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Original Article

Evaluation of the effects of the black mulberry extract against Diazinon induced hepatotoxicity in albino wistar rats.

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Abstract

Background: Diazinon is the globally used organophosphorus compound to which humans are exposed through contamination of food and water. Flavonoids in Morus nigra exhibit a wide range of antioxidant, antimicrobial, anti-inflammatory, anti-cancer, anti-radiation properties and have a protective action against oxidative damage and hepatoprotective effects. The study's objective was to evaluate the effects of black mulberry (Morus nigra) extract against diazinon-induced hepatotoxicity in albino Wistar rats.

Methodology: A quasi-experimental study was conducted from August 2019 to January 2020 at the Department of Pathology, Isra University, Hyderabad, Pakistan. Under standard colony conditions, thirty-six healthy male albino Wistar rats weighing between 200 and 300 grams were randomly divided equally into three groups: Group-1 (Control), Group-2 (given 60 mg/kg Diazinon daily for 4 weeks), Group-3 (administered Diazinon 60 mg/kg with 500 mg/kg of Morus nigra extract daily for 4 weeks through orogastric intubation). Hepatic tissue of all groups was observed under the light microscope. At the same time, hepatic serum markers were also analyzed.

Results: A statistically significant decline in the bodyweight and rise in absolute liver weight of group-3 compared with groups-1 and group-2 ($p<0.05$). Co-administration of Morus nigra significantly lowered serum AST ad ALT levels. Significant histopathological derangement was observed in group-2 hepatic tissues while group-3 rats hepatic tissues had minimal changes and near-normal hepatic parenchyma.

Conclusion: Morus nigra leaf extract has hepatoprotective effects against Diazinon-induced hepatotoxicity in male albino Wistar rats.

Keywords

Antioxidant, Diazinon, Hepatotoxicity, Morus Nigra, Organphospate Poisoning.

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Introduction

Insecticide poisoning is one of the challenging problems that is affecting over a million people worldwide while killing around 100,000 each year[1,2]. For centuries, farmers around the world spray these pesticides in the field pesticides to exterminate organisms that cause diseases in plants and to control insects, weeds, and pests. With the benefits of eliminating pests and insects, these pesticides pose a great threat to the health of farmers, field workers, and people near agricultural farms directly[2]. Moreover, people are also greatly at risk of pesticide toxicity indirectly as these pesticides contaminate the crops and other foods. Consuming these pesticide-contaminated foodstuffs is significantly at risk of pesticide toxicity[2,3].

Diazinon is the widely used organophosphorus compound to which humans are exposed through contamination of food and water[4]. The residues of pesticides stick onto the leafy vegetables and fruits, leading to various health hazards. Diazinon is also used for controlling flies around the animal amenities, public places where food wastes or animal excreta accumulated, and greenhouses. It acts by inhibiting the enzyme acetylcholinesterase and activates cholinergic, muscarinic, and nicotinic receptors[5]. Exposure to organophosphorus insecticides causes salivation, lacrimation, nausea, vomiting, diarrhea, arrhythmias, miosis, involuntary defecation, urination, and arrhythmias[6]. Studies have reported the detrimental effects of Diazinon on many organs, including the brain, gonads, liver, and kidneys[7,8]. Diazinon not only influences the transport of mitochondrial membranes in the rat liver but also disrupts the cytochrome P450 mechanism in the human liver, causing alterations in enzymes levels, biochemical indicators, mitochondrial swelling, and inflicts oxidative stress by generating free radicals[9].

Morus nigra (generally known as Blackberry or Black Mulberry) is farmed all over the world[10]. Morus nigra is a rich amalgam of polyphenols, flavonoids, and anthocyanins, which exert strong antioxidant activity. With the antioxidant properties, Morus nigra causes a protective action against the oxidative damage to the biomolecules as well as membranes resulting from the free radicals[11,12]. Flavonoids in Morus nigra exhibit a wide range of antioxidant, antimicrobial, anti-inflammatory, anti-cancer, anti-radiation properties and have a protective action against oxidative damage and hepatoprotective effects[13]. Morus nigra is a widely grown plant; thus, it can serve as a low-cost and easily available remedy for Diazinon-induced hepatotoxicity and liver diseases.

This study was premeditated to demonstrate the protective effects of Morus nigra on hepatotoxicity induced by Diazinon in albino Wistar rats.

Methodology

This study was a quasi-experimental study that was conducted at the Department of Pathology and Postgraduate Research Laboratory, Isra University, Hyderabad, Pakistan, from August 2019 to January 2020. The study was conducted after getting approved by the ethical and research review committee of Isra University, Hyderabad.

Thirty-six healthy male albino wistar rats weighing between 200 and 300 grams were procured from the animal husbandry of Sindh Agriculture University, Tandojam. The sample size was calculated using the standard power analysis method for animal studies[2,14,15]. The leaves of Morus nigra were also collected from Sindh Agriculture University, Tandojam. After drying the leaves for a week, they were grinding and converted to powder form and then soaked in the methanol aqueous. The solution (Morus nigra leaves powder + methanol) was filtered and evaporated into a gel-like paste. Analytical grade Diazinon PESTANAL® (CAS Number: 336-40-5) was purchased from Sigma-Aldrich.

All the Wistar albino rats were kept for a week in the animal house of Sindh Agriculture University, Tandojam, under standard conditions for acclimatization. All rats were kept in stainless steel cages under standard illumination conditions with 12 hours light-dark cycles at 25±1ºC. The rats were randomly divided into three groups, each having 12

animals. Group-1 (Control) received distilled water ad libitum. Group-2 was given 60 mg/kg BW Diazinon daily using the orogastric tube for 4 weeks. Group-3 was administered Diazinon 60 mg/kg BW daily and 500 mg/kg of Morus nigra extract daily for 4 weeks through orogastric intubation. Towards the end of the experiment. i,e. 24 hours after the last dose, rats were euthanized, and cardiac puncture was done to get blood samples to analyze biochemical liver markers, Alanine transaminase (ALT), and Aspartate transaminase (AST). Liver dissection was done. The liver tissue was processed, and paraffin blocks were prepared. Approximately 5 µm thin sections were sliced, stained with Hematoxylin and Eosin.

The histology of hepatocytes, portal lobule, portal vein, and sinusoids was observed under the light microscope at 200 magnification. The levels of AST and ALT were presented as mean ± SD. SPSS 20 was entering and analyzing data analysis. One-way ANOVA with Post HocTukey's test was applied to establish differences among groups. A statistically significant level was set at a p-value ≤ 0.05.

Results

Table 1 shows the changes in albino Wistar rats' pre-and post-experimental body weights and absolute liver weights. There was a statistically significant decline in the bodyweight of group-2 in comparison with groups-1 and group-3 ($p<0.05$). Similarly, a significant rise in absolute liver weight was observed in group-2 compared to groups-1and group-3 ($p<0.05$). However, the difference between absolute liver weights of groups-1 and group-3 was non-significant (Table 1)

Table 1: Difference in mean body weight and absolute liver weight between experimental groups

Variables	Group 1	Group 2	Group 3	p-value
Initial body weight (gm)	259.7±3.8	262.3±2.4	264.1±3.1	0.380
Final body weight (gm)	276.2±6.4[b,c]	217.9±4.1[a,c]	235.1±2.7[a,b]	0.000*
Absolute liver weight (gm)	1.41±0.07 [b]	1.48±0.09 [a,c]	1.39±0.04[b]	0.000*

[a, b, c] denote the statistical difference between the groups, through post hoc Tukey.
*p<0.05 is considered significant.

Levels of ALT and AST enzymes were significantly raised in the rats belonging to group B. Whereas the co-administration of Morus nigra significantly lowered serum AST ad ALT levels (Table 2).

Table 2: Difference in mean serum Alanine transaminase (ALT) and Aspartate transaminase (AST) levels between experimental groups

Variables	Group 1	Group 2	Group 3	p-value
Serum ALT (U/L)	32.04 ± 11.93[b]	114.49 ± 11.81[a,c]	38.50 ± 2.70[b]	0.000*
Serum AST (U/L)	27.47 ± 10.35[b]	112.8 ± 20.63[a,c]	34.81 ± 4.27[b]	0.000*

[a, b, c] denote the statistical difference between the groups, through post hoc Tukey.
*p<0.05 is considered significant.

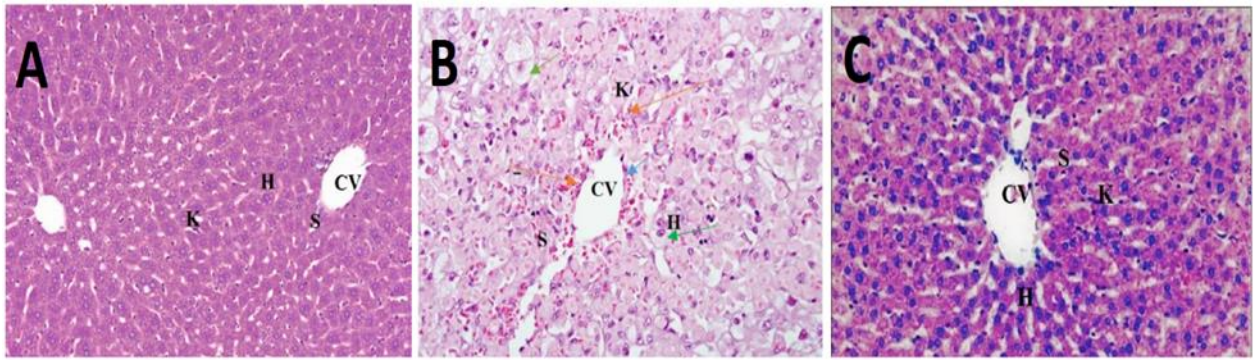

Figure 1. A: Photomicrograph of rat liver (Group-1) showing normal hepatocytes (H), normal central vein (CV), and normal sinusoidal space (S) (H&E, 200x). B: Photomicrograph of rat liver (group-2) showing ballooning and vacuolization of hepatocytes (H) (green arrows), dilated central vein (CV) (blue arrow), and congested sinusoids (S) (orange arrow) (H&E, 200x). C: Photomicrograph of rat liver (group-3) showing normal hepatocytes (H), normal central vein (CV), and normal sinusoidal space (S) (H&E, 200x)

Figures 1 demonstrate the photo-micrographic findings of cut-sections of hepatic tissues of group-1, group-2, and group-3, respectively. Histological examination of liver belonging to control group-1 showed normal hepatocytes and hepatic lobules. Normal Kupffer cells were seen. Liver histology from group-2 revealed vacuolization and ballooning of the majority of the hepatocytes. Some regions of the liver tissue exhibited necrosis. Many intervening hepatic blood sinusoids showed congestion. No congestion was observed in the central vein and sinusoids. Most of Kupffer cells appeared hypertrophied. Congestion was also observed in central veins.

The liver sections of rats belonging to group-3 showed the near-normal histological structure of the hepatic tissue. A near-normal parenchymal arrangement of polygonal hepatocytes was observed. The cytoplasm and the nuclei of the hepatocytes revealed no degenerative changes. Sinusoids also exhibited near-normal features. Kupffer cells appeared near-normal in size, and the central veins were not congested.

Discussion

The injurious effects of pesticides like Diazinonis well have been acknowledged as a serious public health concern during the past decades. Our study revealed histopathological alterations by Diazinon in the liver tissue. Diazinon resulted in damage to the hepatocytes, causes central veinous and sinusoidal congestion. Similar findings were observed by other researchers[7]. Cakici et al. found that Diazinon produces vacuoles in the hepatocytes, increases Kupffer and inflammatory cells, and promotes congestion of the central veins in mice[9]. Ezzi et al. in their research also proved that Diazinon distorts portal triads and congestion of central veins and sinusoids[16].

Diazinon induces histological alterations in the liver by lowering the capacity of antioxidant systems and increasing the levels of free radicals[17]. Research done by Mahmoud et al. revealed that inhalation of Diazinon by male Wistar rats in their study increased the apoptosis in the developing hepatocytes[12].

The liver sections of rats belonging to group-3 showed the normal histological structure of the hepatic tissue. A normal arrangement of hepatocytes was observed. The cytoplasm and the nuclei of the hepatocytes revealed no degenerative changes. Sinusoids exhibited normal features. Kupffer cells appeared normal in size. The central veins were not congested. The results correspond with the findings of Malhi et al., who indicated that

mulberry has protective effects against paracetamol-induced hepatic injury[18].

The increase in serum levels of hepatospecific enzymes like ALT and AST indicates the damage of hepatocytes and severe liver injury. These enzymes are normally stored in the cytoplasm and are released into circulation after the damage of hepatocytes. The current study showed that Diazinon produced a significant rise in the levels of AST and ALT. Haghightjoo et al. also proved that Diazinon causes serious damaging effects in rat's liver. It decreases the total protein in the liver resulting in histological alterations, which result in raised serum levels of hepatic enzymes[19].

However, Morus nigra significantly decreased the AST and ALT levels due to its antioxidant properties. Antioxidants protect the cell membrane integrity and prevent enzyme leakage. They help in scavenging the free radicals[20]. Mulberry extract contains large amounts of tannins, flavonoids, phenols. Therefore, the presence of flavonoids may be responsible for its protective effects on Diazinon-induced liver injury in this study. The results also coincide with the observations of Mallhi et al[18]. Pesticides induce oxidative stress leading to the generation of free radicals and alteration in antioxidants or oxygen-free radical scavenging enzyme systems[6]. Denizet al. proved that Morus nigra protects the liver against carbon tetrachloride, and it can serve as a novel approach for treating various hepatic problems[21]. Free radical scavenging activity is attributed to the flavonoids, mainly quercetin and isoquercitrin found in black mulberry[22].

However, with strengths, this study had certain limitations. The small sample size and shorter duration of the experiment to observe the effects of Morus nigra can be considered as limitations of our study. Another limitation is that the study was conducted on an animal model, so the results cannot be generalized to a human model, so similar studies on humans are recommended.

Conclusion

Morus nigra leaf extract has hepatoprotective effects against Diazinon-induced hepatotoxicity in male albino Wistar rats. Therefore, it can be considered a protective agent against free radical-induced liver damage following exposure to Diazinon. Further studies are needed to investigate and explore the protective mechanism action of Morus nigra extract against Diazinon-induced toxicity. Moreover, studies are needed to evaluate the effect of different doses of Morus nigra leaf, its effects on other organs like kidneys, gastric mucosa, and the use of crude or formulation form of moris nigra leaf extract, etc. in the future.

Conflicts of Interest

The authors have declared that no competing interests exist.

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