR (qPCR). Our results will provide theoretical basis for

92   the research of the ecotoxicity of SDS to aquatic organisms, and provide theoretical reference for

93   the management and protection of freshwater ecosystems.

94   Materials & Methods

95   Materials.

96   SDS was purchased from Biosharp Company of China, and the purity was 99%. Total

97   protein quantitation kit, SOD, CAT, and MDA test kits were purchased from Nanjing Jiancheng

98   Company of China for determination of the activities of SOD, CAT and MDA content. The

99   E.Z.N.A.® Mollusc DNA kit was the product of Omega Bio-Tek Company for extraction of

100   genomic DNA, and 2×Taq PCR StarMix was purchased from GenStar Company. Trizol reagent

101   was purchased from Thermo Fisher Technology Co., Ltd. for extraction of RNA. Reverse

102   Transcription kit and TB Green premix Ex Taq Ⅱ (2×) were purchased from TaKaRa Company.

103   The sequences of 13 random primers and qPCR primers used in this study are shown in

104   supplementary Table S1 and Table S2.

105   Test animals.

106   Planarians used in this experiment were the asexual strain *Dugesia ZB-1*, which were

107   cultured in the laboratory in *Montjuïc* water (1.6 mmol/L NaCl, 1.0 mmol/L CaCl$_2$, 1.0 mmol/L

108   MgSO$_4$, 0.1 mmol/L MgCl$_2$, 0.1 mmol/L KCl, and 1.2 mmol/L NaHCO$_3$) in a biochemical

109 incubator (SPX-2508SH, Shanghai CIMO Medical Instrument Manufacturing Co., Ltd, China) at

110 20℃. Animals were fed with beef liver twice a week and starved for a week before the

111 experiment.

112 **Acute toxicity test.**

113 Based on reference (Li 2008) and pre-experimental results, planarians with body length of

114 about 1 cm were exposed to SDS solutions of 6 different concentrations (3.0 mg/L, 3.5 mg/L, 4.0

115 mg/L, 4.5 mg/L, 5.0 mg/L, and 6.0 mg/L), and the control group were cultured in *Montjuïc* water.

116 10 planarians in each group were treated in Petri dishes with a volume of 10 mL solution. SDS

117 solutions were renewed and the mortality of planarians were calculated at an interval of 24 h.

118 The experiment was repeated three times to prevent accidental error. In order to obtain the

119 relationship between the concentration of SDS and the mortality of planarians, regression

120 equation was obtained by plotting the logarithm of concentration and odds unit. Each time point

121 corresponded to a regression equation. Odds unit with mortality rate of 50% was taken into the

122 regression equation and the LC_{50} of 24 h, 48 h, 72 h, and 96 h were calculated (Hagstrom et al.

123 2015).

124 **Detection of antioxidant enzyme activity.**

125 In order to reflect the oxidative stress response and the oxidative damage to planarians

126 under SDS stress, the activities of antioxidant enzymes and MDA content were detected (Gao et

127 al. 2022; Wang et al. 2020). 10 planarians exposed to 0.5 mg/L and 1.0 mg/L SDS solutions for 1,

128 3, and 5 days were used to detect antioxidant enzyme activities. The planarians cultured in

129 *Montjuïc* water were as control. After the exposure, 10 planarians were put in a precooled mortar

130 and 2 mL PBS buffer was added to grind them to paste. The paste was centrifugated at 4℃,

131 10000 rpm for 10 minutes and the supernatant was used to detect the protein concentration, the

132 activities of SOD, CAT, and MDA content according to the instructions of the corresponding test

133 kits. After adding the corresponding reaction reagents provided by the kits, the absorbance

134 values were measured at the wavelength of 550 nm, 405 nm, and 532 nm, respectively. Finally,

135 the activities of SOD, CAT, and MDA content were calculated based on the absorbance values.

Genomic DNA extraction.

137 Genomic DNAs of planarians exposed to SDS at concentrations of 0.5 mg/L and 1.0 mg/L

138 for 1, 3, and 5 days were extracted according to the instructions of DNA kit. The integrity of

139 DNA was detected by 1% agarose gel electrophoresis, and the purity and concentration were

140 measured by detecting OD_{260}/OD_{280} with a micro-spectrophotometer (K5600, Beijing Kaiao

141 Technology Development Co., Ltd, China).

RAPD amplification.

143 RAPD can be applied to detect the changes in genomic DNA at the molecular level (Zhang

144 et al. 2016b), so the method was used for the genotoxicity assay in this study. Each polymerase

145 chain reaction (PCR) was conducted in a mixture of 25 μL containing 20 ng genomic DNA, 0.2

146 μmol/L primer, and 12.5 μL 2×Taq PCR StarMix. Amplifications were carried out in a DNA

147 thermocycler (TC-XP, Hangzhou Bioer Technology Co., Ltd, China).The PCR program was

148 94°C for 5 min, 40 consecutive cycles including 94°C for 1 min, 37°C for 1 min, and 72°C for 2

149 min, then followed by 72°C for 10 min as the final extension (Zhang et al. 2016b). After

150 amplification, the PCR products were analyzed by electrophoresis on 1% agarose gel at a voltage

151 of 100 V and a current of 200 mA for 60 min. Then the electropherograms were photographed

152 under an AlphaImager HP system (Alpha2200-5, Alpha Innotech, USA).

Estimate of GTS.

154 Genomic template stability (GTS) is an indicator of genotoxicity, and its value can reflect the

155 degree of genotoxicity. GTS is calculated according to the formula: $GTS(\%) = \left(1 - \frac{a}{n}\right) \times 100$,

156 where "a" represents the number of polymorphic bands detected in each treatment sample; "n"

157 represents the number of total bands in the control (Atienzar et al. 1999; Zhang et al. 2016b). The

158   GTS of the control group is set to 100%, and the GTS of each treatment group is expressed as a

159   percentage of the control group.

160   **RNA extraction and qPCR.**

161   After exposure to 1.0 mg/L SDS for 1, 3, and 5 days, the RNAs of planarians of the

162   treatment groups and the control group were extracted. Reverse transcription and qPCR were

163   conducted to study the expression level of genes (Liang et al. 2022). Total RNA was extracted by

164   Trizol reagent and was reverse transcribed into cDNA with the reverse transcription mixtures of

165   20 μL containing 2 μg RNA and 2.5 μmol/L oligo(dT)$_{15}$ as primer. The qPCR was performed in

166   a Light Cycler 480 System (Roche Diagnostics, Switzerland) with the PCR program was 95°C

167   for 30 s followed by 40 consecutive cycles consisting of 95°C for 5 s, 58°C for 10 s, and 72°C

168   for 15 s. Each group was performed in triplicate. The relative expression levels were calculated

169   using the $2^{-\Delta\Delta Ct}$ method with *Dj-Actin* gene as the endogenous standardization.

170   **Statistical analysis.**

171   Statistical analysis and regression analysis were performed using SPSS 26.0 software. The

172   odds unit with the mortality rate of 50% was taken into the regression equation to calculate the

173   LC$_{50}$ and 95% confidence interval (95% CI) of SDS to *D. japonica*. R^2 represents the coefficient

174   of determination. The activities of SOD and CAT, MDA content, and the levels of gene

175   expression were presented as mean ± SD. One-way ANOVA was used to compare the

176   differences between each treatment group and the control group. The value of $p < 0.05$ was

177   considered statistically significant, and $p < 0.01$ represents highly significant (Gao et al. 2022;

178   Liang et al. 2022).

179   **Results**

180   **Acute toxicity of SDS to planarians.**

181   After acute toxicity experiment, the regression equation, R^2, LC$_{50}$ and 95% confidence

182 interval (95% CI) of SDS to *D. japonica* were calculated. As shown in Table 1, the LC_{50} of SDS

183 to *D. japonica* at 24 h, 48 h, 72 h, and 96 h were 4.29 mg/L, 3.76 mg/L, 3.45 mg/L, and 3.20

184 mg/L respectively. Results showed that the acute toxicity of SDS to planarians increased with the

185 extension of exposure time.

186 **Effects of SDS on oxidative stress.**

187 After treatment with 0.5 mg/L and 1.0 mg/L SDS solutions for 1, 3, and 5 days, the

188 activities of SOD, CAT, and MDA content in planarians were detected. Results showed that the

189 activities of SOD, CAT, and MDA content in the treatment groups were higher than that of the

190 control group. With the extension of SDS exposure time, SOD activity in 0.5 mg/L SDS

191 treatment groups increased significantly, reached the highest on the fifth day. In the 1.0 mg/L

192 SDS treatment groups, SOD activity highly significantly increased on the first day, continued to

193 increase on the third day, and returned to the same level as the control group on the fifth day

194 (Figure 1A). The CAT activities of the 0.5 mg/L and 1.0 mg/L SDS treatment groups

195 significantly increased firstly from the first to the third day, and then decreased from the third to

196 the fifth day (Figure 1B). The MDA content in the 0.5 mg/L SDS treatment groups showed an

197 increasing trend from the first to the fifth day, while in the 1.0 mg/L SDS treatment groups, it

198 was highest on the first day, and then decreased, reaching the lowest level on the fifth day

199 (Figure 1C). These results suggested that the treatment of SDS might cause oxidative stress and

200 lipid peroxidation in planarians.

201 **Effects of SDS on RAPD profiles.**

202 The OD_{260}/OD_{280} value of each group of the planarians genomic DNA was between 1.7 and

203 2.2, and a single band was obtained by 1% agarose gel electrophoresis (Figure 2A), indicating

204 that the purity and integrity of DNA was good and no degradation. The bands of PCR products

205 amplified with the same template and the same primer were consistent (Figure 2B), indicating

206 that this technique is repeatable.

207   The amplified products of RAPD were subjected to agarose gel electrophoresis, and

208   obvious bands were obtained. A total of 56 bands were amplified from the genomic DNAs of the

209   control group with 13 random primers, and 1~9 bands were amplified with each primer (Table 2,

210   Figure 2C-F). The number of polymorphic bands in 0.5 mg/L and 1.0 mg/L SDS treatment

211   groups were 18, 20, 23, and 20, 22, 29 after 1, 3, and 5 days of exposure (Table 2), indicating

212   that the RAPD patterns of SDS treated groups were different from that of the control group, and

213   changed with the SDS concentrations and the exposure time.

214   GTS is the percentage of the number of polymorphic bands in the RAPD maps to the total

215   number of bands in the control group. The polymorphic bands amplified with each primer varied

216   with concentration of SDS (0.5 mg/L, 1.0 mg/L) and exposure time (1, 3, and 5 days). The

217   RAPD maps were analyzed and the GTS values were calculated. Results showed that the GTS

218   values of planarians exposed to 0.5 mg/L and 1.0 mg/L SDS for 1, 3, and 5 days were 67.86%,

219   64.29%, 58.93%, and 64.29%, 60.71%, 48.21%, respectively (Figure 3). GTS decreased with the

220   increase of SDS concentration and the extension of exposure time, indicating that SDS has

221   genotoxicity to planarians in a dose and time-related manner.

222   **Effects of SDS on gene expression.**

223   The changes of gene expression in planarians were detected by qPCR after treatment with

224   1.0 mg/L SDS solution for 1, 3, and 5 days. Results showed that the expression level of apoptosis

225   marker gene *Dj-caspase3* was highly significantly upregulated, reaching the highest on the fifth

226   day (Figure 4A). The expression levels of cell proliferation related gene *Dj-piwiA* and neoblast

227   differentiation related gene *Dj-piwiB* had no significant difference with the control on the first

228   day, but decreased significantly on the third and the fifth days (Figure 4B-C). The cell

229   proliferation marker gene *Dj-PCNA and* cell cycle related gene *Dj-cyclinB* significantly

230   decreased from the first day, and reached the lowest on the fifth day (Figure 4D-E). The

231   expression level of DNA damage related gene *Dj-RAD51* significantly downregulated, and

232   reached the lowest on the third day (Figure 4F). The qPCR results showed that SDS might

233   induce apoptosis, affect cell proliferation, differentiation, normal progression of cell cycle, and

234   DNA repair ability in planarians.

Discussion

236   In this study, LC_{50} of SDS to planarian *D. japonica* was determined by acute toxicity test.

237   Previous study showed that LC_{50} of SDS to planarian *D. japonica* was 0.36 mg/L, the same value

238   from 24 h to 96 h (Li 2008). In order to accurately measure the LC_{50} of SDS to *D. japonica* at

239   different times, we conducted this acute toxicity experiment and obtained the corresponding

240   values at different time points. Our results showed that LC_{50} was 3.20 ~ 4.29 mg/L from 24 h to

241   96 h, and it was getting lower with the extension of SDS exposure time. Li (2008) determined

242   LC_{50} of many surfactants to *D. japonica*, and the toxicity rank of 96 h LC_{50} was as follows: SDS >

243   CTAB > NP > LAS > Hyamine 1622 > Triton X-100 > PFOS > PFOA. SDS had different

244   toxicity to different aquatic organisms. Nunes et al. (2005) determined LC_{50} of SDS to three

245   aquatic species including the euryhaline fish *Gambusia holbrooki*, the hypersaline crustacean

246   *Artemia parthenogenetica*, and the marine algae *Tetraselmis chuii*. LC_{50} of SDS to *A.*

247   *parthenogenetica* at 48 h was 12.2 mg/L, and that to *T. chuii* and *G. Holbrooki* at 96 h were 30.2

248   mg/L and 15.1 mg/L (Nunes et al. 2005). In our study, LC_{50} of SDS to planarian *D. japonica* at

249   48 h and 96 h were 3.76 mg/L and 3.20 mg/L, indicating that the toxicity of SDS to planarian *D.*

250   *japonica* was higher than that to the above three organisms.

251   When organisms are subjected to environmental stress, oxidative stress reactions usually

252   occur and reactive oxygen species (ROS) is produced (Lushchak 2016; Yang et al. 2020). ROS is

253   a substance with active properties and strong oxidizing power, its excessive accumulation can

254   destroy the spatial structure of biological macromolecules such as DNA, proteins, and lipids,

255   causing DNA damage or cell death (Finkel 2011; Sachdev et al. 2021; Tang et al. 2019; Tsikas

256   2017). The disequilibrium between the ROS formation and the neutralization by antioxidant

257   enzymes can lead to the reactions of oxidative stress (Bhattacharya et al. 2022; Kurutas 2016).

258   SOD is the key enzyme that catalyzes the conversion of superoxide anion free radicals into

259   hydrogen peroxide, while CAT converts hydrogen peroxide into water and molecular oxygen

260   (Ding et al. 2009; Li et al. 2021). Therefore, SOD and CAT are the first line of defense for the

261   organisms against oxygen toxicity, and the increase of their activities indicates that the

262   antioxidant defense ability of organisms is enhanced (Wang et al. 2020; Zagal & Mazmanci

263   2011). MDA is one of the degradation products of lipid peroxidation, its content can be

264   measured by the reaction with thiobarbituric acid. An increase in free radicals could cause

265   overproduction of MDA (Gaweł et al. 2004). Therefore, MDA can be assessed as an oxidative

266   stress marker to detect the degree of oxidative stress of organisms (Amin et al. 2018; Tsikas

267   2017). Previous studies have shown that the changes of antioxidant enzyme activities and MDA

268   content are closely related to the reactions of oxidative stress. Some pollutants, such as

269   microplastics, imidazolyl, and copper, could induce significant changes of antioxidant enzyme

270   activities and MDA content in planarians (Gao et al. 2022; Wang et al. 2020; Zhang et al. 2016a).

271   In this study, the activities of SOD, CAT, and MDA content increased after SDS treatment,

272   indicating that SDS might cause the production of ROS, which in turn activated the

273   corresponding antioxidant enzymes and led to an increase in their activities. Due to the inability

274   of ROS to be completely cleared, it caused oxidative damage to cells, leading to lipid

275   peroxidation in planarians.

276   In addition to the changes of antioxidant enzyme activities, many compounds can cause

277   damages to the DNA of organisms. These damages include DNA strand breaks, base

278   modifications, and substitutions (Pandey et al. 2018). RAPD is a sensitive method for detecting

279   DNA damage at the molecular level, and the degree of DNA damages can be reflected by the

280   GTS values (Aksakal & Esim 2015). In this study, SDS led to the changes of GTS values of

281   planarians, which might be related to DNA damages caused by the changes of oligonucleotide

282   sites, and the breakage, insertion, or deletion of DNA fragment (Tofalo & Corsetti 2017; Zhang

283 et al. 2017; Zhang et al. 2016b). The GTS values of the treatment groups decreased with the

284 increasing of SDS concentrations and the extension of exposure time, indicating that SDS had

285 genotoxicity to planarians in a time and dose-related manner. Some similar studies regarding

286 genotoxicity of 8-hydroxyquinoline to *Misgurnus anguillicauatus*, furacilin to *Euplotes vannus*,

287 and 1,3-methylimidazole to *D. japonica* also showed that the genotoxicity caused by drugs to

288 organisms also had certain correlation with time and dose (Nan et al. 2013; Zhang et al. 2016b;

289 Zhou et al. 2011).

290   Apoptosis is a strictly controlled cell suicide characterized by nuclear condensation, cell

291 shrinkage, membrane blebbing, and DNA fragmentation (Bertheloot et al. 2021; Majtnerová &

292 Roušar 2018). Caspase3 is a member of the cysteine family and plays a vital role in the progress

293 of apoptosis (Gong et al. 2022; Lei et al. 2022). Studies have shown that aspirin could reduce the

294 level of caspase3 protein and inhibit cell apoptosis, microplastic could induce the expression of

295 *caspase3* gene and promote cell apoptosis in planarians (Gao et al. 2022; Liang et al. 2022). In

296 the present study, the expression level of *Dj-caspase3* was upregulated in the treatment groups,

297 and gradually increased with the extension of treatment time, suggesting that SDS could induce

298 apoptosis in planarians. PIWI proteins have broader functions in many vital biological processes

299 including cell proliferation, differentiation, and survival. PiwiA and piwiB are members of the

300 PIWI protein family (Kashima et al. 2020; Ponnusamy et al. 2017). It has been reported that

301 *piwiA* and *piwiB* are specifically expressed in neoblasts and encode PIWI proteins in cytoplasm

302 and nucleus respectively (Kashima et al. 2020). The main function of *PiwiA* is to maintain cell

303 proliferation, and *piwiB* is involved in the regulation of neoblasts differentiation (Reddien et al.

304 2005; Shibata et al. 2016; Tharp & Bortvin 2016). PCNA is a key factor in the process of DNA

305 replication, and the expression of *PCNA* in all organisms is related to cell proliferation and DNA

306 synthesis during genome replication in S phase of cell cycle (Orii et al. 2005; Strzalka &

307 Ziemienowicz 2011). Therefore, *Dj-piwiA*, *Dj-piwiB* and *Dj-PCNA* can be used as marker genes

308 to detect the effects of pollutants on the proliferation or differentiation of planarian cells. Here,

309   our results showed that the expression levels of *Dj-piwiA*, *Dj-piwiB* and *Dj-PCNA* were

310   downregulated in the treatment groups, especially on the third and the fifth day. We speculated

311   that with the extension of exposure time, SDS might decrease the proportion of mitotic stem cells

312   and consequently restrain the proliferation or differentiation of planarian cells. CyclinB is an

313   important regulator of cell cycle, which is responsible for the transition from G2 phase to M

314   phase in the cell cycle (van Wolfswinkel et al. 2014; Zhong et al. 2019). The inhibition of

315   proliferation in the regenerating planarians treated by aspirin might be related to the abnormal

316   cell cycle caused by the reduced expression of cyclinB (Liang et al. 2022). A similar study

317   showed that downregulation of G2/mitotic-specific cyclinB could constrain proliferation, induce

318   apoptosis, and trigger autophagy in nasopharyngeal carcinoma cells (Xie et al. 2019). In this

319   study, the expression level of *Dj-cyclinB* significantly decreased in the treatment groups,

320   indicating that SDS might interfere with cell cycle progression via downregulation of *cyclinB*,

321   and then lead to the inhibition of cell proliferation. DNA integrity is crucial for maintaining the

322   homeostasis of planarian tissues. RAD51 is an essential component in maintaining the genomic

323   stability and repairing DNA double strand break, so its encoding gene can be used as a marker

324   gene for detecting the degree of DNA damages (Barghouth et al. 2019; Bonilla et al. 2020; Prado

325   2021). In this experiment, the expression level of *Dj-RAD51* was significantly downregulated in

326   the treatment groups, suggesting that SDS could reduce the ability of DNA repair, leading to

327   gene mutation and genomic instability in planarians.

328   Conclusions

329   Taken together, SDS has acute toxicity and genotoxicity to planarian *D. japonica*. SDS of

330   0.5 mg/L and 1.0 mg/L could induce oxidative stress and genetic toxicity in planarians.1.0 mg/L

331   SDS upregulated the expression of apoptosis-related gene, downregulated the expression of

332   genes related to cell cycle, cell proliferation and DNA repair ability. These results indicate that

333   SDS has toxic effects on freshwater planarians and potential hazards to the aquatic environment.

334   Our study provides a theoretical basis for the risk assessment and management of SDS, as well

335   as for the protection of aquatic organisms.

Abbreviation index

337   SDS: Sodium dodecyl sulfate; LC_{50}: Semi-lethal concentration; SOD: Superoxide dismutase;

338   CAT: Catalase; MDA: Malondialdehyde; RAPD: Random amplified polymorphic DNA; PCR:

339   Polymerase chain reaction; GTS: Genomic template stability; ROS: Reactive oxygen species;

340   qPCR: Fluorescent quantitative PCR; *Dugesia japonica: D. japonica*.

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Semi-lethal concentration (LC$_{50}$) of SDS to *D. japonica* at different time points.

Notes: x is the logarithm of concentration, y is the odd unit; R^2 is the "coefficient of determination"; 95% CI is 95% confidence interval.

Exposure time (h)	Regression equation	R^2	LC_{50} (mg/L)	95% CI (mg/L)
24	y=11.6x-2.36	0.961	4.30	4.09 ~ 4.51
48	y=13.5x-2.76	0.941	3.76	3.61 ~ 3.93
72	y=11.5x-1.19	0.998	3.45	3.28 ~ 3.63
96	y=13.3x-1.71	0.955	3.20	3.06 ~ 3.34

Analysis and statistics of different bands in RAPD profiles of control group and treatment groups.

Notes (a) appearance of new bands; (b) disappearance of normal bands; (c) increase in band intensities; (d) decrease in band intensities; (a+b) polymorphic bands; (a+b+c+d) varied bands.

Primer	Control	0.5 mg/L												1.0 mg/L											
		1d				3d				5d				1d				3d				5d			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
S5	3	3	0	2	1	3	0	2	1	2	1	2	0	3	0	2	1	3	0	2	1	2	1	2	0
S8	2	3	0	1	0	3	0	1	0	2	0	1	1	3	0	1	0	3	0	1	0	3	0	1	0
S10	5	2	0	2	1	2	0	2	2	2	0	3	1	2	0	3	0	2	0	3	0	0	1	2	2
S15	2	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	0
S17	5	2	0	2	0	2	0	1	1	3	0	1	1	0	0	1	1	0	0	1	1	3	1	3	1
S18	5	0	1	2	0	0	1	2	0	0	1	2	0	0	2	1	0	0	1	3	0	0	1	2	1
S20	5	0	3	0	1	0	1	2	1	0	2	1	1	1	1	1	0	0	4	0	0	0	4	0	0
S64	4	0	0	1	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	3	0	1
S75	6	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	1	0	2	0	2	0	2
S78	4	0	0	1	0	0	0	3	0	0	1	2	0	0	0	4	0	0	0	2	0	0	2	0	2
S80	5	1	0	3	0	1	0	2	0	0	3	1	2	2	0	2	0	3	0	0	2	0	0	2	1
S83	4	0	0	3	0	1	1	2	0	1	0	0	3	0	1	2	0	1	1	1	0	0	1	0	3
S84	6	0	2	2	0	0	3	1	0	0	3	2	0	0	2	3	0	0	3	3	0	0	4	0	2
Total	56	11	7	19	3	13	7	19	5	10	13	17	10	13	7	21	2	12	10	17	6	8	21	12	15
a+b		18				20				23				20				22				29			
a+b+c+d		40				44				50				43				45				56			

Figure 1

The effects of SDS on oxidative stress of *D. japonica*.

(A) SOD, (B) CAT activities and (C) MDA content of the planarians exposed to 0.5 mg/L and 1.0 mg/L SDS for 1, 3, and 5 days. * $p < 0.05$; ** $p < 0.01$.
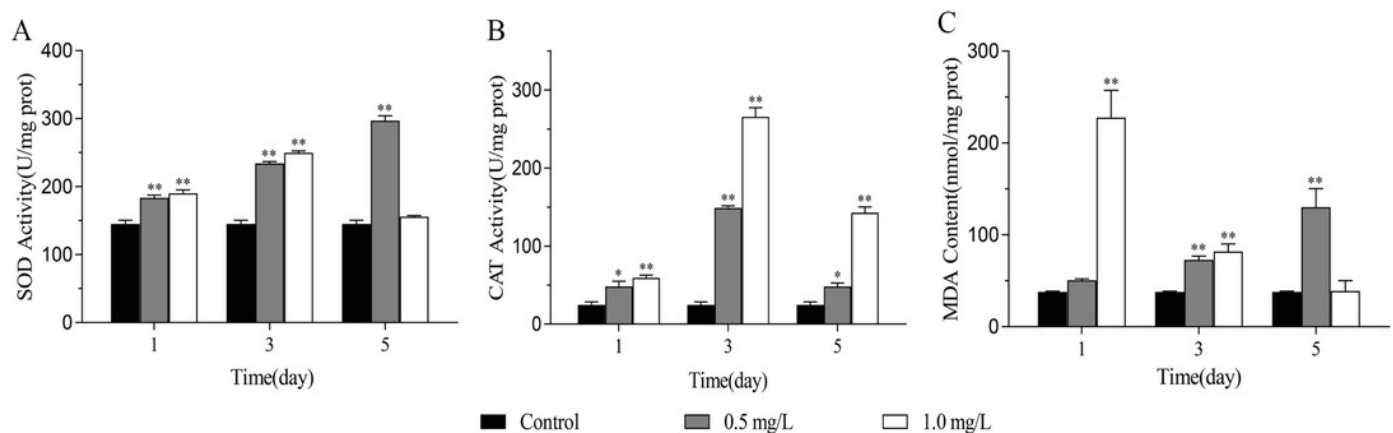
Figure 2

Genomic DNAs and RAPD profiles of planarian *D. japonica*.

(A) Genomic DNAs isolated from *D. japonica* exposed to 0.5 mg/L and 1.0 mg/L SDS for 1, 3, and 5 days. M is 1kb DNA ladder (10 000, 8 000, 6 000, 5 000, 4 000, 3 000, 2 000, 1 500, 1000, 500 bp from top to bottom). 0 is control. (B) Reproducibility of RAPD profiles generated from *D. japonica* of the control group DNAs. M is DL2000 DNA marker (2 000, 1 000, 750, 500, 250, 100 bp from top to bottom). (C)-(F) RAPD profiles of genomic DNAs from *D. japonica* exposed to SDS using primers S5, S8, S10, and S17. M is DL2000 DNA marker.
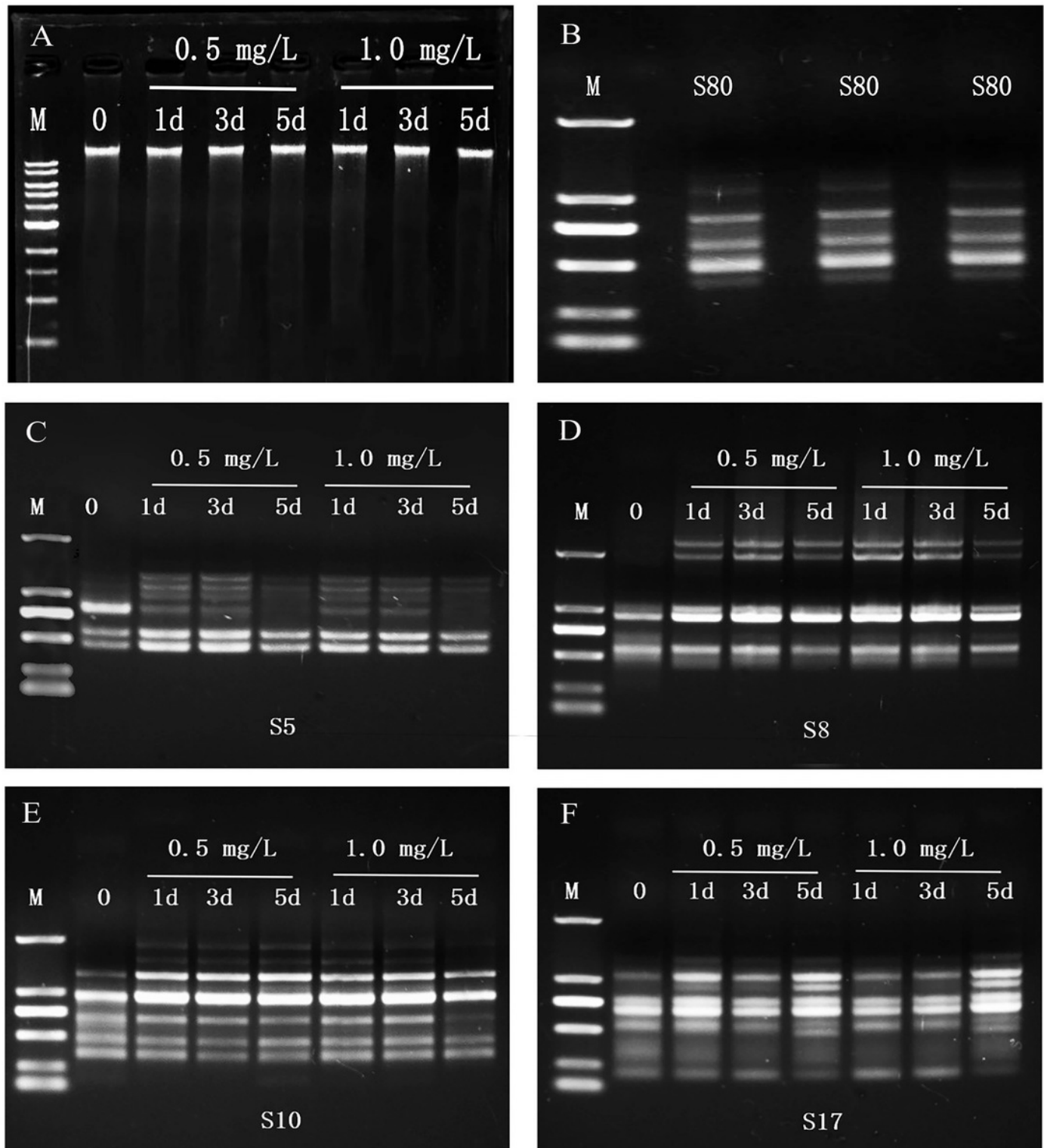
Figure 3

Genomic template stability (GTS) of *D. japonica* exposed to 0.5 mg/L and 1.0 mg/L SDS for 1, 3, and 5 days.
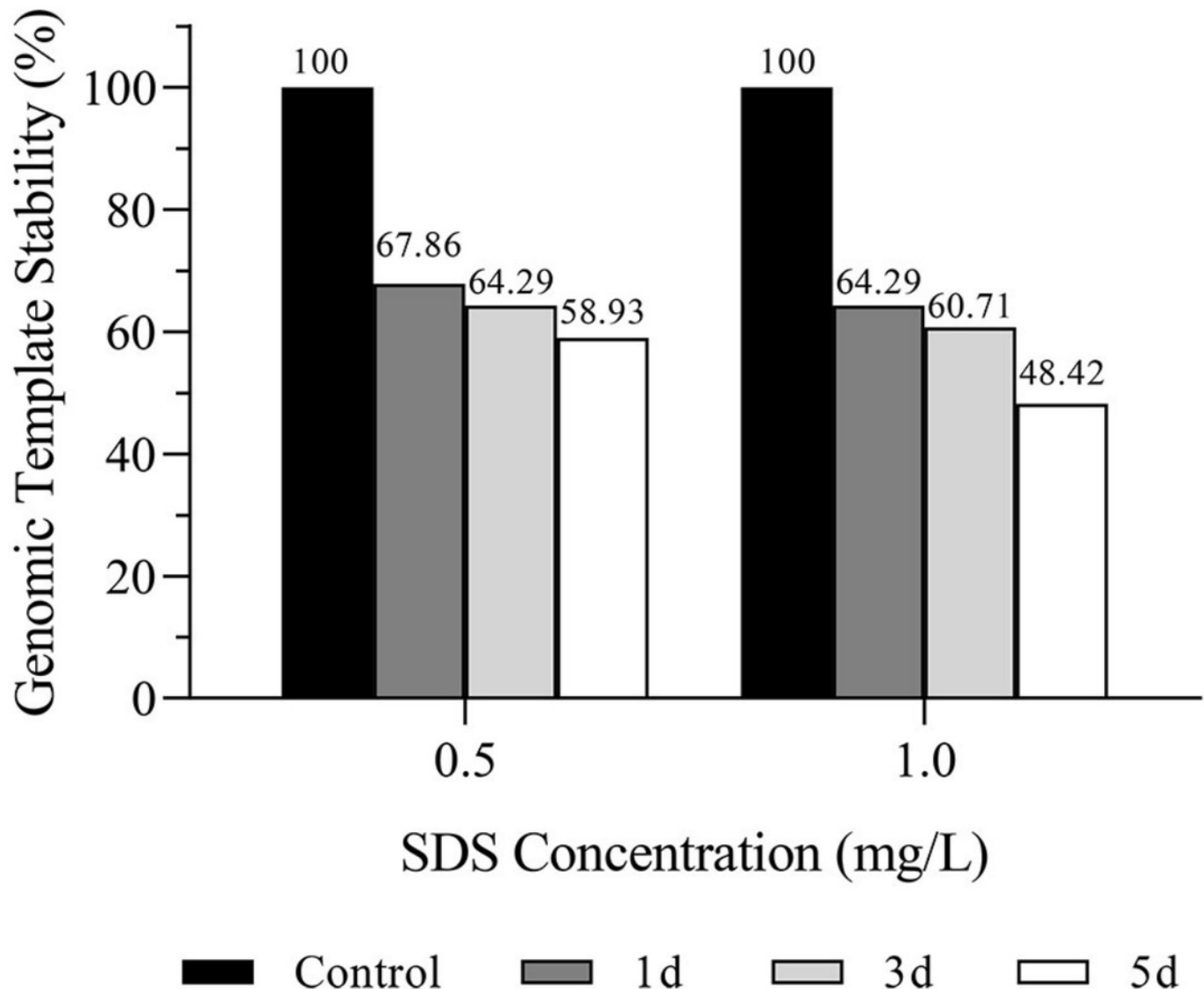
Figure 4

Expression levels of mRNA by qPCR after exposure to 1.0 mg/L SDS for 1, 3, and 5 days in *D.japonica*.

The expression levels of (A) apoptosis related genes *Dj-caspase3*; (B) cell proliferation related gene *Dj-piwiA*, (C) neoblast differentiation related gene *Dj-piwiB*; (D) cell proliferation marker gene *Dj-PCNA*; (E) cell cycle related gene *Dj-cyclinB*; (F) DNA damage related gene *Dj-RAD51*. * $p < 0.05$; ** $p < 0.01$.