

# Histological Effect of Ursolic Acid, Hyperinsulinemia and Vitamin B Complex on Sciatic Nerve Repair and Lipid Profile of Alloxan induced Diabetic Rabbits

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## Abstract

This study aimed to evaluate the effects of ursolic acid, hyperinsulinemia and vitamin B complex on sciatic nerve repair in diabetes mellitus male rabbits, determination of their effects on lipid profile and histological structures of the sciatic nerve. The results of our study showed that alloxan induced diabetes caused a significant increase ( $p<0.05$ ) in serum cholesterol and triglyceride while treatment of extracted and standard ursolic acid, hyperinsulinemia and vitamin B complex caused a significant decrease ( $p<0.05$ ) in serum cholesterol and triglyceride. There are significantly decreased ( $p<0.05$ ) in serum high density lipoprotein (HDL) and significantly increase ( $p<0.05$ ) in serum low density lipoprotein (LDL) is found in induced diabetic and hyperinsulinemia groups while there is a significant increase ( $p<0.05$ ) of HDL in all treated groups except hyperinsulinemic group, on the other hand, there is a significant decrease ( $p<0.05$ ) in LDL, especially in extracting ursolic acid group ( $p<0.05$ ). Histological sections of sciatic nerve confirmed there is a degeneration of nerve fibers appears as areas of cloudy swelling and fatty degeneration of the nerve fibers due to nerve crush. There are various improvement changes due to the different treatment type.

**Keywords:** Histological, Ursolic Acid, Hyperinsulinemia, Vitamin B Complex, Sciatic Nerve, Lipid Profile, Alloxan, Diabetic, Rabbits.

## الخلاصة

تهدف هذه الدراسة إلى تقييم تأثير حامض الأورسوليك، فرط الأنسولين وفيتامين ب المركب على إصلاح العصب الوركي في ذكور الأرانب المصابة بمرض السكري، وتحديد آثارها على الدهون في الدم وكشف آثارها على التركيب النسيجي للعصب الوركي. وأظهرت نتائج هذه الدراسة أن السكري المستحدث بالآلوكسان تسبب في زيادة نسبة الكوليسترول والدهون الثلاثية في الدم في حين العلاج باستخدام حامض الأورسوليك المستخلص والقياسي، فرط الانسولين وفيتامين ب المركب سبب انخفاضاً كبيراً في نسبة الكوليسترول والدهون الثلاثية في الدم ( $p<0.05$ ).

هناك انخفاض ملحوظ في البروتين الدهني عالي الكثافة (HDL) وزيادة كبيرة في البروتين الدهني منخفض الكثافة (LDL) في الدم وجد في مجموعات السكري وفرط الانسولين، في حين أن هناك زيادة كبيرة في (HDL) في جميع مجاميع العلاج باستثناء مجموعة فرط الانسولين. من ناحية أخرى، هناك انخفاض ملحوظ في (LDL)، خاصة في مجموعة حامض الأورسوليك المستخلص ( $p < 0.05$ ). لقد بينت المقاطع النسيجية للعصب الوركي انحلال الألياف العصبية يبدو كمناطق تورم غير واضحة وتحلل دهني للألياف العصبية بسبب عملية سحق العصب. بعد العلاج هناك العديد من التغييرات المختلفة التي دلت على وجود تحسن في العصب الوركي اختلفت هذه التغييرات وفقا لنوع العلاج.

**الكلمات المفتاحية:** الأنسجة، حامض الاورسوليك، فرط الأنسولين، فيتامين ب المركب، العصب الوركي، مستوى الدهون، الألوكسان، السكري، الأرانب.

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## 1-Introduction

Diabetes Mellitus (DM) is metabolic variation categorized by the incidence of chronic hyperglycemia accompanied in a different degree, by alterations in the metabolism of protein, lipids and carbohydrate [1] as an effect of disorder on insulin secretion, insulin activity or both. The diabetes type depends on the suppose etiology. It consists of two types of diabetes mellitus widely prevalent that are diabetes mellitus type1 and type 2 [2]. The traditional signs of diabetes are polyuria, polydipsia, and polyphagia [3]

Ursolic acid (UA) ( $3\beta$ -hydroxyurs-12-en-28-oic acid) is a pentacyclic triterpenoid that exists in various plants and is a component of numerous herbal medicines [4]. It is the major effective component among the other triterpenoids in apple peels [5]. Ursolic acid has antibacterial, antifungal, insecticidal, anti-HIV, complement inhibitor, diuretic, antidiabetogenic and gastrointestinal system adjusting actions [6] [7]. It has antitumor action [8], and produces anti-inflammatory influence in cells with definite inflammation, but in normal cells, it can be pro-inflammatory [9].

Hyperinsulinemia is a situation with a level of blood insulin greater than normal level in individuals without diabetes. Although hyperinsulinemia is not diabetes, it is usually accompanied with diabetes type2. [10] [11]

The sciatic nerve, innervating the hind limb; it involves mixed of motor and sensory axons and is usually used as a model for studying regeneration of the nerve and its regeneration is associated with a variety of changes in the dorsal root ganglion (DRG) neuron cell bodies and regenerates is associated with the expression of new genes and proteins [12]

## 2-Materials and Methods

### 3-Animals

Fifty healthy-mature male local rabbits (*Lepus cuniculus*) obtained from the local market- places of Basrah, their weights ranged from (900 g – 1.900 g) and ages between 5–8 months were adapted for one week in an animal house of the College of Veterinary Medicine/ Basrah University. The experimental period lasts for seven weeks included (one week – adaptation period, three weeks – DM induction, one week – sciatic nerve crush surgery and two week treatment period). The animals were distributed into five groups (ten animals in each group). Diabetes Mellitus was induced in all groups by 200 mg/ kg intravenous injection of Alloxan [13]; each group divided into two subgroups without and with sciatic nerve crush operation by an incision made over the lateral aspect of the hind limb. The sciatic nerve of the left limb was crushed at the mid-thigh level using a small hemostatic forceps for a period of 60 Second [14]; then each group except (G1+ve control/ diabetic rabbits) treated with different treatment for two weeks, the treatments include extracted ursolic acid (50 mg/ kg) [15], standard ursolic acid (50 mg/ kg) [15], hyperinsulinemia (1.2 IU/ kg Insulin) [16] and Vitamin B complex (2 mg/ kg) [17], respectively.

### 4-Lipid Profile Measurements:

#### 4.1-Determination of Serum Cholesterol

Serum Cholesterol level in the blood was measured by the enzymatic method of a commercial kit (BioMerieux; France) using the enzymatic method of [18]

##### 4.1.1 - Determination of Serum Triglyceride

Serum triglyceride was measured by (BioMerieux; France) Kit depending on the enzymatic method of [19].

**Determination of Serum HDL and LDL** The measurement was done by the (BioMerieux; France) Kit depending on the enzymatic method of [20] .

### 5-Histological parameter

The sciatic nerve was isolated and fixed in 10% formalin. The fixed tissues were processed routinely for paraffin embedding; the sections were deparaffinized using xylene and dehydrated in a gradient of alcohol solutions. The Sciatic nerve sections were stained with Hematoxylin and Eosin stain as a routine histological stain used to demonstrate the general composition of the tissue [21].

## 6-Statistical Analysis

All data were expressed as means $\pm$ SD. Significant differences among the experimental groups were determined by one way ANOVA method analysis of variance. Statistical significance was considered significant at  $p<0.05$  [22].

## 7-Results

## 8-Lipid Profile Measurements

The Lipid Profile Measurements for serum cholesterol, triglyceride, HDL and LDL were clarified in the tables listed below respectively.

**Table (1):** The effect of ursolic acid, hyperinsulinemia and vitamin B complex on serum cholesterol and serum triglyceride of alloxan induced diabetic rabbits.

Groups	Subgroups	Parameters	
		Cholesterol mg/dl	Triglyceride mg/dl
G1 +Ve control (Diabetes only)	With Crush	235.200 $\pm$ 12.029 B	128.400 $\pm$ 3.507 C
	Without Crush	253.000 $\pm$ 37.536 C	180.880 $\pm$ 19.243 D
G2 Extracted UA	With Crush	51.333 $\pm$ 0.419 D	66.338 $\pm$ 0.247 E
	Without Crush	22.048 $\pm$ 0.157 E	89.328 $\pm$ 0.315 A
G3 Standard UA	With Crush	49.476 $\pm$ 0.106 F	142.386 $\pm$ 0.169 F
	Without Crush	174.149 $\pm$ 0.155 G	152.145 $\pm$ 0.123 F
G4 Hyperinsulinemia	With Crush	156.000 $\pm$ 31.200 H	134.600 $\pm$ 39.966 G
	Without Crush	292.600 $\pm$ 5.029 I	79.800 $\pm$ 1.303 H
G5 Vitamin B complex	With Crush	70.324 $\pm$ 19.713 K	128.127 $\pm$ 0.027 K
	Without Crush	84.637 $\pm$ 0.030 L	101.708 $\pm$ 0.013 L
LSD		12.99	10.66

Means bear different letters differs significantly at the 5 % level.

The statistical analysis of our study shows significantly ( $p<0.05$ ) increases serum cholesterol and triglycerides of both crushed and non-crushed sciatic nerve. It is also clear from the table that treatment with Ursolic Acid either extracted or standard shows significantly decrease these two parameters more than diabetic group despite the crush injury. Hyperinsulinemia significantly decreases cholesterol of crushed injury sciatic nerve rabbits, but increases non-crushed rabbits cholesterol; the result of triglycerides for hyperinsulinemia is vice versa. Vitamin B complex treatment significantly decreases serum cholesterol of both crushed and non-crushed diabetic rabbits. But it significantly decreased triglycerides of only non-crushed sciatic nerve diabetic male rabbits.

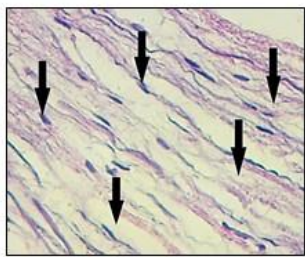
**Table (2):** Effect of ursolic acid, hyperinsulinemia and vitamin B complex on serum HDL and serum LDL of alloxan induced diabetic male rabbits.

Groups	Subgroups	Parameters	
		HDL    mg/ dl	LDL    mg/ dl
G1 +Ve control (Diabetes only)	With Crush	32.698± 1.629    C	175.600± 10.114    C
	Without Crush	18.020± 3.721    D	201.000± 66.215    D
G2 Extracted UA	With Crush	102.628± 4.135    E	77.653± 0.356    E
	Without Crush	112.008± 0.076    F	160.977± 0.082    F
G3 Standard UA	With Crush	97.865± 0.081    G	137.741± 0.088    G
	Without Crush	117.324± 0.083    H	173.448± 0.078    H
G4 Hyperinsulinemia	With Crush	18.360± 2.941    I	111.400± 26.178    I
	Without Crush	37.100± 0.158    J	244.400± 2.073    J
G5 Vitamin B complex	With Crush	100.412± 0.172    M	132.585± 0.025    G
	Without Crush	95.388± 0.024    N	218.161± 0.014    K
LSD		1.562	17.23

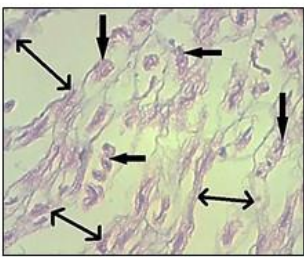
Means bear different letters differs significantly at the 5% level.

It is clear from the table that in induced diabetes group there is significantly decreased ( $p<0.05$ ) serum HDL and significantly increase ( $p<0.05$ ) serum LDL. It is also clear from the table that all treatment groups cause such increase in HDL except hyperinsulinemia which causes a significant decrease in HDL ( $p<0.05$ ). In the case of serum LDL treatment with extracted ursolic acid cause a significant decrease in LDL. Standard ursolic acid caused a significant increase ( $p<0.05$ ) in this parameter. Hyperinsulinemia treatment caused a highly significant increase in LDL in non-crushed sciatic nerve diabetic rabbits but causes a significant decrease ( $p<0.05$ ) in crushed sciatic nerve diabetic rabbits while treatment with Vitamin B complex causes a significant increase ( $p<0.05$ ) in LDL.

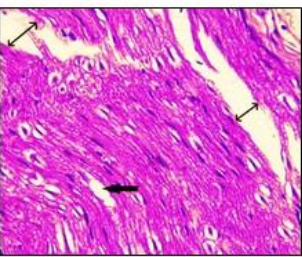
9-Histological parameter



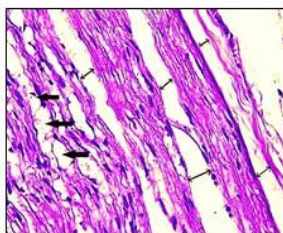
**Figure (1):** Longitudinal section of rabbit sciatic nerve of G1-positive control group (diabetic only) without crush, showing clear separation of Neurofibrils and loose connection between them with prominent Schwann cells (Black arrows) (H&E 400x).



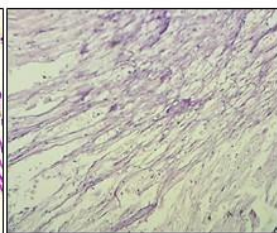
**Figure (2):** Longitudinal section of rabbit sciatic nerve of G1-positive control group (diabetic only) after 14 days of crush injury, showing clear deficient and loose nerve fibers ( ↔ arrows) with clear enlarged Schwann cells nuclei of myelinated axons (Black arrows) (H&E 400x).



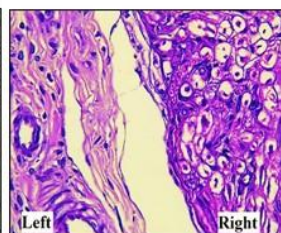
**Figure (3):** Longitudinal section of rabbit sciatic nerve of G2-diabetic treated with extracted UA after 14 days of crush injury, showing clear improvement and regeneration of Neurofibrils but there some vacuolation still found and some separations of Neurofibrils (Black arrows) (H&E 400x).



**Figure (4):** Longitudinal section of rabbit sciatic nerve of G3-diabetic treated with standard UA after 14 days of crush injury, showing there is a cloudy degeneration (Black arrows) and separations and loosening of Neurofibrils (↔ arrows) still found (H&E 400x).



**Figure (5):** Longitudinal section of rabbit sciatic nerve of G4-diabetic treated with Hyperinsulinemia after 14 days of crush injury, showing clear loose nerve fibrils and cloudy degeneration with clear increased number of Schwann cells of axons (H&E 100x).



**Figure (6):** Longitudinal section (left) and transverse section (Right) of rabbit sciatic nerve of G5-diabetic treated with Vit. B complex after 14 days of crush injury, showing good regeneration of neurofibrils and disappearance of cloudy and fatty degenerations but there is some enlargement of Schwann cells of axons (H&E 100x).

## 10-Discussion

Lipid Profile Measurements were investigated in our study in order to examine the effect of diabetes and different treatments to the rabbit's serum cholesterol, triglyceride, HDL and LDL.

The outcome of serum lipid profile laboratory investigations done on the studied rabbits demonstrated in the tables (1 and 2). It is clear that there is a significant elevation ( $p < 0.05$ ) of serum cholesterol of alloxan induced rabbits and highly significant, rise ( $p < 0.05$ ) of triglyceride (TG). While there is a more significant decrease ( $p < 0.05$ ) of high-density lipoprotein (HDL) in alloxan induced diabetic rabbits. In addition, there is a significant increase ( $p < 0.05$ ) of low-density lipoprotein (LDL) of diabetic rabbits.

All these measurement results resemble those found by [23] on alloxan induced diabetic rabbits. These findings also are comparable with that reported by [24] [25] [26] [27].

The reason for these findings is that in normal conditions there is suppression of the release of free fatty acids into the circulation due to inhibition of lipolysis because of the active effect of insulin which inhibits the lipase enzyme activity that is responsible for the lipolysis process [28].

In diabetes mellitus, there is insufficient insulin leads to the activation of lipase enzyme and enhancement of lipolysis and subsequently discharges more free fatty acids into the blood circulation [29]. Moreover, the presence of extra fatty acids in plasma due to alloxan induced hyperglycemia enhances liver to convert some of them into phospholipids and cholesterol [30].

Results in the table (1) showed a significant reduction ( $p < 0.05$ ) of cholesterol and triglyceride when treated with ursolic acid are coming to an agreement with results found by [31] when found that ursolic acid decrease the level of triacyl glycerol in the plasma via the inhibition of pancreatic lipase and by stimulating lipolysis in adipocytes. Furthermore, [32] found the same result that this study found when the stated that Ursolic Acid has the ability to reduce the level of plasma total cholesterol, free fatty acids and triglycerides in diabetic mice.

It is obvious from the table that hyperinsulinemia causes a significant increase ( $p < 0.05$ ) in the cholesterol and triglyceride levels if compared with other treatment groups as well as with the diabetic group (positive). These results are assisted by those found by [33] and [34] when stated that insulin resistance is commonly associated with hyperinsulinemia and hypertriglyceridemia, also [35] found that it is associated with an increase in LDL-TG production. Because of the close correlation between TG production rate and hyperinsulinemia, it has generally been assumed that insulin facilitates LDL production, particularly when plasma free fatty acids and glucose levels are elevated [36]. It is also clear from the table (1 and 2) that treatment with Vitamin B complex decreases serum cholesterol and triglyceride of non-crushed sciatic nerve diabetic rabbits, but it significantly increases HDL and little effect on LDL.

Different degrees of histological changes in the sciatic nerve after examination of several histological slides from all the experimental groups which clearly appeared in (Figures 1-6) stained with Hematoxylin Eosin stain. After crush injury of alloxan induced diabetes rabbits and after treatments that are used in this study, several histological changes were found in those slides as shown in the figures mentioned; those changes could be noticed four days after crush injury and after two weeks after the treatments as mentioned by several workers such as [37] and [38]. These changes included degeneration of axons when started as a cloudy swelling and fatty degenerations followed by myelin sheath destruction and the proliferation of a larger number of Schwann cells, all such changes are found in slides taken from the groups of our experiment and also mentioned by the above-mentioned workers.

To encourage the phenomenon of healing, regeneration, and recovery of damaged nerve by crush injured many drugs have been used, these drugs can help in nerve regeneration or healing or pain relief. Such drugs involve systemic steroid or local ones [39] [40] [41]. But in our study, we used ursolic acid extracted from green apple peels compared with standard imported one as well as using insulin and vitamin B complex. The results we obtained are very good results of healing using extracted ursolic acid compared to insulin. Vitamin B complex gave very good results which are documented by the results found by and [42]. When concluded that vitamins B was considered as the best drugs which can be used in the regeneration of damaged nerves. [43] and [44] stated that the vitamin B complex has shown to promote regeneration and functional recovery of injured sciatic nerve and regeneration of nerve fibers with their myelin sheath.

Very good results of sciatic nerve regeneration obtained by using Ursolic Acid in this study are agreeing with what is found by [45] when they stated that Ursolic Acid can promote repair of peripheral nerves, can repair damaged neurons and promote neural regeneration after peripheral nerve injury.

## 11-Conclusions

From the results of this experiment, we can conclude that Alloxan induced diabetes causes degeneration of the peripheral nerve represented by the sciatic nerve. Treatment with ursolic acid that extracted from apple peels can overcome degenerative processes in the sciatic nerve and has positive effects on lipid profile measurement. Physiologic hyperinsulinemia has less effect on studying parameters compared to ursolic acid. Treatment with vitamin B complex gave positive results and can overcome bad results which occur due to diabetes and crush injury to the sciatic nerve.

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