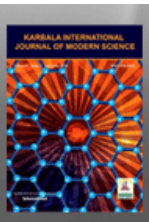

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Imidazole derivative improves antioxidant status and causes differential alteration of redox-status in Drosophila melanogaster

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Imidazole derivative improves antioxidant status and causes differential alteration of redox-status in Drosophila melanogaster

Abstract

This study investigated the toxicity of a new imidazole compound, 1-(1,4,5-triphenyl-1*H*-imidazol-2-yl)-naphthalen-2-ol, through the evaluation of selected oxidative stress markers and antioxidants. Both male and female *D. melanogaster* (3–5 days old) were fed a diet containing the imidazole derivative (20, 50, and 100 mg IMZ/kg diet) for five days. After cessation of imidazole treatment, half the population of the imidazole-exposed flies was homogenized for biochemical assays, while the other half of the flies was allowed to have a normal diet for an additional five days to see if any induced effect would be resolved. Imidazole derivatives did not significantly affect the survival rate of flies. Furthermore, there was a significant increase in reduced glutathione level (GSH) ($p < 0.05$) when compared to the control group. Also, the activity of catalase was significantly increased in flies fed with the 50 mg IMZ/kg diet. An increase was observed in the levels of glutathione peroxidase, glutathione transferase, total protein level, nitric oxide, and lipid peroxidation, but this elevation was not statistically significant compared to the control group. Flies fed the 100 mg IMZ/kg diet showed a significant increase in protein carbonyl level and DNA fragmentation, implying that the imidazole derivative is toxic to the flies at higher concentrations. However, this toxic effect was resolved within five days after treatment cessation. The results support the safety prospects of 1-(1,4,5-triphenyl-1*H*-imidazol-2-yl)-naphthalen-2-ol as an alternative anti-parasite therapy. The present findings represent a portion of the critical pre-clinical data needed to warrant further experiments and development of the imidazole-based compound as a prospective anti-parasite therapy.

Keywords

Antiparasitic, antioxidant, Drosophila melanogaster, imidazole derivative (1-(1, 4, 5-triphenyl-1H-imidazol-2-yl)-naphthalen-2-ol), oxidative stress, toxicity

RESEARCH PAPER

Imidazole Derivative Improves Antioxidant Status and Causes Differential Alteration of Redox-status in *Drosophila Melanogaster*

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Abstract

This study investigated the toxicity of a new imidazole compound, 1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol, through the evaluation of selected oxidative stress markers and antioxidants. Both male and female *Drosophila melanogaster* (3—5 days old) were fed a diet containing the imidazole derivative (20, 50, and 100 mg IMZ/kg diet) for five days. After cessation of imidazole treatment, half the population of the imidazole-exposed flies was homogenized for biochemical assays, while the other half of the flies was allowed to have a normal diet for an additional five days to see if any induced effect would be resolved. Imidazole derivatives did not significantly affect the survival rate of flies. Furthermore, there was a significant increase in reduced glutathione level (GSH) ($p < 0.05$) when compared to the control group. Also, the activity of catalase was significantly increased in flies fed with the 50 mg IMZ/kg diet. An increase was observed in the levels of glutathione peroxidase, glutathione transferase, total protein level, nitric oxide, and lipid peroxidation, but this elevation was not statistically significant compared to the control group. Flies fed the 100 mg IMZ/kg diet showed a significant increase in protein carbonyl level and DNA fragmentation, implying that the imidazole derivative is toxic to the flies at higher concentrations. However, this toxic effect was resolved within five days after treatment cessation. The results support the safety prospects of 1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol as an alternative anti-parasite therapy. The present findings represent a portion of the critical pre-clinical data needed to warrant further experiments and development of the imidazole-based compound as a prospective anti-parasite therapy.

Keywords: Antiparasitic, Antioxidant, *Drosophila melanogaster*, Imidazole derivative (1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol), Oxidative stress, Toxicity

1. Introduction

Imidazole compounds are well-identified and well-known compounds which possess several pharmacological properties and play crucial roles in diverse biochemical processes. These properties of imidazole allow it to easily bind to a wide range of therapeutic targets, resulting in broad pharmacological activity [1,2]. Also, imidazole derivatives are exceptionally versatile and appear in large numbers of clinically active drug molecules such as antiulcer, antihistaminic, analgesic, anticancer, anti-HIV, antibacterial, antifungal, antihypertensive, antiviral, including antiparasitic, and other therapeutic drugs with high therapeutic effectiveness and high market significance [3].

Imidazole is significantly present in some biological skeletons, such as histamine, amino acids (histidine), vitamin B12, purines, and biotin. Many natural or

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synthetic drug molecules, such as cimetidine, azomycin, ketoconazole and metronidazole, also contain an imidazole ring. Imidazole rings are also among the chemical structures of antibiotics such as Megazol, which was first reported to possess antimicrobial activity and was later identified as an excellent antiparasitic agent against trypanosomes [4].

Furthermore, recent *in vitro* and *in silico* studies have shown that the imidazole derivative 1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol has potential value in drug development against toxoplasmosis [5]. Overall, the prospective properties of this imidazole derivative as an alternative antiparasitic agent underscore the need to evaluate the toxicity profile of the compound vis-à-vis oxidative stress markers and DNA fragmentation. The present study will contribute data toward determining whether the new imidazole-based compound, 1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol, can be considered safe.

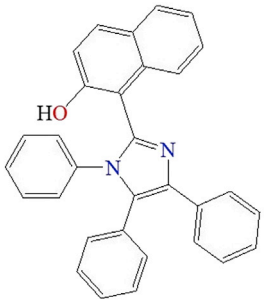
Drosophila melanogaster was the model organism used in this study because these flies respond to drugs in a similar way to mammals [6]. They also have a short life span and are inexpensive to maintain in the laboratory. Additionally, *D. melanogaster* fulfils the 3Rs (Reduction, Refinement, and Replacement) of laboratory animal usage as an alternative method for toxicity testing as described by the European Centre for the Validation of Alternative Methods (ECVAM) standard [7].

2. Materials and methods

All the reagents and chemicals used were of analytical grade and were prepared in glassware. Diphenylamine, iodide, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), Ehrlich reagent, Griess reagent, and Tris–HCl buffer were products of Sigma–Aldrich (St. Louis, MO, USA). The imidazole derivative 1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol (Scheme 1) was generously provided by the Institute of Inorganic and Analytical Chemistry, Friedrich-Schiller-Universität Jena, Germany. The experimental synthesis and characterization have been previously reported [5,8].

2.1. Culture media for Drosophila melanogaster

D. melanogaster (Harwich strain) was provided by the *Drosophila* Laboratory, University of Ibadan. The flies were maintained and reared in the Biochemistry Laboratory, Biochemistry Department, Landmark University Omu-Aran, Nigeria. The flies were sustained on corn meal medium containing brewer's yeast, agar, and nipagin at a constant temperature (22–25 °C), under 12 h dark/light cycle conditions, and at 60–70% relative humidity.



1-(1,4,5-triphenyl-1H-imidazol-2-yl)naphthalen-2-ol

Scheme 1. Molecular structure of 1-(1,4,5-triphenyl-1H-imidazole-2-yl)-naphthalen-2-ol.

2.2. Experimental design and treatment

The experimental design and treatment took place in two phases.

2.2.1. Phase 1 experimental design and treatment

D. melanogaster flies (Harwich strain, 3–5 days old) were randomly distributed into 4 groups. Each group had fifty (50) flies per vial and three (3) replicates. The flies were exposed to different concentrations of the imidazole-based compound (1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol) in their diet for five days as follows:

Group 1 served as the control group and received a normal diet without the imidazole derivative.
Group 2 was fed a 20 mg/kg diet containing the imidazole compound.
Group 3 was fed a 50 mg/kg diet containing the imidazole compound.
Group 4 was fed a 100 mg/kg diet containing the imidazole compound.

The selection of doses was made based on a predicted oral LD50 of ≥2000 mg/kg in mice [5].

2.2.2. Phase 2 experimental treatment

In the phase 2 of the experimental design and treatment, the remaining flies (25 per vial) from the phase 1 treatment were removed on day 5 and introduced to fresh corn meal media lacking the imidazole compound for the following five days.

2.3. Harvesting of D. melanogaster and homogenate preparation

The collection of flies for preparation of homogenate was done as previously described elsewhere [9]. After five days of exposure to an imidazole derivative,

twenty-five (25) flies per vial from each group were randomly collected (the remaining 25 flies per vial were carried forward to the phase 2 experiment). They were then anaesthetized on ice, weighed, and homogenized in 0.1 M phosphate buffer, pH 7.4 (1:10 flies/volume). The homogenate was centrifuged at 4000 g for 10 min at 4 °C, and the supernatant was separated into Eppendorf tubes and kept frozen until it was used for evaluation of the biochemical assays.

2.4. Survival rate curve

The survival of experimental flies was analyzed as described by Ref. [10]. The number of dead flies was recorded daily and was thereafter used to plot the survival curve by Kaplan–Meier statistical analysis.

2.5. Sample preparation for evaluation of biochemical assays after treatment of Drosophila melanogaster with a diet containing imidazole

Several biochemical assays were carried out on the *D. melanogaster* homogenate. The total protein was determined using the method described by Gornall et al. [11], while reduced glutathione (GSH) concentration was assayed by the method of Jollow et al. [12]. Catalase activity was assessed according to the method described elsewhere by Beers and Sizer [13]. Lipid peroxidation level was determined according to the method of Vashney and Kale [14], and protein carbonyl level was assayed according to the method of Categna et al. [15]. The glutathione transferase level was determined by the procedure described by Habig et al. [16]. The nitric oxide level (NO) was determined as the amount of nitrite/nitrate concentration in the homogenates using the method described by Green et al. [17]. DNA fragmentation was determined according to the procedure described by Perandones [18]. Determination of total antioxidant capacity (TAC) was based on the procedure described by Saha et al. [19]. Glutathione peroxidase (GPx) activity was assayed by employing the procedure described by Rotruck et al. [20]. The kynurenine level was assayed according to the method described by Adeyemi et al. [21].

2.6. Statistical analysis and data presentation

Statistical analysis was carried out using GraphPad Prism 8.0 software (San Diego, California, USA). The values are expressed as the mean value plus or minus the standard error of the mean (SEM) of three replicates. The treatment and control groups were compared using one-way analysis of variance (one-way ANOVA), and Dunnett's multiple comparison was used for the post-hoc test. The survival rate (%) was analysed using the Kaplan–Meier statistical graph. *P*-values less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Results

3.1. Determination of survival rate of Drosophila melanogaster exposed to a diet containing imidazole

Imidazole treatment after five days caused a decrease in the survival rate of flies fed with 20, 50 and 100 mg of IMZ/kg by 6.1%, 11.7% and 11.1%, respectively, although these decreases were not statistically significant (Fig. 1, Supplementary Table 1).

Five days after the cessation of imidazole treatment, a slight decrease in the survival rate of flies was also observed—specifically, 2%, 2.6% and 4.6%, respectively, for diets with 20, 50 and 100 mg of IMZ/kg, although again the differences were not statistically significant (Fig. 2, Supplementary Table 2).

3.2. Imidazole did not alter the total protein level in Drosophila melanogaster

After 5 days of treatment, imidazole administration elicited a slight though not significant ($p > 0.05$) reduction in the total protein level of flies across the treatment group compared to the control (Fig. 3A). There was no significant difference in the total protein level of flies fed a normal diet after cessation of treatment compared to the control group (Fig. 3B).

3.3. Imidazole caused a differential alteration in the level of antioxidant parameters in Drosophila melanogaster

The effect of the imidazole derivative on antioxidant parameters was determined using GST, GSH,

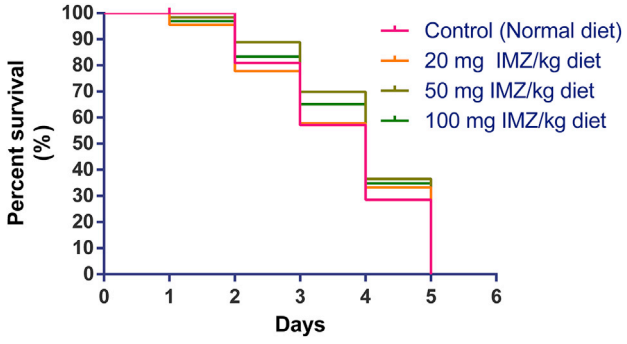


Fig. 1. Survival rate (%) of Drosophila melanogaster after exposure to 20, 50 and 100 mg imidazole (IMZ) derivative/kg for 5 days. Values are presented as mean ± SEM.

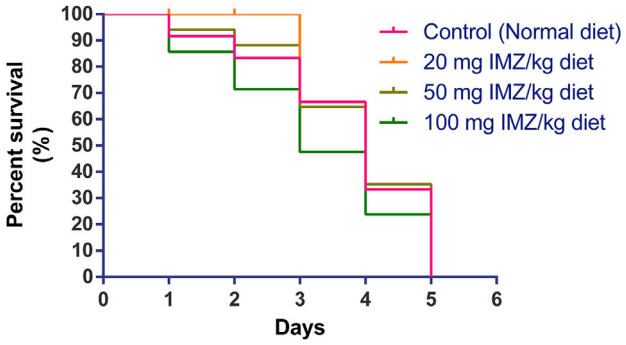


Fig. 2. Survival rate (%) of Drosophila melanogaster 5 days after cessation of treatment. Values are presented as mean ± SEM.

GPx, and CAT activities as indices. Our results indicated that imidazole administration caused an increase in glutathione transferase activity (Fig. 4A), although it was not statistically significant ($p > 0.05$) compared to the control group. Also, there was no significant difference in the group that was fed a normal diet after cessation of treatment (Fig. 4B). Furthermore, imidazole administration increased the GSH level significantly ($p < 0.05$) across the treatment groups compared with the control group (Fig. 5A). For the group fed a normal diet after cessation of treatment, there was no significant ($p > 0.05$) difference relative to the control group (Fig. 5B). In addition, imidazole administration increased the glutathione peroxidase activity of flies fed with 20 and 50 mg of IMZ/kg (Fig. 6A), although the difference was not statistically significant ($p > 0.05$) compared to the control group. There was no significant difference ($p > 0.05$) in the glutathione peroxidase activity of flies fed a normal diet after cessation of treatment when compared to the control group (Fig. 6B). Furthermore, imidazole administration led to a significant increase ($p > 0.05$) in the catalase activity of flies fed 50 mg of IMZ/kg (Fig. 7A) when compared to the control group. There was also a significant increase ($p < 0.05$) in the catalase activity of flies fed 50 mg of IMZ/kg and then fed a normal diet after cessation of treatment compared to the control group (Fig. 7B).

Imidazole administration caused a dose-dependent, though non-significant ($p < 0.05$), increase in the nitric oxide level of flies across all treatment groups (Fig. 8A), when compared to the control group. The nitric oxide level of flies fed 100 mg of IMZ/kg decreased after cessation of treatment, although not significantly ($p < 0.05$).

Imidazole administration caused a non-significant ($p > 0.05$) decrease in the total antioxidant capacity of flies (Fig. 9A) compared to the control group. In addition, there was a dose-dependent increase in the level of TAC of flies across the treatment groups after cessation of treatment compared to the control group (Fig. 9B).

3.4. Imidazole caused a differential alteration in the level of oxidative stress parameters in Drosophila melanogaster

Our results showed that imidazole administration increased malondialdehyde levels of flies fed 50 and 100 mg IMZ/kg (Fig. 10A), although the differences were not statistically significant ($p > 0.05$) compared

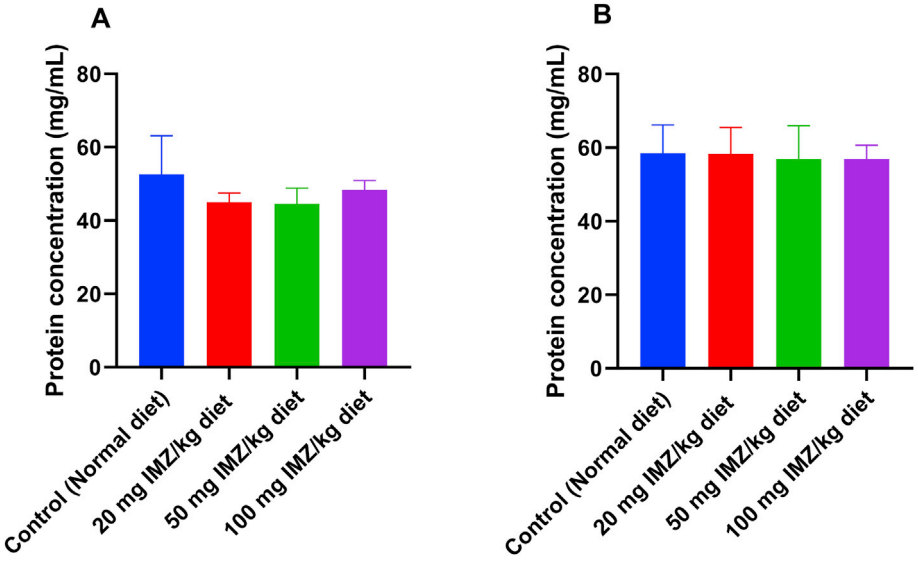


Fig. 3. Effect of administration of imidazole (IMZ) derivative on total protein concentration in Drosophila melanogaster (A) Total protein concentration after exposure of flies to 20, 50 and 100 mg of imidazole/kg for 5 days (B) Total protein concentration after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.

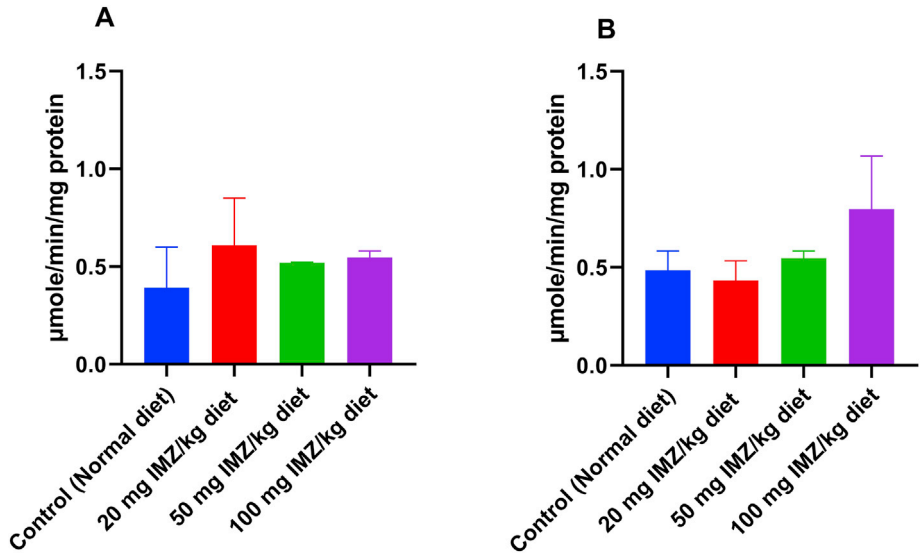


Fig. 4. Effect of administration of imidazole (IMZ) derivative on glutathione transferase (GST) concentration in Drosophila melanogaster (A) Glutathione transferase concentration after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days (B) Concentration after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.

to the control group. Also, there was no significant difference ($p > 0.05$) in the malondialdehyde level of flies fed a normal diet after cessation of treatment when compared to the control group (Fig. 10B). Furthermore, imidazole treatment after five days increased the protein carbonyl level of flies fed 100 mg IMZ/kg when compared to the control group, but the difference between the carbonyl levels of flies fed 20 or 50 mg IMZ/kg (Fig. 11A) was not significant compared to the control group. Also, there was no significant difference in the group fed a normal diet after cessation of treatment when compared to the control group (Fig. 11B). Imidazole administration increased the DNA fragmentation of the flies fed 100 mg IMZ/kg (Fig. 12A). This increase was statistically significant ($p < 0.05$) when compared to the results for the control group. Furthermore, there was no significant difference in the percentage of DNA fragmentation of flies fed a normal diet after treatment cessation ($p > 0.05$) compared to the control group (Fig. 12B).

3.5. Effect of imidazole treatment on kynurenine levels in D. melanogaster

The effect of imidazole on kynurenine levels in *D. melanogaster* was evaluated. After five days of administration, the results revealed that imidazole

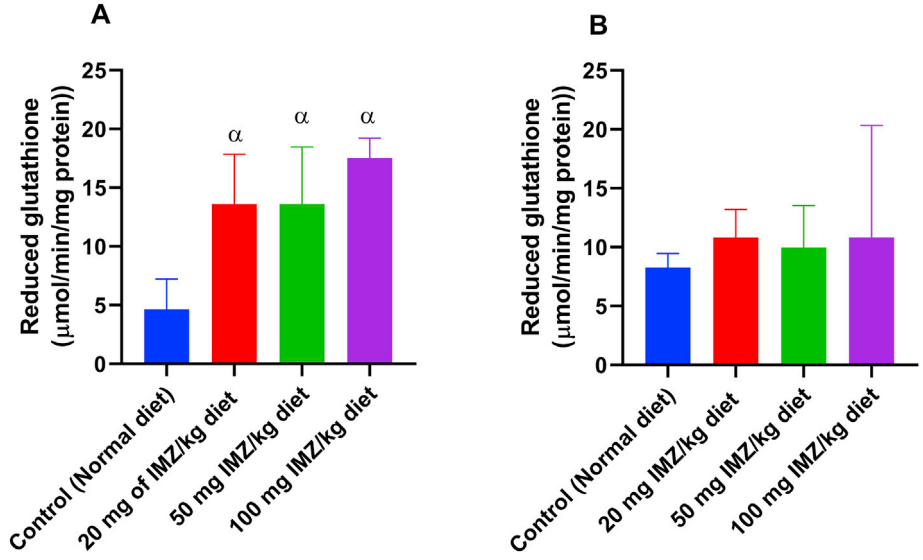


Fig. 5. Effect of the administration of imidazole (IMZ) derivative on reduced glutathione (GSH) concentration in Drosophila melanogaster (A) Reduced GSH concentration after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days (B) Reduced concentration after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM. α is significant at $p < 0.05$ versus the control.

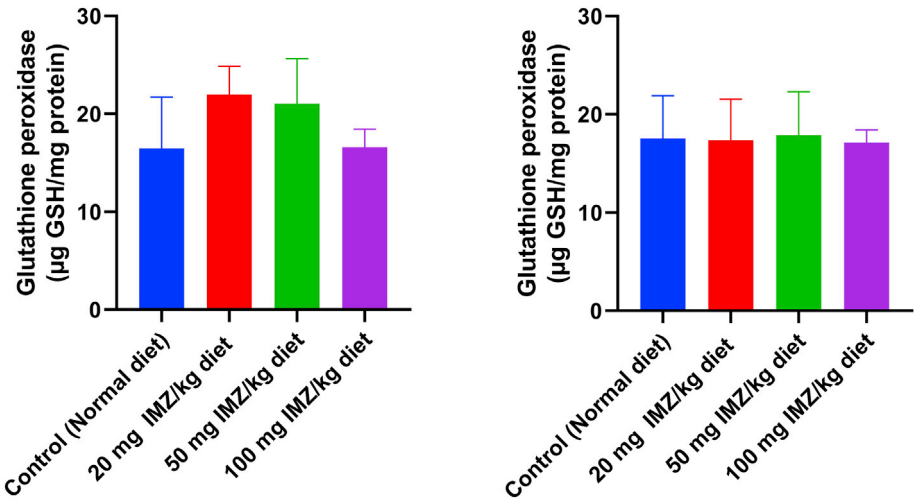


Fig. 6. Effect of administration of imidazole derivative on glutathione peroxidase (GPX) concentration in Drosophila melanogaster **(A)** *GPX concentration after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days* **(B)** *GFX concentration after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.*

caused a decrease in the kynurenine level of flies fed a 50 mg IMZ/kg diet when compared to the control group, but there was not a significant difference ($p > 0.05$) in the kynurenine level of flies fed 20 or 100 mg IMZ/kg (Fig. 13A) when compared to the control group. There was no significant difference ($p > 0.05$) in the group fed a normal diet after cessation of treatment, compared to the control group (Fig. 13B).

4. Discussion

Imidazole has been reported to possess antibacterial effectiveness [22], anticancer [23,24], antioxidant [25], anti-tubercular [26,27], analgesic, anti-inflammatory [28], and antiparasitic activity [5,29] This study evaluated the toxicity of various doses of the imidazole derivative 1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol in *D. melanogaster*.

Imidazole treatment might not have adversely affected the survival of the flies. Only a few deaths (averaging 10%) were recorded following imidazole treatment compared with the control group. In addition, after the cessation of imidazole treatment, fewer deaths (average < 4%) were recorded in the next five days compared to the control. Taken together, these results may suggest the relative safety of the compound. Meanwhile, the exposure of

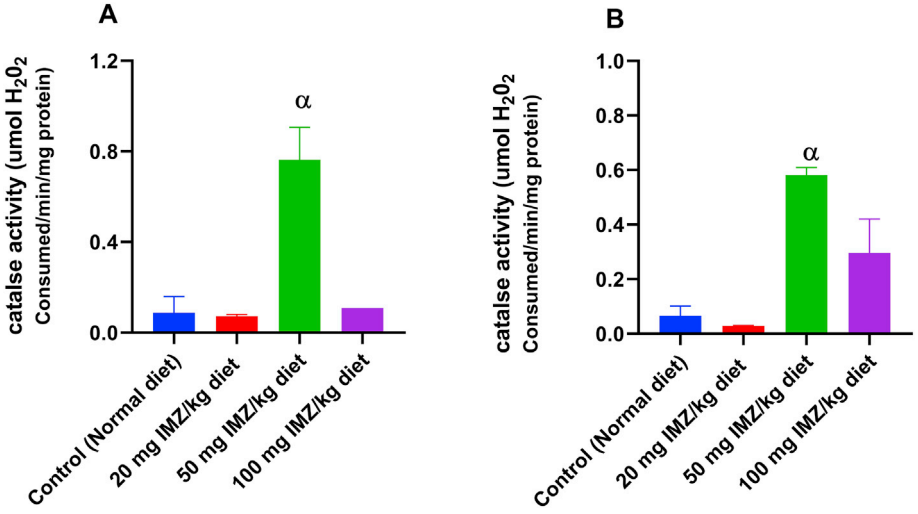


Fig. 7. Effect of administration of imidazole derivative on catalase activity in Drosophila melanogaster **(A)** *Catalase activity after exposure to 20, 50 and 100 mg of imidazole/kg diet for 5 days* **(B)** *Catalase activity after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.* **α** *is significant at $p < 0.05$ versus the control.*

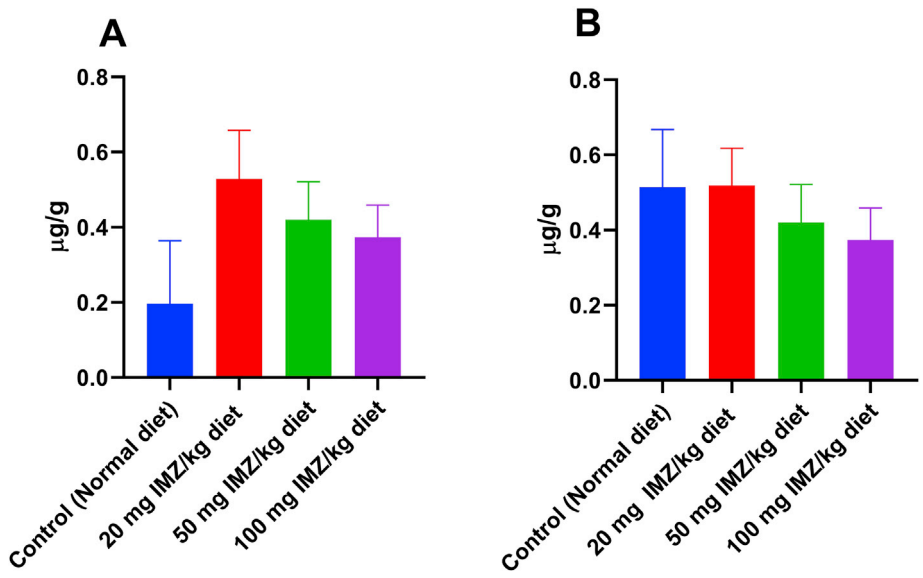


Fig. 8. Effect of administration of imidazole (IMZ) derivative on nitric oxide concentration in Drosophila melanogaster ***(A)*** *Nitric oxide level after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days* ***(B)*** *Nitric oxide level after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.*

flies to the imidazole derivative significantly raised GSH levels across the treatment groups. GSH is a cellular non-enzymatic antioxidant known to protect against assault by free radicals. The increased GSH level could be the result of an adaptive response mechanism to cope with the exposure to imidazole. The elevated GSH level may help to counteract likely oxidative stress due to the imidazole treatment. Therefore, increased GSH levels in *D. melanogaster* might serve to protect the organism against oxidative stress resulting from the imidazole exposure.

Glutathione S-transferase (GST) belongs to the phase II family of detoxifying enzymes and consists of a cysteine-rich domain. This family of enzymes play an important role in xenobiotic detoxification by conjugating GSH with electrophilic molecules that are endogenous. This antioxidant enzyme also shields the cell against harmful effects caused by oxidative stress [30] and helps in regulating cellular processes involved in oxidative stress. Under a diseased condition, GST levels are very low, but they are high under normal conditions [31]. In our study, a non-significant increase was observed in

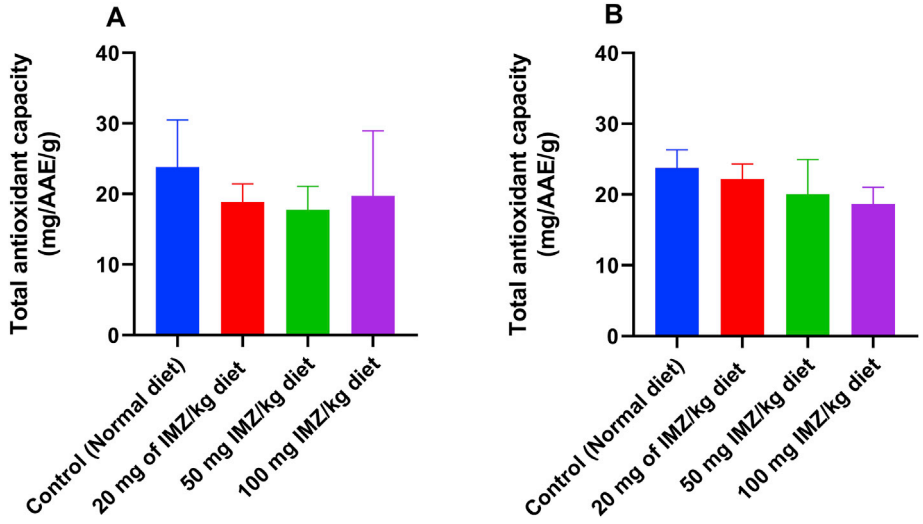


Fig. 9. Effect of administration of imidazole (IMZ) derivative on total antioxidant capacity in Drosophila melanogaster ***(A)*** *Total antioxidant capacity after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days* ***(B)*** *Total antioxidant capacity after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.*

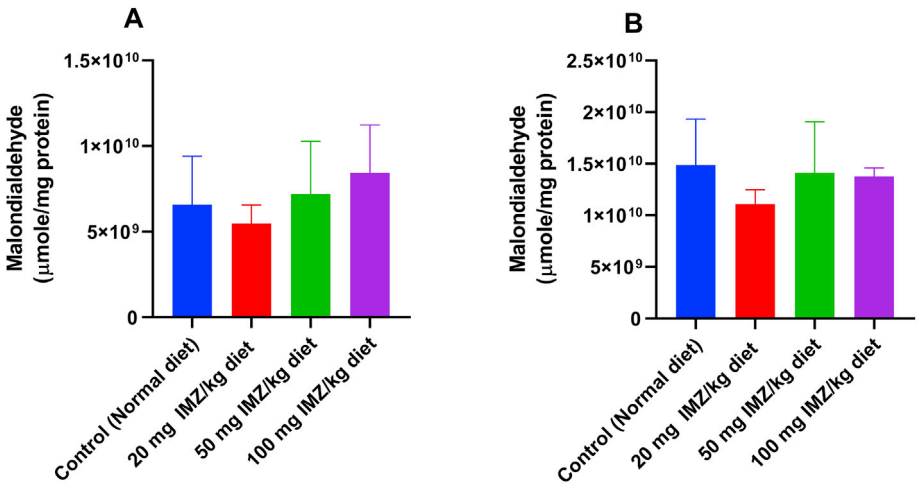


Fig. 10. Effect of administration of imidazole (IMZ) derivative on malondialdehyde (MDA) concentration in Drosophila melanogaster ***(A)*** *MDA concentration after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days* ***(B)*** *MDA concentration after 5 days of exposure to normal diet after cessation of treatment. Values are presented as mean ± SEM.*

total activity of GST, which may suggest the activation of the phase II detoxification system under this experimental condition. This may indicate an attempt by the flies to detoxify the imidazole-based compound. This finding may further corroborate the suggestion above that the elevated GSH levels represent an adaptive response mechanism by the flies.

In addition, reactive oxygen species (ROSs) such as superoxide anion and hydrogen peroxide free radicals are detoxified by the antioxidant enzymes glutathione peroxidase (GPx) and catalase (CAT) [32]. Although ROSs play a physiological role in diverse biological processes, including cellular signaling, intracellular accumulation of ROS concentrations might occur as a result of exposure to cytotoxic agents, thereby inducing oxidative damage to cellular macromolecules [33]. ROSs also have the ability to indirectly or directly damage biological molecules, including DNA, lipids, carbohydrates and proteins.

GPx is a cytosolic enzyme that reduces hydrogen peroxide to water. GPx also protects the cell against free radical effects and oxidative stress, but its role in *D. melanogaster* is not fully understood [34]. In this study, imidazole administration caused an increase in the glutathione peroxidase level, although it was not statistically significant. Furthermore, imidazole

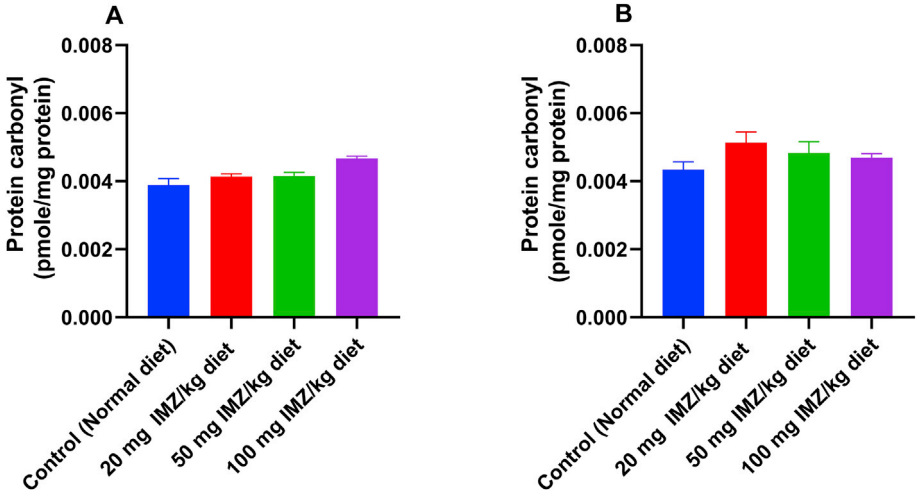


Fig. 11. Effect of administration of imidazole (IMZ) derivative on protein carbonyl level in Drosophila melanogaster ***(A)*** *Protein carbonyl level after exposure to 20, 50 and 100 mg of imidazole/kg diet for 5 days* ***(B)*** *Protein carbonyl level after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.* α *is significant at* $p < 0.05$ *versus the control.*

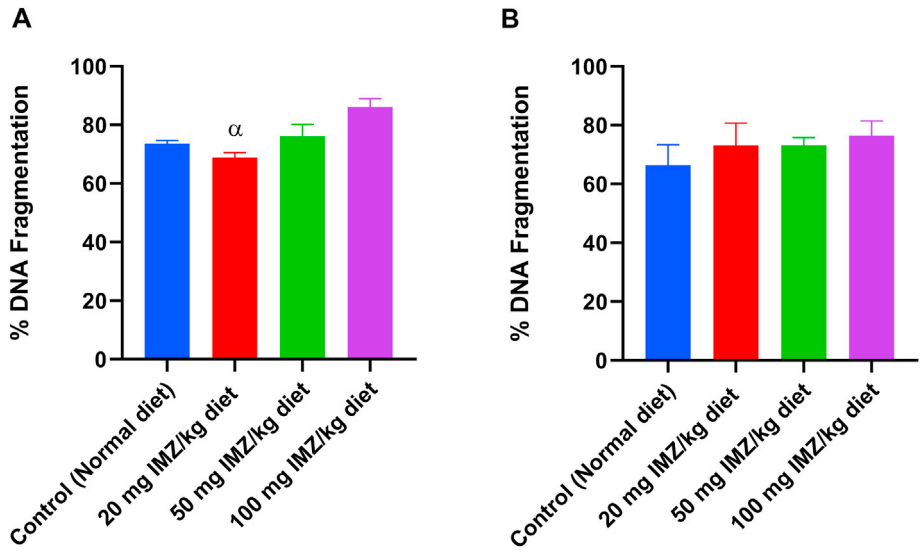


Fig. 12. Effect of administration of imidazole (IMZ) derivative on DNA fragmentation level in Drosophila melanogaster ***(A)*** *DNA fragmentation after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days* ***(B)*** *DNA fragmentation after 5 days of exposure to normal diet after cessation of treatment. Data are presented as mean ± SEM.* ***α*** *is significant at* $p < 0.05$ *versus the control.*

administration after five days caused a significant increase in the catalase activity of flies fed 50 mg IMZ/kg. The increase in catalase activity may suggest the election of adaptive mechanisms to tackle an increased production of hydrogen peroxide (H_2O_2). Increased activity of CAT has been reported to be an adaptive response to oxidative stress challenges [35]. The increase in the antioxidant enzymes can be linked with the induction of ROSs produced in *D. melanogaster* by imidazole derivatives. This result is, however, in agreement with [36], where increased levels of antioxidant enzymes in *D. melanogaster* were attributed to the diet. The increased ROS level in *D. melanogaster* suggests a state of oxidative stress and redox imbalance. This may indicate that the increased antioxidant enzymes are an adaptive response mechanism to oxidative stress. An adaptive response has been identified in *D. melanogaster* [37], showing the organism's ability to resist damage to cellular tissues induced by exposure to cytotoxic agents. Such an increase in the activity of antioxidant enzymes in *D. melanogaster* as

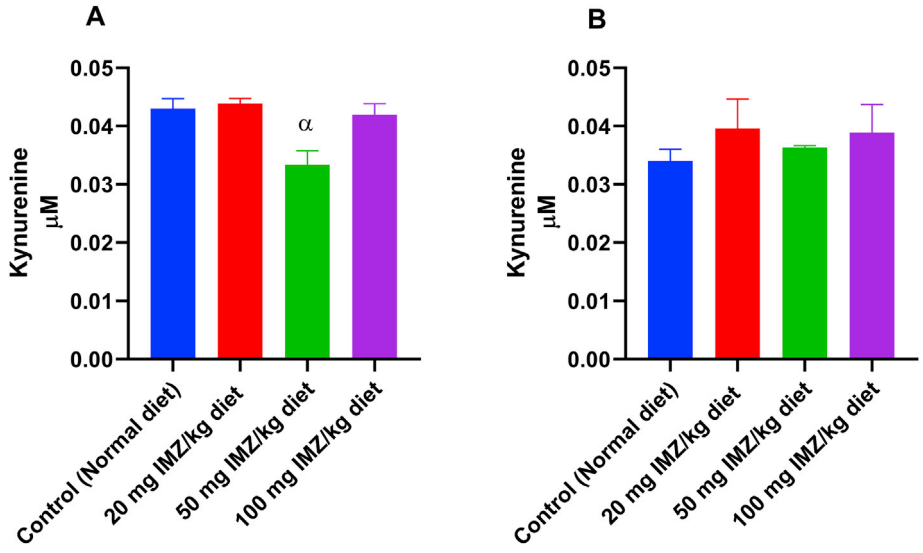


Fig. 13. Effect of administration of imidazole (IMZ) derivative on kynurenine level in Drosophila melanogaster ***(A)*** *Kynurenine level after exposure to 20, 50 and 100 mg of imidazole/kg for 5 days* ***(B)*** *Kynurenine level after 5 days of exposure to normal diet following cessation of treatment. Values are presented as mean ± SEM.* ***α*** *is significant at* $p < 0.05$ *versus the control.*

an adaptive response to cytotoxic agents has been reported previously [38]. Moreover, our results revealed a non-significant differential in TAC. The TAC, which measures the capacity to scavenge free radicals, is considered one of the useful antioxidant status markers [39].

Nitric oxide (NO) is a diffusible gas with a short half-life that recombines quickly to form the stable metabolites nitrite and nitrate [40]. It is known to be a pro-inflammatory mediator because of its ability to react with superoxide anion to form a harmful anion, leading to various disease conditions [41]. The NO radical also plays an important role as a physiological messenger, but its accumulation could result in a reaction with superoxide anion to generate a nitrite anion, thereby causing damage to tissues. NO is formed from L-arginine by nitric oxide synthase, which exists in several isoforms such as *i*NOS, *n*NOS and *e*NOS. NO reduces oxidative stress and increases the endogenous potential of antioxidants. NO may also cause a predisposition to inflammation [42]. Studies have further indicated that NO plays a role in the immune response of flies to parasites and pathogens [43]. Though there is no information on the role NO plays in the physiology of adult flies, NO production has been reported to be more narrowed to their brains [36]. In this present study, there was a non-significant increase in NO level compared to the control group. Thus, imidazole administration did not affect the nitric oxide level of flies at the administered doses. These observations could also imply that the non-significant increase in NO may protect the antioxidant enzymes.

Lipid peroxidation, which can be measured by the level of malondialdehyde (MDA), a mutagenic end product of the peroxidation of lipids [44], is used to assess redox imbalance as it is one of the common markers of oxidative stress. Elevated levels of MDA suggest alteration to and changes in membrane structure and function. In this study, the administration of imidazole did not have any significant effect on the MDA level in *D. melanogaster*. Also, protein carbonyl is known to be one of the most important and commonly used oxidative stress markers [45]. Protein carbonyl is a result of protein oxidation. In the present study, our data suggest that the effects of the imidazole derivative on protein carbonyl and DNA fragmentation might be related to oxidative stress. Moreover, DNA fragmentation may be used to assess the extent of oxidative stress. The administration of the imidazole-based compound at the lower doses of 20 and 50 mg of IMZ/kg did not significantly affect protein carbonyl and DNA fragmentation, but at the highest concentration (100 mg of IMZ/kg), a significant increase was observed. This could indicate that *D. melanogaster* was experiencing oxidative stress. The results may also suggest that imidazole treatment at the highest dose (100 mg of IMZ/kg) temporarily caused toxicity by elevating the levels of DNA fragmentation and protein carbonyl of *D. melanogaster* after five days of administration compared to the control group. However, the toxicity caused by imidazole exposure was ameliorated following cessation of treatment (Fig. 12B).

The kynurenine level of flies fed a 50 mg IMZ/kg diet was reduced significantly compared to the control group. The kynurenine pathway breaks down the amino acid L-tryptophan into kynurenine, kynurenic acid and quinolinic. The end product of the route is NAD^+. The metabolic reaction is mediated by the enzyme indolamine-2, 3-dioxygenases (IDO). During adaptive immune response, pro-inflammatory cytokines increase, leading to an elevated level of IDO activity. This increase in turn leads to activation of the kynurenine pathway, which is thought to play a significant role in several disorders [46,47].

In this study, administration of an imidazole derivative significantly decreased the kynurenine level of flies fed a 50 mg IMZ/kg diet, but it did not cause any change in the kynurenine level of flies fed a 20 or 100 mg IMZ/kg diet (Fig. 13A).

5. Conclusion

The findings from this study revealed that the imidazole derivative 1-(1,4,5-triphenyl-1*H*-imidazole-2-yl)-naphthalen-2-ol did not alter the levels of antioxidant enzymes, nitric oxide, or lipid peroxidation of flies, thereby indicating a lack of serious toxicological effects. The imidazole-based compound might have caused a mild oxidation-related toxicity by elevating the levels of protein carbonyl and DNA fragmentation of *D. melanogaster* at the highest concentration tested (100 mg IMZ/kg). However, this toxicity was ameliorated after five days following cessation of treatment. Taken together, the present findings represent a portion of the critical pre-clinical data needed to warrant further experiments and development of the imidazole-based compound as a prospective anti-parasite therapy.

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Supplementary data

Supplementary Table 1. Survival rate (%) of Drosophila melanogaster.

Days	Control	20 mg IMZ/kg diet	50 mg IMZ/kg diet	100 mg IMZ/kg diet
1	100 ± 0.00	98.9 ± 0.56	94.4 ± 0.57	98.9 ± 0.56
2	97.8 ± 0.55	95.0 ± 1.47	96.7 ± 0.00	95.0 ± 0.96
3	97.2 ± 1.11	95.0 ± 2.22	93.3 ± 0.00	93.3 ± 1.92
4	96.7 ± 1.67	93.9 ± 2.22	88.3 ± 2.54	88.9 ± 2.94
5	96.7 ± 1.67	91.7 ± 3.85	87.2 ± 3.64	87.2 ± 3.37
Average survival %	**97.6 ± 1.37**	**94.7 ± 2.27**	**91.9 ± 4.01**	**92.6 ± 2.10**
Average death %	**2.4%**	**5.3%**	**8.1%**	**7.4%**

The significance of the 'bold' is to emphasize the percentage survival and death.

Supplementary Table 2. Survival rate (%) of flies after cessation of treatment.

Days	Control	20 mg IMZ/kg diet	50 mg IMZ/kg diet	100 mg IMZ/kg diet
1	98.9 ± 0.78	100.0 ± 0.00	98.9 ± 0.78	96.7 ± 1.36
2	98.9 ± 0.78	100.0 ± 0.00	98.9 ± 0.78	96.7 ± 1.36
3	97.8 ± 1.57	96.7 ± 0.00	95.6 ± 0.78	94.4 ± 0.78
4	95.6 ± 2.08	96.7 ± 0.00	94.4 ± 0.78	94.4 ± 0.78
5	94.4 ± 2.80	96.7 ± 0.00	93.3 ± 0.00	94.4 ± 0.78
Average survival %	**97.1 ± 0.90**	**98.0 ± 0.80**	**96.2 ± 1.44**	**95.3 ± 0.54**
Average death %	**2.6%**	**2%**	**3.8%**	**4.7%**

The significance of the 'bold' is to emphasize the percentage survival and death.

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