



## The Neuro-protective Role of Vitamin B<sub>12</sub> in Diazinon Poisoned Male Wistar Rats: Histopathological and Biochemical Evaluation



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THE protective role of Vit.B<sub>12</sub> on neurotoxic effect of diazinon has been studied and conducted on 24 adults male wistar rats were divided equally into 4 groups, the group I was used as normal control; group II was orally given 1/10 LD<sub>50</sub> (3.8mg/kg.bw) of diazinon for one month; group III was orally given 1/10 LD<sub>50</sub> (3.8mg/kg.bw) of diazinon plus systemic injection (I.M route) of Vit.B<sub>12</sub> (4 mg/kg.bw) for one month; group IV was given systemic injection (I.M route) of Vit.B<sub>12</sub> (4 mg/kg.bw) only for one month. Biochemically it revealed a significant (P≤0.05) increase in GSH, CAT and SOD values in both Vit.B<sub>12</sub> groups (group III and group IV) as well to control group when compared to diazinon group (group II); while the values of MDA and peroxynitrite revealed a significant (P≤0.05) decreases in the both Vit.B<sub>12</sub> groups (group III and group IV) as well to control group when compared to diazinon group (group II); moreover, the histopathological results of the diazinon group (group II) revealed a serious neurologic changes included necrotized neurons, diffuse neuronal edema, perivascular edema and hyperplasia of glial cells; while the Vit.B<sub>12</sub> groups showed no effective histopathological changes were more or less to control group. There are a little information has been provided concerning the relation between diazinon and Vit.B<sub>12</sub>, therefore, the current study concluded that the Vit.B<sub>12</sub> has a significant protective role against the neurological toxic effects of diazinon on basis of histopathological and biochemical patterns.

**Key words:** Histopathological, Neuron, Vitamin B<sub>12</sub>, Diazinon, Toxicity.

### Introduction

The vitamin B<sub>12</sub> (Vit.B<sub>12</sub>) is a water soluble vitamin almost important for hematopoiesis, nervous system homeostasis, gastrointestinal maintenance and Vit.B<sub>12</sub> metabolically regulation [1].

The hematopoietic as well as the neurological disorders are important clinical features therefore, it links to severe and life threatened diseases; besides the hematopoietic aspect is the highly pathognomonic, thus the primary indicator for Vit. B<sub>12</sub> insufficiency was the disorders of neurological aspects show a variation range and don't receive due a recognition as the effect of the Vit.B<sub>12</sub> insufficiency clinically, as well as the neurological

disorders are the earliest clinical signs of Vit.B<sub>12</sub> deficiency [2,3].

The neurological and the hematological disorders occur together, overall, it may assume that near to 60% of patients with pernicious anemia were manifested a signs of funicular myelosis. About 25% of patients with deficiency of Vit. B<sub>12</sub> as well as the neurological disorders that do not show any hematological disorders, there is a correlation between the severity of the neurological and hematological variation [4].

Diazinon is an organic phosphorous pesticides, it is a synthetic chemical substances characterized by broad-spectrum insecticide activity [5].

The diazinon was released to experimental estimation in the 1950's, therefore, nowadays it is used extensively in commercial and indoor application for control cockroach, lice and flies on sheep as well as the insect of plants, food crops, parasites and insects in fields [6, 7].

Diazinon causes a serious damage to different tissues either in human and animals such as neurotoxicity, myocardial injury, cytotoxicity and other systemic dysfunctions by inhibition of acetyl cholinesterase and up regulation of its receptors due to acetylcholine accumulation [8].

The wildlife animals and even the domesticated cattle and birds may expose to diazinon via drinking water or by absorbing it via feet and legs, through consume grass and grains, or by ingesting the toxic agent that impregnated in a carrier particles [9]. The widely spreading of pesticides are connected with problems of pollutions and health hazards [10]. Thus, this study aimed to investigate the neurological protective role of Vit.B<sub>12</sub> against the neurological toxic effects of diazinon.

### Material and Methods

The current study was performed on 24 adult male Wistar rats weighing 200-250 grams at animals laboratory house of veterinary medicine college at University of Basrah, the animals were divided to 4 equal groups: group I was served as normal control; the group II was orally given 1/10 LD<sub>50</sub> (3.8mg/kg.bw) of diazinon daily for 30 days according to Ahmed and Alwan [5]; the group III was orally given 1/10 LD<sub>50</sub> (3.8mg/kg.bw) of diazinon, and a systemic (I.M route) of (4 mg/kg) injections of vitamin B<sub>12</sub> daily for 30 days according to [11]; the group IV was administered only a systemic (I.M route) of (4 mg/kg) injections of vitamin B<sub>12</sub> daily for 30 days.

### Blood samples collection

The samples of blood were collected through cardiac punctures by 5ml disposable syringes, then the sera were prepared via centrifugation of blood in 3x10<sup>3</sup> rpm for ten minutes, then frozen at -20C° till it used for biochemical analysis.

### Serum biochemical analysis

The glutathione concentration (GSH) was analyzed according to Burtis and Ashwood [12]. The catalase (CAT) activity according to Goth [13], the superoxide dismutase (SOD) activity according to Winter bournm et al. [14], the malondialdehyde (MDA) concentration according to Buege and Aust [15]. The peroxy nitrate according to Vanuffelen et al.[16].

### Histopathological examination

The histological processes of brain were performed according to Finkbeiner et al. [17].

### Statistical analysis

The statistical analysis was done according to one-way analysis basis in variance (ANOVA); thus, the significant (P≤0.05) difference was determined by used a least significant difference [18].

### Results

#### Biochemical findings

The serum GSH, CAT were significantly (P≤0.05) increased in group III (Diazinon + Vit.B<sub>12</sub>) which showed 4.070.9± and 4.050.9± when compared to group II (Diazinon group) which showed 2.820.3± and 2.850.18± respectively; while the SOD values showed significant (P≤0.05) increases among group I (control group), group III (Diazinon + Vit. B<sub>12</sub>) and group IV (Vit.B<sub>12</sub> group) which showed 5.630.9±5.59 ,0.1± and 5.610.3± respectively as compared to group II (Diazinon group only) was 3.1±.2 (Table 1).

TABLE 1. The serum oxidant & antioxidant biomarkers.

	GSH (nmol/g) Mean ± SE	CAT (u/mg) Mean ± SE	SOD (u/mg) Mean ± SE	MDA (nmol/ ml) Mean ± SE	Peroxy nitrite (m/l) Mean ± SE
Group I Control	4.33±0.4 a	4.67±0.3 a	5.63±0.1 a	7.16±0.1 b	9.18±0.5 b
Group II Diazinon	2.82±0.3 c	2.85±0.18 c	3.1±0.2 b	15.05±0.1 a	14.12±0.9 a
Group III Diazinon + Vit.B <sub>12</sub>	4.07±0.9 b	4.05±0.9 b	5.59±0.9 a	7.37±0.3 b	9.14±0.1 b
Group IV Vit.B <sub>12</sub>	4.41±0.3 a	4.62±0.9 a	5.61±0.3 a	7.18±0.4 b	9.1±0.2 b

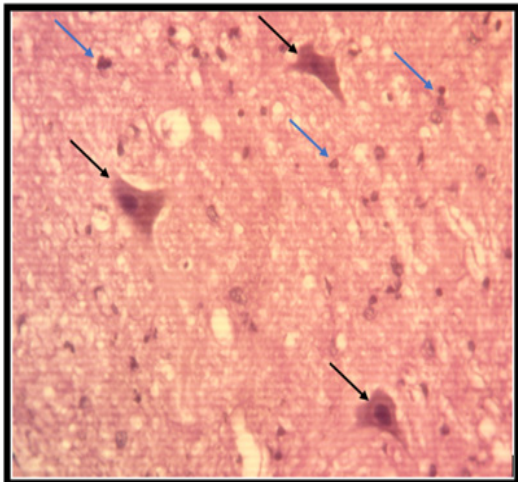
\* letters vertically when differs that referred to a presence of significant differences among groups.

Besides, the MDA and peroxynitrite mean values showed significant ( $P \leq 0.05$ ) increases in group II (Diazinon group) that showed  $15.1 \pm 0.03$  and  $14.120.9 \pm$  respectively when compare to other groups  $7.160.4 \pm 7.18$ ,  $0.3 \pm 7.37$ ,  $0.1 \pm$  and  $9.180.2 \pm 9.1$ ,  $0.1 \pm 9.14$ ,  $0.5 \pm$  of MDA and peroxynitrite values in the group I (control group), group III (Diazinon + Vit.B<sub>12</sub>) and group IV (Vit. B<sub>12</sub> group) respectively (table 1).

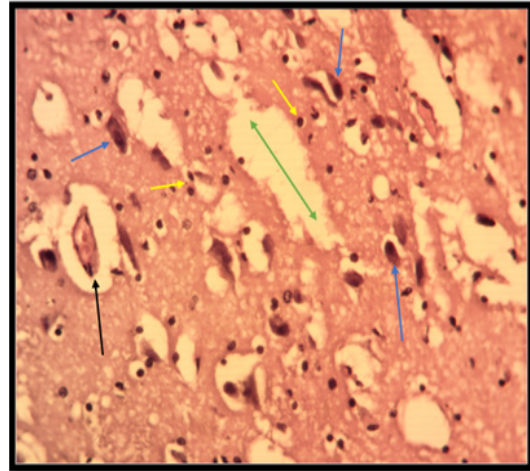
#### *Histopathological findings*

The histopathological results of control of cerebrum showed a normal neuronal parenchyma

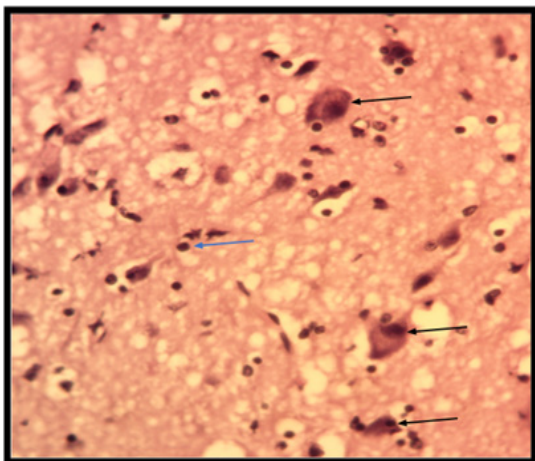
consisted intact neurons and other glial cells as in fig.1; whereas, the histopathological examination of cerebrum of group II (Diazinon group) showed a perivascular edema, necrotized neurons, hyperplasia of glial cells and diffuse interneuronal edema as in fig.2; moreover the group III (Diazinon + Vit.B<sub>12</sub>) showed normal cerebral parenchyma consisted from normal neurons, and mild hyperplasia of glial cells as in fig.3; while the group IV (Vit.B<sub>12</sub>) revealed normal cerebral parenchyma consist from normal neurons, and normal glial cells as in fig.4.



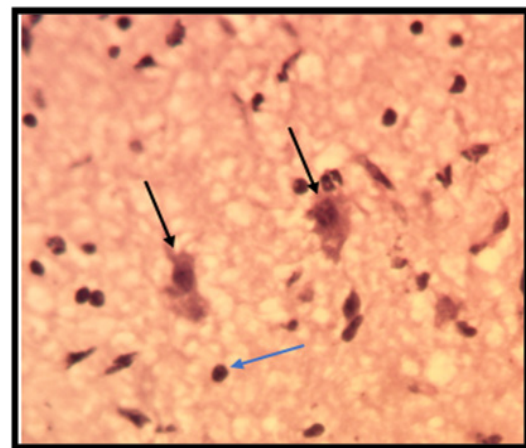
**Fig.1.** Histologic view of brain (cerebrum) of group I shows normal architectures of brain parenchyma consist from normal neuron (black arrows), and normal glial cells (blue arrows). H. &E. Stain. 400X.



**Fig.2.** Histologic view of brain (cerebrum) of group II shows perivascular edema (black arrows), necrotized neurons (blue arrows), hyperplasia of glial cells (yellow arrows), and diffuse interneuronal edema (green arrows). H. &E. stain. 400X.



**Fig.3.** Histologic view of brain (cerebrum) of group III shows normal architectures of brain parenchyma consist from normal neuron (black arrows), and mild hyperplasia of glial cells (blue arrows). H. & E. Stain. 400X.



**Fig.4.** Histologic view of brain (cerebrum) of group IV shows normal architectures of brain parenchyma consist from normal neuron (black arrows), and normal glial cells (blue arrows). H.&E. Stain. 400X.



## Discussion

The main scope of the current study is that Vit. B<sub>12</sub> can significantly decrease the diazinon induced neuronal damage in animal model; in which the diazinon may inhibit the acetylcholine esterase activity through a phosphorylation the hydroxyl serine group that resulted in acetylcholine accumulation [19]. The current study revealed that the significant increases in serum GSH, CAT and SOD in Vit.B<sub>12</sub> treated animals, this may due to the Vit.B<sub>12</sub> enriched the antioxidant activities in the body leading to ameliorating the toxic effects of diazinon therefore, the Vit.B<sub>12</sub> contributes significant myelin sheath synthesis as well as the epithelial tissues regeneration as a coenzyme principal of fatty acid in addition, to carbohydrate and nucleic acid metabolism [2].

Besides, the MDA and peroxy nitrite mean values appeared significantly elevated in diazinon treated animals in which the diazinon can generate and increasing oxidative stress then leading to imbalance among the oxidants and antioxidants [20]. In addition, it reported that the organic phosphorus compound could cause serious oxidative stress by inhibiting enzymatic and non-enzymatic antioxidant balance [21]. Moreover, the diazinon acts as neurotoxins in the environment that suggested causing lipid peroxidation [22]. Diazinon is one of the most insecticide that it can induce a serious oxidative stress, which leads to generate a free radicals that altered the antioxidants and the enzymes of reactive oxygen species scavenging [23].

The histopathological results of brain of group II (Diazinon group) revealed a perivascular edema, necrotized neurons, hyperplasia of glial cells and diffuse interneuronal edema, these changes were occurred due to the effects driven by the reactive oxygen species that generated by the oxidant action of diazinon on neuronal tissues lead to serious histopathological changes, this idea agree with Sonei et al.[24] who found the exposure to the pesticide may initiate a effective changes to the system of antioxidants and lead to oxidative damages in treated mice brain, associated with their study on diazinon which showed a decreases brain acetyl choline activity in mice. Besides, the Vit.B<sub>12</sub> treated animals showed normal brain parenchyma consist of normal neurons that attribute to the protective role of Vit.B<sub>12</sub> on ameliorating the toxic effects of diazinon by reducing the oxidative stress to the neuronal tissue, this investigation agrees with

recent studies reported that Vit.B<sub>12</sub> may promote an axonal growth of neurons after injury, which is used to treatment particularly the peripheral nerves damages clinically [25]. Also, the Vit.B<sub>12</sub> is used in patients suffered from nerve damage, it was mentioned that Vit.B<sub>12</sub> promoted when it used experimentally in rats a recovery processes in peripheral nerve damage [26]. The Vit.B<sub>12</sub> was showed as a scavenger for superoxide that contributed for axonal growth of the neurons [27]. Thus, the current study at an idea for the role of Vit.B<sub>12</sub> in enhancement the axonal formation of neurons by reducing neuronal necrosis and / or apoptosis as well as the stabling the microtubule structures.

## Conclusions

There are a little information has been provided concerning the relation between diazinon and Vit. B<sub>12</sub>, therefore, the current study concluded that the Vit.B<sub>12</sub> has a significant protective role against the neurological toxic effects of diazinon on basis of histopathological and biochemical patterns.

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## Ethical consideration

This study was carried out in accordance to the ethical rules for samples handling and animal's managements and researches, Veterinary Medicine College, Basrah University, Iraq.

## Conflict of interest

The authors declare that they have no competed interest.

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### الدور الوقائي للأعصاب ليفيتامين ب ١٢ في ذكور الجرذان المعرضة للديازينون: تقييم نسيجي مرضي وكيميائي حيوي

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تم دراسة التأثير الوقائي للأعصاب ليفيتامين ب ١٢ ضد تأثير الديازينون من خلال استخدام ٢٤ جرذ ذكر تم تقسيمهم الى اربع مجاميع بصورة متساوية حيث كانت المجموعة الأولى مجموعة سيطرة سالبة اما المجموعة الثانية فأعطيت ١٠/١ من نصف الجرعة القاتلة للديازينون (٣,٨ ملغم لكل كغم من وزن الجسم) فمويًا لمدة شهر واحد، بينما المجموعة الثالثة أعطيت ١٠/١ من نصف الجرعة القاتلة للديازينون (٣,٨ ملغم لكل كغم من وزن الجسم) فمويًا مع (٤ ملغم لكل كغم من وزن الجسم) من فيتامين ب ١٢ بالعضلة لمدة شهر واحد ، اما المجموعة الرابعة فأعطيت (٤ ملغم لكل كغم من وزن الجسم) من فيتامين ب ١٢ بالعضلة لمدة شهر واحد. أظهرت النتائج الكيموحيوية وجود زيادة معنوية في قيم GSH و CAT و SOD في المجاميع المستخدمة بها فيتامين ب ١٢ ومجموعة السيطرة مقارنة مع مجموعة الديازينون بينما أظهرت النتائج وجود انخفاض معنوي في قيم MDA و peroxynitrite في المجاميع المستخدمة بها فيتامين ب ١٢ ومجموعة السيطرة مقارنة مع مجموعة الديازينون. وأظهرت النتائج النسيجية المرضية لمجموعة الديازينون وجود تغيرات عصبية تضمنت نخر الخلايا العصبية وترسب سوائل منتشر (وذمة عصبية) حول الخلايا العصبية للدماغ وكذلك الأوعية الدموية الدماغية مع تكاثر للخلايا الدبقية في المخ، بينما لم تلاحظ أي تغييرات نسيجية في الدماغ في المجاميع التي استخدم فيها فيتامين ب ١٢. لذلك استنتجت الدراسة الحالية ان ليفيتامين ب ١٢ دورا واقيا حيويًا للنسيج العصبي في الدماغ ضد التأثير السمي للديازينون.

**الكلمات المفتاحية:** الانسجة المرضية، الخلية العصبية، فيتامين ب ١٢، ديازينون، التسمم.