Research Article

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Histological and Biochemical Investigation of Hepato-protective Role of Vitamin B12 in Male Wistar Rats Intoxicated by Diazinon

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Abstract In order to estimate the hepato-protective activity of Vit.B_{12} in diazinon hepatotoxic effect, the current study was performed on twenty-four male adult Wistar rats. They'd divided into four equal groups for 30 days: group A (control group) served as control; group B (Dzn group) administered an oral daily dose of 1/10 LD_{50} (3.8mg/ kg.bw) from diazinon; group C (Dzn + Vit.B_{12} group) administered an oral daily dose of 1/10 LD_{50} (3.8 mg / kg bw) from diazinon, in addition to the systemic intramuscular doses of (4 mg/kg) from Vit.B_{12} in daily basis; group D (Vit.B_{12} group) administered daily systemic intramuscular doses of (4 mg/kg) Vit.B_{12} only. The results showed that group C had a significant improvement in the antioxidant indicators compared with group B in which the CAT, GSH, and SOD serum activities showed significant increasing ($P \leq 0.05$) in group C, in addition, it revealed a significant decreasing ($P \leq 0.05$) in the values of MDA and peroxynitrite comparing to the same levels of the group B. The histological results of the liver of group C showed a great improvement compared with group B which showed normal hepatic architecture consisting of normal hepatocytes, and normal sinusoids with normal other hepatic components except mild central vein dilation. The current study concludes that vitamin B_{12} helps to ameliorate the diazinon hepatotoxic effects as a model for a wide spread of organophosphorus compounds in agriculture and veterinary sectors by ameliorating its harmful oxidation on the liver since vitamin B_{12} is cheap and available as a commercial supplement therefore we recommend its uses

Keywords: Histological, Biochemical, Hepato-protective, Vitamin B_{12}, Diazinon

Introduction

Pesticide uses have been felt to improve plant production, particularly to prevent pests and weeds in agriculture (1). The intensive use of pesticides is toxic to end consumer, like animal being by oral intake, air inhalation and direct contact with the skin (2). Hence, diazinon is a pesticide that is used commonly for the purpose of improving production but it may cause harmful effects on both animals and humans. Moreover, diazinon is pesticide belong organophosphorus family characterized by its inhibitory mechanism to the enzyme cholinesterase activities (3). It has less effect on those non-target life organisms and is most toxic to the vertebrate animal compared to those agents of the organochlorine family (4). Diazinon may be neutralized through the liver via hepatocytes by the mechanism of detoxification, but it is capable to produces free radicals as a result of cellular damage; thus the continuous exposure to diazinon lead to triggering liver damage in spite of this organ plays a role in the process of detoxification mechanisms (5). Diazinon and other organophosphorus compounds are metabolized by the liver and may inhibit the acetylcholinesterase enzyme resulting in the increasing of acetylcholine in both nervous and neuromuscular tissues. These increments cause cellular ions to be unstable and lead to the initiations of reactive oxygen radicals, which will be triggering the production of pro-inflammatory cytokines (6).

Vitamin B_{12} (Vit.B_{12}) scientifically noun as cyano-cobalamin. It is a vitamin of watery soluble origin that is important for the body functions and maintenance of both type of nervous organs either central or peripheral nervous systems, it has a considerable role in the white matter and nerves myelination (7). It has crucial antioxidative roles besides its capabilities to the regulation of inflammatory cytokines (8). The synthetic Vit.B_{12} is originate from the cobalamin forms which's commercially available as a supplementary agent to eliminate reactive species as well as its other therapeutic features (9). This work aims to estimate the hepatoprotective effects of Vit.B_{12} ameliorating diazinon toxicity based on histological and biochemical criteria.

Materials and Methods

The study was achieved on twenty-four male adult Wistar rats, which their weights ranged between (200-250) grams and their age between (2-3) months which they housed in the animal's house of veterinary medicine college \ Basrah University. The experimental animals were divided equally into 4 groups: group A (control group) as control given normal saline only; group B (Dzn group) administered an oral daily dose of 1/10 LD_{50} (3.8 mg / kg. bw) from diazinon for 30 days according to (4).; the group C (Dzn + Vit.B_{12} group) administered an oral daily dose of 1/10 LD_{50} (3.8 mg / kg. bw) from diazinon, in addition to intramuscular systemic doses of (4 mg/kg) from Vit.B_{12} in daily basis for 30 days according to (6); the group D (Vit.B_{12} group) given intramuscular systemic doses of (4 mg/kg) from Vit.B_{12} in daily basis for 30 days.

After the experiment was achieved, the samples of blood were collected throughout the cardiac punctures by 5ml syringes, which the sera were prepared at ($3x10^3$) rpm centrifugation for 10 minutes, and then kept at (-20) Cº till it used in the analysis of biochemical criteria. The concentration of glutathione (GSH), the catalase activity (CAT), the superoxide dismutase activity (SOD), the malondialdehyde concentration (MDA), and the peroxynitrite analyzed according to (10-14). The Histological preparation of the liver was achieved as described previously by (15). The statistical analysis was done by one-way ANOVA, and significant differences ($P \leq 0.05$) were analyzed by least's differences (16).

Results

The current results revealed significant decreasing ($P \leq 0.05$) in the CAT, GSH and SOD serum activities in group B (Dzn group) were (2.85±0.18), (2.82±0.3) and (3.1±0.2) respectively among other groups. Besides the levels of MDA and peroxynitrite seems significant increasing ($P \leq 0.05$) were (15.05±0.1) and (14.12±0.9) respectively among other groups as in (Table 1).

Group C (Dzn + Vit.B_{12} group) revealed significant improvement in these antioxidant indicators compared to group B (Dzn group) in which the serum CAT, GSH, and SOD activities revealed significant increasing ($P \leq 0.05$) in group C (Dzn + Vit.B_{12} group) were (4.07±0.9), (4.05±0.9) and (5.59±0.9) respectively compared with group B (Dzn group). In addition, it revealed significant decreasing ($P \leq 0.05$) in MDA and peroxynitrite levels which showed (7.37±0.3) and (9.14±0.1) in the group C (Dzn + Vit.B_{12} group) comparing to the same values in the group B (Dzn group) as in (Table 1).

In addition, the values of GSH, CAT, SOD, peroxynitrite and MDA showed non-significantly differences ($P \geq 0.05$) in the both group A (control group) and group D (Vit.B_{12} group) when they compared closely each other as in (Table 1).

The histological results revealed that group A (control group) had the normal architecture of liver parenchyma which consisted of normal central vein, normal hepatocytes, and normal sinusoids as in (Figure 1). While group B (Dzn group) showed dilation of the central vein, and severe infiltration of inflammatory cells in the hepatic parenchyma and in the area behind the central vein revealed coagulative necrosis and pericentral vein necrosis as in (Figure 2); group C (Dzn + Vit.B_{12}) revealed a normal sinusoid normal hepatocytes architecture with mild central vein dilation, otherwise all other hepatic structures were near to those of normal group as in (Figure 3). Moreover, the histological investigation in group D (Vit.B_{12}) showed normal architecture of hepatocytes, sinusoids, central vein, and other hepatic structures as in (Figure 4).

Table (1): Biomarkers of serum oxidants and antioxidants (Mean±SE)

Group	Peroxynitrite (m\l)	MDA (nmol\ml)	SOD (u\mg)	CAT (u\mg)	GSH (nmol\g)
Group A (control)	9.18±0.5 b	7.16±0.1 b	5.63±0.1 a	4.67±0.3 a	4.33±0.4 a
Group B (Dzn)	14.12±0.9 a	15.05±0.1 a	3.1±0.2 b	2.85±0.18 c	2.82±0.3 c
Group C (Dzn+Vit.B_{12})	9.14±0.1 b	7.37±0.3 b	5.59±0.9 a	4.05±0.9 b	4.07±0.9 b
Group D (Vit.B_{12})	9.1±0.2 b	7.18±0.4 b	5.61±0.3 a	4.62±0.9 a	4.41±0.3 a

- **Small letters when different mean a significant difference between groups.**

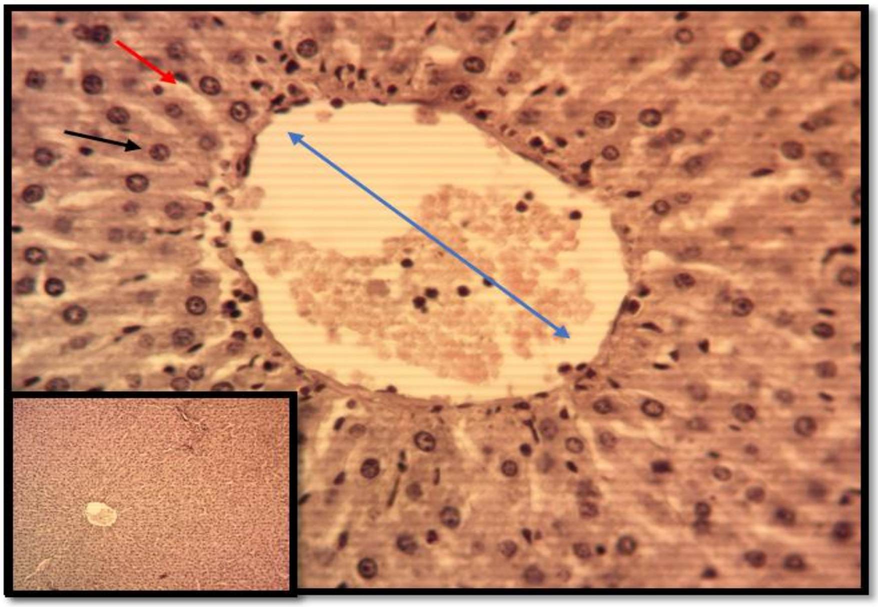

Figure (1): Histological section of liver of group A (control) showing the normal architecture of the central vein (double-headed blue arrow), normal hepatocytes (black arrow), and normal sinusoids (red arrow). H&E stain. 400X.

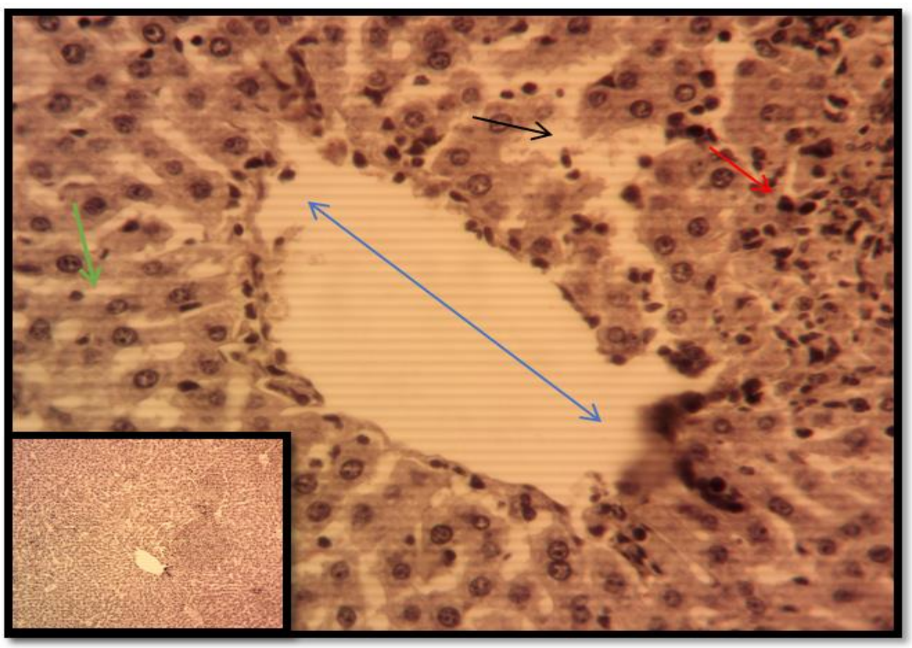

Figure (2): Histological section of liver of group B (Dzn group) showing the dilatation of the central vein (double-headed blue arrow), inflammatory cells infiltration in the hepatic parenchyma (red arrow), pericentral vein necrosis (black arrow), and sinusoidal dilation (green arrow). H&E stain. 400X.

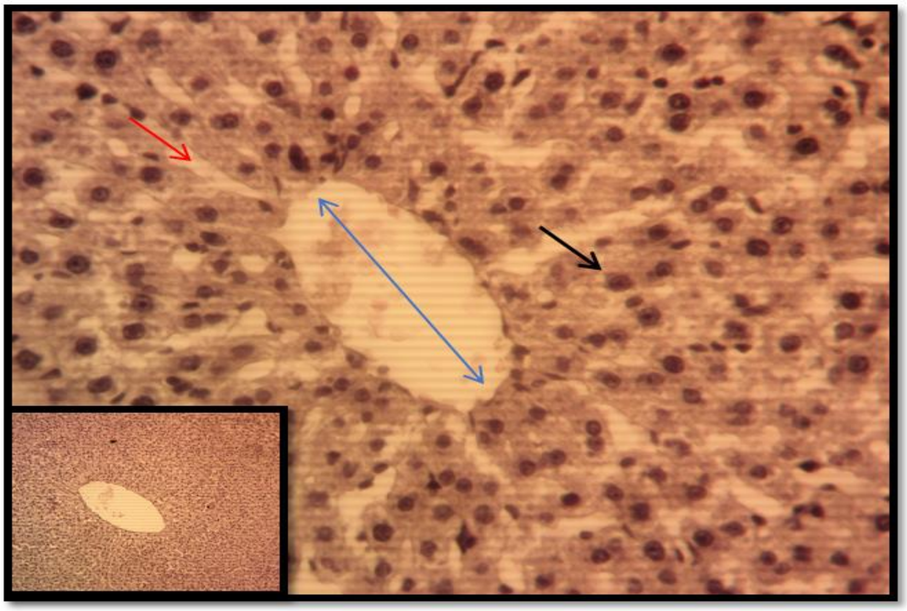

Figure (3): Histological section of liver of group C (Dzn + Vit.B_{12} group) showing mild dilatation of the central vein (double-headed blue arrow), normal hepatic sinusoids (red arrow), normal hepatocytes architecture (black arrow). In addition there are no areas of necrosis or inflammatory cells infiltration in the hepatic parenchyma. H&E stain. 400X.

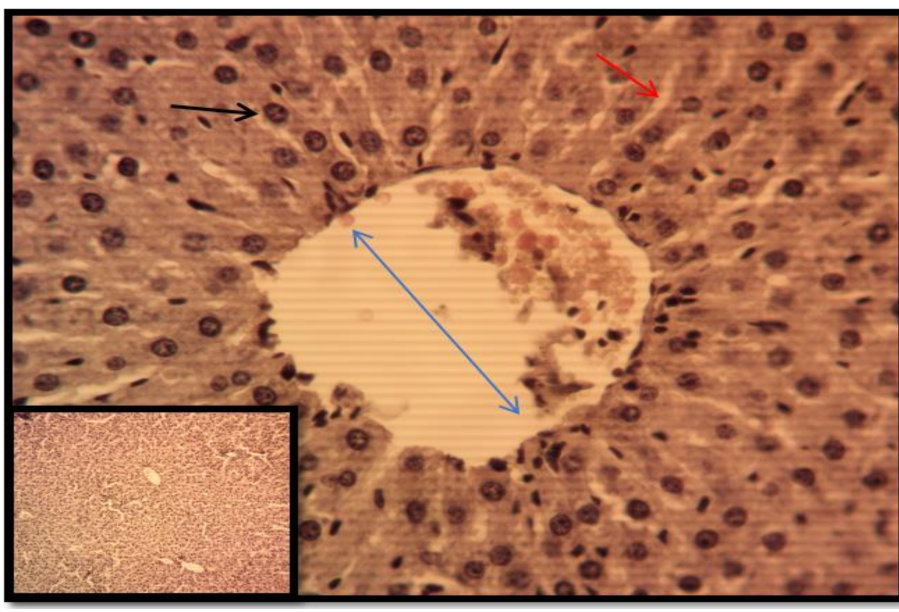

Figure (4): Histological section of liver of group D (Vit.B_{12} group) showing normal central vein (double-headed blue arrow), normal hepatic sinusoids (red arrow), normal hepatocytes architecture (black arrow). H&E stain. 400X.

Discussion

It was reported from many studies that the use of diazinon can cause harmful effects on vital organs such as the liver, brain, kidney, and gonads (17). It affects the transportation of the mitochondrial membrane of the rat's liver thus disturbing the cytochrome P_{450} balancing system in the liver and causing disturbances in liver enzymes as well as the biochemical index and causing mitochondria swelling, via generating oxidative stress and free radicals provoking (18). In the present study, ameliorating the harmful effect of diazinon with vitamin B_{12} revealed significant decreases in peroxynitrite and MDA levels which looked like the normal values mean the vitamin B_{12} can lowering the reactive oxygen species that induced lipid peroxidation and then generated the peroxynitrite, it regards a powerful nitrating oxidant agent which it essentially modifies cellular components and depletion of mitochondrial ATP that lead to hepatocellular necrosis and other sinusoidal affection, these findings agreed with the previous study of (19). Who they reported that the vitamin B_{12} can be lowering MDA and peroxynitrite levels. On other hand, the levels of SOD, CAT, and GSH were significantly improved via using of vitamin B_{12} able to scavenge the oxygen radicals initiated through the effects of diazinon uses and thus leading to improve the mentioned biomarkers by vitamin B_{12} possible ability to reducing the harmful effects of reactive oxygen species, these findings agreed with (20), who reported the beneficial ameliorating effects of vitamin B_{12} against reactive oxygen species. the augment roles of GSH in the protection of tissue from harmful oxidation which act as antioxidant was mentioned previously (21). Besides, it showed a significant improvement in SOD value in vitamin B_{12} group compared with diazinon group, the SOD is an excellent defense mechanism against cellular reactive oxygen species, in which the SOD inhibits the peroxidation of lipids via analysis of the conversation of superoxide's into H_2O_2 and oxygen, so it aligned with (22), who they documented the role of SOD as a protective agent to cells from superoxide oxidation by removing superoxide radicals.

On the basis of histological evaluation of diazinon hepatotoxic effects it seems many changes like dilatation of the central vein, inflammatory cells infiltration in the hepatic parenchyma, precentral vein necrosis, and sinusoidal dilation; it has been found these harmful effects occur due to the metabolism of diazinon occur mainly in the liver and then it generates serious nitrogen and oxygen reactive species that act to increase cellular lipid peroxidation and causing mitochondrial damage leading to cellular swelling and necrosis, these also mentioned by (23), who reported that diazinon causes hepatotoxicity via the mechanism of increasing level of reactive oxygen species. Other researchers aligned with the current evaluation which mentioned that the diazinon causing severe sinusoid's dilation and hepatocellular vacuolar changes, as well as in the portal area inflammatory events and around the central hepatic vein (24). Vitamin B_{12} act as an antioxidant by chelating the metal ions and reactive oxygen species in the liver thus can ameliorating diazinon hepatotoxic effects, therefore the current study showed the liver seems as those of the control group in which there isn't inflammation, and necrosis even the uses of diazinon, these findings agreed with previous study of (25), who they mentioned the antioxidants act on several mechanisms like electrons donation or metal ions chelation, in addition, vitamin B_{12} mainly act by donation of electrons to the free radicals and thus leading to the elimination of reactive oxygen or nitrogen species. Moreover, the cobalt complex in vitamin B_{12} has effectively been shown to inhibit liver inflammation and also liver fibrosis, as well as to the protective effect of vitamin B_{12} in experimental hepatic injuries induced by heavy hepatotoxic agents like carbon tetrachloride (26).

Conclusion

The current study concluded vitamin B_{12} uses helps in ameliorating the hepatotoxic effects of diazinon as a model for a wide spread of organophosphorus compounds in agriculture and veterinary sectors by ameliorating its harmful oxidation on the liver since vitamin B_{12} is cheap and available as a commercial supplement.

Acknowledgments

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التحري النسيجي والكيميائي الحيوي للدور الواقي لفيتامين ب 12 في ذكور الجرذان المختبرية

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الخلاصة

لغرض تقييم الدور الواقي لفيتامين ب 12 ضد التأثير السمي الكبدي للديازنون فقد أجريت هذه الدراسة على (24) جرذ مختبري ذكر وقد تم تقسيمهم الى اربع مجاميع متساوية ولمدة (30) يوم متتالية، حيث كانت المجموعه (أ) كمجموعة سيطرة، المجموعة (ب) اعطيت جرع فموية يومية من الديازنون بمقدار (1\10) من نصف الجرعة القاتلة والبالغة (3.8) ملغم لكل كغم من وزن الجسم ، اما المجموعة (ج) فاعطيت جرع فموية يومية من الديازنون بمقدار (1\10) من نصف الجرعة القاتلة والبالغة (3.8) ملغم لكل كغم من وزن الجسم اضافة الى جرع عضلية يومية من فيتامين ب (12) بمقدار (4) ملغم \ كغم من وزن الجسم ، اما مجموعة (د) فقد اعطيت فقط جرع عضلية يومية من فيتامين ب (12) بمقدار (4) ملغم \ كغم من وزن الجسم. اظهرت النتائج وجود تحسن معنوي في معايير قيم المؤشرات المضادة للاكسدة عند استخدام فيتامين ب 12 في مجموعة ج مقارنتا مع المجموعة ب الحاوية على الديازنون فقد لوحظ زيادة معنوية بقيم GSH , CAT , SOD بينما شوهد نقصان معنوي بقيم MDA و peroxynitrite عند مقارنتها مع مجموعة ب الحاوية على الديازنون. اما نتائج الفحص النسيجي للكبد فقد سجل تحسن ملحوظ لمجموعة ج الحاوية على فيتامين ب 12 مقارنتا بمجموعة ب الحاوية على الديازنون فقد كانت النتائج قريبة لمثيلاتها في مجموعة السيطرة حيث اشارت الى مظهر نسيجي طبيعي للكبد متمثلا بمظهر سليم للخلايا الكبدية و الجيبانيات الكبدية و الوريد المركزي عند مقارنتها بمجموعة ب الحاوية على الديازنون. تستنتج الدراسة الحالية ان لاستخدام فيتامين ب12 دورا حاميا للكبد من استخدام المركبات العضوية الفسفورية كالديازنون نتيجة لكثرة استخدامها في القطاعين الزراعي والبيطري واضافة الى كون فيتامين ب 12 متوفر بشكل مكمل غذائي و رخيص الثمن لذلك تنصح هذه الدراسة بأستخدامة.

الكلمات المفتاحية: علم الانسجة، الكيمياء الحيوية، الحامي الكبدي، فيتامين ب12 ، الديازنون