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Molecular Insights into Diazinon-Mediated DNA Damage and Oxidative Stress: Impact on Hepatic Enzyme Activity in Albino Rats.

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Abstract:

Diazinon, a toxic organic pesticide, is extremely dangerous when discharged into the environment, particularly to aquatic life and terrestrial animals. This study examines the toxicological consequences of diazinon on albino rats were used in a controlled laboratory setting for the investigation. Rats were split into three 3 groups, a control group and two LD50 concentrations, to assess the sub-chronic concentration of LD50. (60 and 300 ppm) over a period of 30 days, where the LD50 value was 588 ppm. The oxidative stress enzymes (CAT and SOD), as well as environmental stress enzymes (MDA), also liver function enzymes (ALT, AST, and ALP), were examined at 1, 15, and 30 days. Within the last day, DNA damage was evaluated using the comet assay. The findings indicated that both MDA and CAT had significantly increased, in contrast with SOD, which showed a significant decrease after exposure to Diazinon. There was a noticeable rise in DNA damage, as seen by the longer comet tail. These findings highlight diazinon's capacity to harm antioxidant defense mechanisms and to induce oxidative stress and genotoxicity even at sublethal concentrations.

Keywords: Genotoxicity, Oxidative Stress, DNA Damage, Antioxidant Enzymes, Lipid Peroxidation.

Introduction:

Diazinon is an organophosphate pesticide that is frequently used to control insect pests in both urban and agricultural environments. But because of its widespread use, the environment has become seriously polluted, endangering both aquatic and terrestrial life (1). Diazinon's toxic effects are mostly caused by the suppression of the enzyme acetylcholinesterase (AChE), which is necessary for healthy nervous system operation (2). This inhibition can promote acetylcholine accumulation, resulting in nervous system overstimulation and physiological issues in exposed animals (3).

Non-target animals in terrestrial habitats, such as mammals, are particularly sensitive to Diazinon's toxic effects. Albino rats (Rattus norvegicus) are commonly used as model organisms in toxicological studies due to their physiological similarities with humans and vulnerability to environmental pollutants (4). The term "genotoxicity" describes a chemical agent's capacity to alter DNA and cause mutations, cellular dysfunction, and potentially carcinogenic effects (5). Conversely, oxidative stress is brought on by an imbalance between the body's antioxidant defense systems and the generation of reactive oxygen species (ROS), which damages cells. In 2017,

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Pizzino et al. The comet test, a sensitive technique for detecting breaks in DNA strands, is used to assess genotoxicity (6). Oxidative stress and lipid peroxidation are assessed using biomarkers such as malondialdehyde (MDA) levels, catalase (CAT) activity, and superoxide dismutase (SOD) activity (7,8). To understand the dangers that environmental pollutants pose, the study emphasizes the significance of assessing chronic exposure conditions at sub-lethal dosages. by elucidating how pesticides harm cells.

Materials and methods

Albino Rats (*Rattus norvegicus var albinus*) weighed 150 ± 5g. Rats were brought in cages to the laboratory and placed in many plastic containers for 14 days to acclimate to the new circumstances. The lab temperature remained at 19 ± 2 °C. During the acclimatization and testing phase, the rats were given commercial rat food. Following 14 days of acclimatization, a static acute toxicity test was performed. Al Fares Company sold diazinon with a purity of 60%, which was used to make diazinon test solutions. Rats were exposed to five different concentrations of diazinon (0, 60, 300, 600, 800, 1000 ppm) to ascertain the test animals' LD50 values at 1, 24, 48, 72, and 96 hours.

The LD50 value was determined using the Probit Analysis test. The 96-hour acute toxicity test was followed by the sub-lethal toxicity test. In addition to the control group, which received no treatment, rats were given two sub-lethal dosages of diazinon for 30 days: 60 ppm, or 10% of the LD50, and 300 ppm, or 50% of the LD50 value.

The liver function parameters of AST, ALT, and ALP in serum were assessed using the DRI-CHEM NX500 Fujifilm biochemistry analyzer (9).

Comet Assay

Rats' DNA damage was measured using a modified Singh-based alkaline comet test. To put it briefly, PBS was used to dilute blood samples taken straight from the heart. Slides that had previously been coated with 1% normal agarose and 0.1% LMPA were coated with a combination of 15 µL of cell suspension (about 15,000 cells) and 85 µL of 0.5% LMPA. After gel solidification, cells were lysed for 60 minutes at 4 °C using a solution of 2.5 M NaCl, 100 mM Na2-EDTA, 10 mM Tris (pH 10), 10% DMSO, and 1% Triton X-100. To encourage DNA unwinding and the conversion of alkali-labile sites, slides were treated for 25 minutes in an alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA, 0.2% DMSO, pH 13.5). 4 degrees Celsius. Electrophoresis was performed at 70 V for 60 minutes. Slides were then neutralized with 0.4 M Tris (pH 7.4) for 10 minutes at 4 °C and stained with 75 µL ethidium bromide (10–20 µg/mL) to see and quantify DNA damage. The comet tail form was quantitatively analyzed using the comet score tool following the acquisition of pictures using fluorescence microscopy.

The enzyme catalase

The CAT enzyme's activity was measured using the Aebi technique. The reaction was initiated by adding 30 mM H2O2 to an adequate amount of blood in a 50 mM sodium phosphate buffer at pH 7. The absorbance was then measured at 240 nm for 10 minutes. The particular activity was evaluated using the U/ml.

SOD, or superoxide dismutase

The SOD enzyme activity was measured using the Winterbourn method. In a cuvet, serum was mixed with 0.1 M EDTA, 0.3 mM sodium cyanide, and 1.5 mM NBT. The mixture was then incubated at 37ºC for five minutes. Following that, 0.12 mM riboflavin was combined with 0.067 M potassium phosphate buffer (pH=7.8) and left to stand at room temperature for ten minutes. The specific activity was computed using the unit per milligram of protein following five minutes of monitoring the absorbance at 560 nm.

MDA

Lipid peroxidation was measured using a spectrophotometer. Using 5% TCA, reactions were halted at predetermined incubation times. After

centrifugation, mix 700 µL TBA (thiobarbituric acid) at 0.67% with 350 µL of supernatant. The solution was cooked in a boiling water bath for fifteen minutes. After cooling, the mixture's absorbance was measured at 535 nm. Microsomal lipid peroxidation is measured in malondialdehyde (MDA) nanomoles per milligram of protein. At 535 nm, MDA levels were determined to have a molar extinction value of 156 mM/cm. Each figure is the mean of three computations. TCA, along with 0.02% butylated hydroxytoluene (BHT), was employed to end incubations in certain studies. BHT did not affect the amount of MDA detected (10).

3. Results and Discussion

The LD50 was found to be 588 ppm after exposure to Diazinon

Aspartate aminotransferase (AST/GOT)

The result of AST shows the highest value in the control group (262±3) U/L in the first day and lowest value in the 15th days with (300 ppm) (67±5) U/L, the results also show that there was a decreasing of AST in the (60ppm) concentration in the 1st and 15th days of experiment, and significant increasing in the 30th days compering with control group. The (300 ppm) concentration shows a significant decrease compared with the control group on all the days of the experiment. These results agree with (11) and disagree with (12).

Elevated transaminase (AST) activity in the blood has been considered a sign of tissue damage (13,14). Also, the AST might be elevated by other factors, such as environmental factors, stress, lack of nutrition, during hepatocellular damage, and these enzymes are released into the bloodstream, leading to a rise in serum levels (15).

Alanine Aminotransferase (ALT/GPT)

The ALT data show the highest value in the 60 ppm group (401±3) U/L on the 15th day, and the lowest value in the 300 ppm group (16±3) U/L on the first day. The results show a significant increase in ALT levels for the (60 ppm) concentration on the 15th day compared to the control group (29±2), followed by a reduction to (26±6) U/L on the 30th day compared with the control group.

The (300 ppm) group demonstrates a significant reduction in ALT levels compared to the control group across all days of the experiment, with values of (16±3) U/L on the 1st day, (322±3) U/L on the 15th day, and (36±3) U/L on the 30th day. The current study disagrees with (16).

Diazinon, upon metabolic activation, generates reactive oxygen species (ROS) and oxidative stress, which compromise hepatocellular integrity (17). The liver, being the primary organ for xenobiotic metabolism, accumulates toxic metabolites of Diazinon, leading to lipid peroxidation, mitochondrial dysfunction, and eventually cell necrosis or apoptosis (18). This cellular damage results in the leakage of ALT into circulation, explaining the significant increase in ALT levels observed.

Alkaline phosphatase (ALP)

The ALP results showed that the (300 ppm) concentration had the highest value on the 1st day, (529±4) U/L, while the control group had the lowest value (2021±3) U/L. On the first day, the (60 ppm) concentration showed intermediate levels (367±2) U/L, which gradually decreased to (315±4) U/L on the 15th day, and then slightly increased to (324±4) U/L on the 30th day in both concentrations compared with the control group.

The (300 ppm) concentration showed a significant decrease in ALP levels from the 1st day (529±4) U/L to the 15th day (319±4) U/L, then elevated compared to the control group (285±7) U/L. By the 30th day. The current study agrees with (19,20).

Diazinon has been shown to induce oxidative stress and cell cycle alterations in liver cells, suggesting hepatotoxic effects (21). Liver injury can disrupt normal hepatobiliary transport, potentially leading to bile duct and biliary epithelial cell damage (22). So that the significant increase in alkaline phosphatase (ALP) following Diazinon exposure is primarily associated with liver damage.

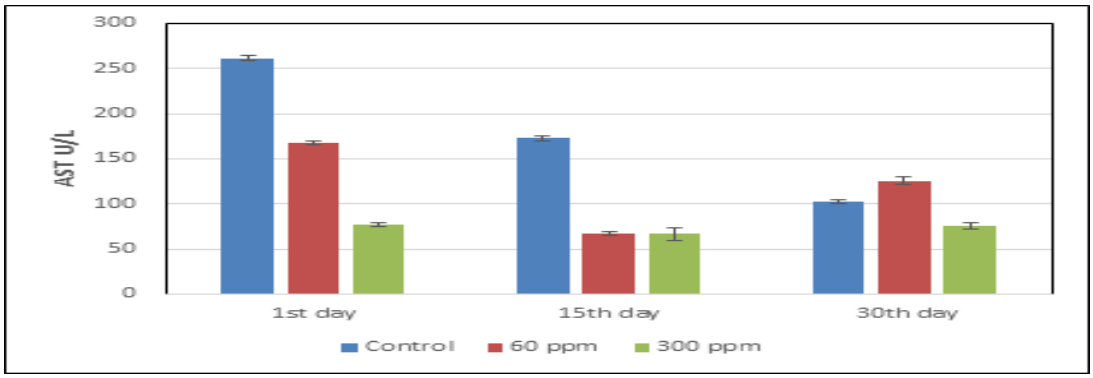

Fig. 1 The AST enzyme activity

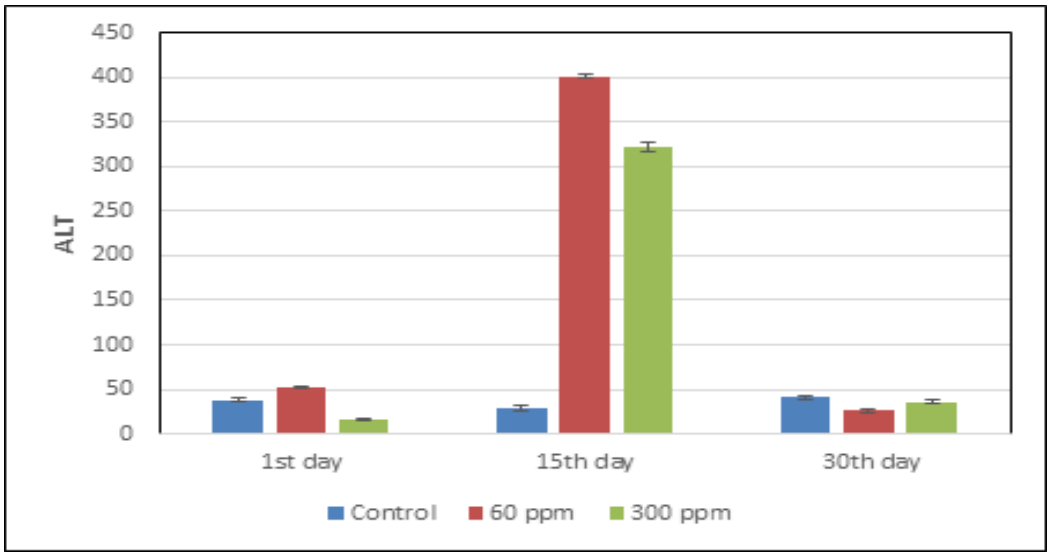

Fig. 2 The ALT enzyme activity

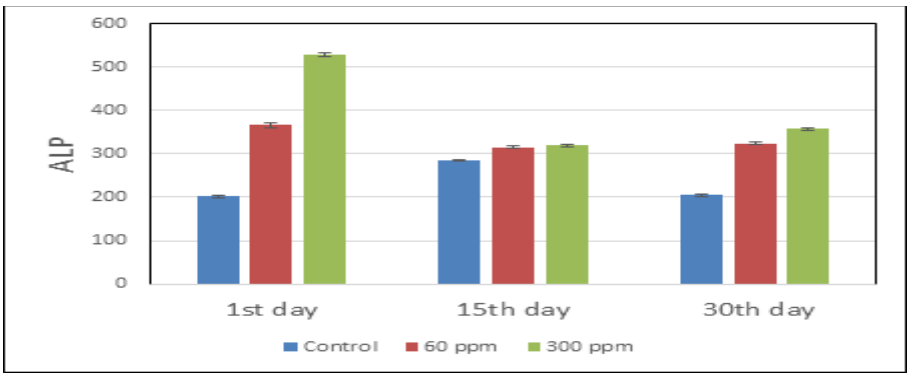

Fig. 3 The ALP enzyme activity

Catalase activity (CAT)

The catalase results show that the (300 ppm) group (160±5) U/mL on the first day, and the lowest value was in the control group (40±3) U/mL on the 30th. The (60 ppm) concentration had intermediate activity (120±4) U/mL on the first day, but decreased to (80±5) U/mL by the 30th day compared to the control group.

The (60 ppm) concentration showed a decreasing pattern but remained higher than the control throughout the experiment. The (300 ppm) concentration showed a significant decrease in CAT activity over the experiment, declining from (160±5) U/mL on the 1st day to (100±4) U/mL by the 30th day, though remaining higher than the control group (40±3) U/mL. The current study agrees with (23).

The metabolism of diazinon can lead to the generation of reactive oxygen species (ROS), including superoxide radicals (O_2^-). These radicals are produced early in the metabolic process, often within hours of exposure (24). Superoxide radicals are turned into hydrogen peroxide by superoxide dismutase (SOD), an enzyme essential for managing oxidative stress. The accumulation of (H_2O_2) triggers the upregulation of catalase (25).

Superoxide Dismutase (SOD)

The results of SOD activity show the highest value in the control group (36.190 ± 3.299) U/mL on the 15th day and the lowest value in the (300 ppm) concentration (16.000 ± 6.928) U/mL on the 30th day. The (60 ppm) showed intermediate activity (35.008 ± 4.593) U/mL on the 1st day, decreasing to (24.556 ± 2.365) U/mL by the 30th day, compared with the control group.

The SOD activity decreased significantly over the excrement, from (33.222 ± 6.669) U/mL on the first day to (16.000 ± 6.928) U/mL by the 30th day, compared with the control group (15.259 ± 2.281) U/mL. The current study agrees with (26) and disagrees with (27).

Diazinon, an organophosphate insecticide, is metabolized into reactive oxygen species (ROS), including superoxide radicals (O2−), which contribute to oxidative stress shortly after exposure. This process is linked to the oxidative metabolism of diazinon and its metabolites, such as diazoxon, which are known to induce oxidative damage in biological systems (28). Superoxide dismutase (SOD) serves as a critical antioxidant enzyme that rapidly converts (O2−) into hydrogen peroxide (H_2O_2) to mitigate oxidative damage. This enzymatic activity represents a first-line defense against ROS-induced cellular damage. The initial rise in SOD activity after diazinon (29).

Malondialdehyde (MDA) activity

The results of MDA levels show the highest value in the (60 ppm) concentration (9.391 ± 0.090) nmol/mL on the 15th day and the lowest value in the control group (1.175 ± 0.015) nmol/mL on the 1st day. The (60 ppm) concentration showed a progressive increase, reaching its maximum (9.391 ± 0.090 nmol/mL) on the 15th day and maintaining high (8.518 ± 1.638 nmol/mL) on the 30th day. Compared with the control group.

The (300 ppm) concentration showed the highest activity level (7.487 ± 1.7) nmol/mL on the 1st day, decreasing to (5.411 ± 1.912) nmol/mL by the 30th day.

Diazinon induces oxidative stress by generating reactive oxygen species (ROS) in the body. ROS are highly reactive molecules that can damage cellular components, including lipids, proteins, and DNA (30).

The increase in ROS leads to lipid peroxidation, where unsaturated fatty acids in cell membranes are oxidized, resulting in the formation of malondialdehyde (MDA). MDA is a stable end product of lipid peroxidation and serves as a reliable marker for oxidative damage (31).

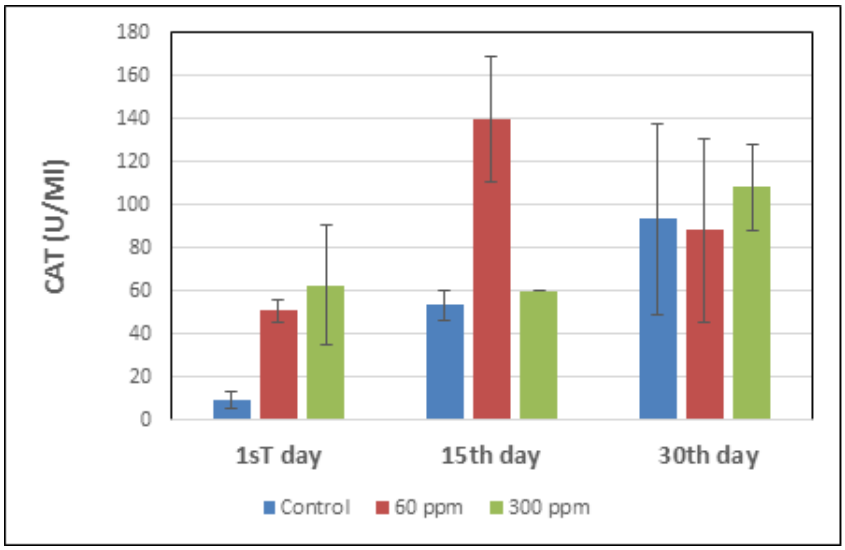

Fig. 4 The CAT activity

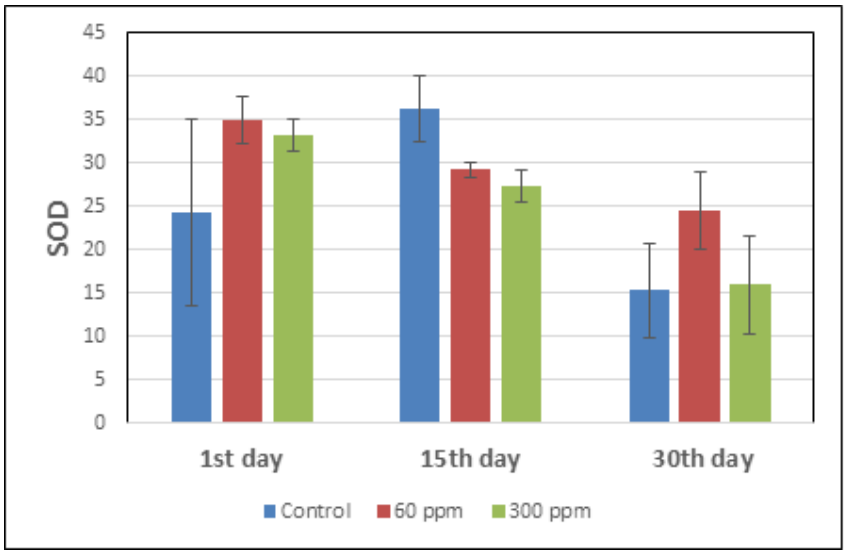

Fig. 5 The SOD activity

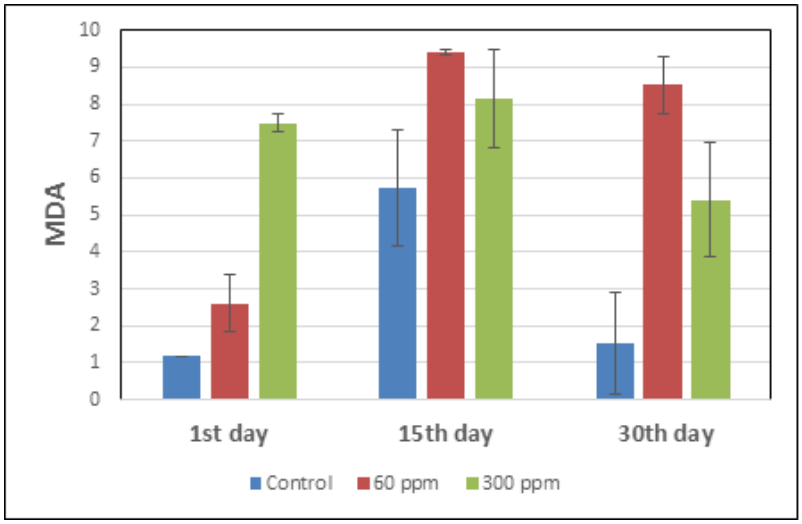

Fig. 6 The activity of MDA

DNA damage (comet assay)

The comet assay analysis showed that the control group had the highest percentage of intact DNA (low comet%: 93.000 ± 4.583%) and the lowest DNA damage (high comet%: 2.667 ± 3.786%). The (60 ppm) concentration shows considerable DNA, with low comet% decreasing to 60.000 ± 6.557%, medium comet% increasing to 26.000 ± 3.606%, and high comet% rising to 12.000 ± 2.000%.

The (300 ppm) concentration had higher damage in the high comet assay (23.000 ± 9.165%), although having a slightly lower median comet percentage (19.333 ± 2.517%) than the (60 ppm) concentration. The current study agrees with (32,33).

The Comet assay results demonstrate a clear dose-dependent increase in DNA damage (measured as tail length) following exposure to diazinon, with the highest damage observed at 300 ppm. This happened due to oxidative stress, represented by ROS, which directly attaches to the DNA and causes a strand break (34,35).

Oxidative stress is a recognized mechanism of DNA damage, and the comet assay is widely used to quantify this effect. ROS can lead to alkali-labile sites and strand breaks, which are effectively detected by this method (36,37).

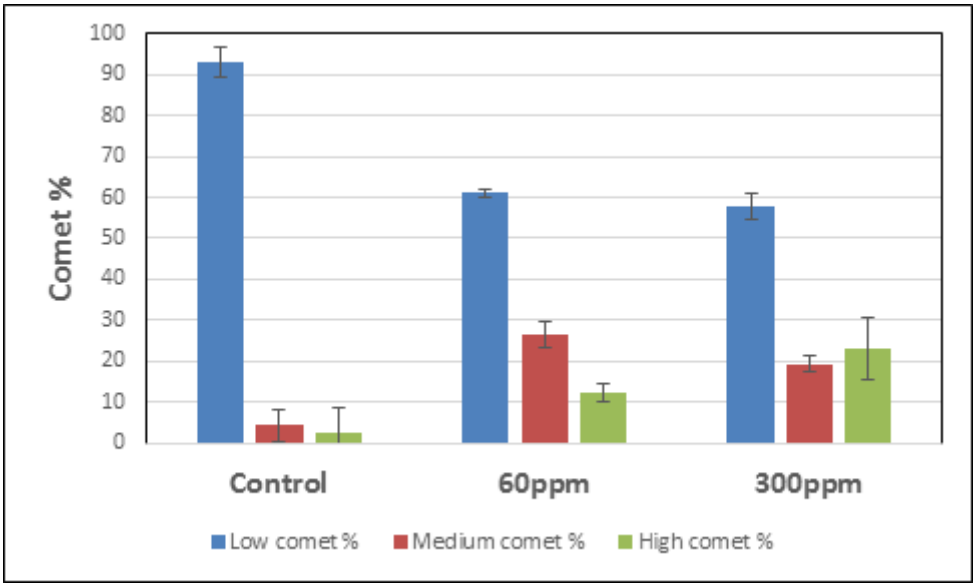

Fig:7 DNA damage (comet assay)

Conclusion

In conclusion, Diazinon has an effect on the liver function antioxidant activities, which shows the toxicity of Diazinon that affects the vital activities, also the genotoxicity of Diazinon, which was vividly clear in the results of the comet assay test.

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Conflict of Interest: NIL

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