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## **Estimation of some biomarkers of liver damage and its tissue in male albino rats treated with isotretinoin at different periods**

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**Abstract---**Isotretinoin, commonly known as 13-cis-retinoic acid, is a vitamin derivative used to treat severe acne and several types of skin, head, and neck cancer. According to certain research, isotretinoin causes apoptosis in a number of different types of bodily cells. In instances of clinically obvious acute liver injury with jaundice, isotretinoin has not been definitively linked. It is unknown how isotretinoin elevates serum aminotransferase levels, however, given that this effect seems to occur more frequently with a greater dose of medication, it may be a direct harmful impact. Methods: This study was conducted in the animal house in the College of Science / University of Babylon for the period from 22/11/2021 to 22/12/2021. The study included 40 white male albino rats (*Rattus rattus*) aged 2-3 months, weighing 100 to 150 g, and at a temperature of (25+3C) and 12 u (light-dark cycle) and then divided into eight groups, each group containing five rats. The animals were left before treatment for two weeks to acclimatize. After that, the rats were treated with Isotretinoin and they were given free water and aration. On the last day of dosing, it was left for a day, then the animals were sacrificed after being anesthetized with chloroform, and livers were taken for histopathological study. Blood was collected for liver functional enzymes evaluation. Liver functional enzymes include (ALT, AST, and ALP) and Biomarkers of liver injury include (FABP1 and Kallistainen). Results: According to the findings, there was a considerable increase in liver enzymes, which indicates that isotretinoin caused tissue damage. The biomarker FABP1 result showed a substantial decline, indicating that the more tissue damage, the lower this biomarker, whereas the biomarker kallistainen result showed a significant increase, indicating that the more this biomarker, the more kallistinen value. Isotretinoin alters the texture of the liver tissue, with the main

changes being sinusoid congestion and inflammatory cell infiltration around the central vein. Conclusion: According to the findings of the current study, high doses of isotretinoin, especially over a long period of time, can cause liver damage, alter the texture of the liver, and lower levels of functional liver enzymes.

**Keywords**---Isotretinoin, 13-cis-retinoic acid, liver enzyme, kallistainen, FABP1.

## Introduction

Isotretinoin (ISO) is the most effective treatment currently available for acne. Isotretinoin or 13-cis-retinoic acid is recommended for severe inflammatory acne of the streptococcus or lumpy types and for acne that has proven resistant to previous treatments with antibiotics or topical medications (1). Isotretinoin works by normalizing sebaceous gland keratinization by binding to specific retinoid receptors and altering gene transcription (2). The keratinization of hair follicles, sebaceous gland activity, and size, as well as the activity of inflammatory cytokines, are all decreased by isotretinoin (3, 4). Additionally, isotretinoin reduces the prevalence of *Propionibacterium acne* (5). Utilizing this form of vitamin A derivative may impact the liver by raising serum levels of liver enzymes and altering lipid levels by increasing triglyceride, total cholesterol, and low-density lipoprotein (LDL) cholesterol levels while lowering high-density lipoprotein (HDL) cholesterol levels (6,7,8).

## Material and Methods

40 white male albino rats (*Rattus rattus*) aged 2-3 months, weighing 100 to 150 g, were used in this study divided into eight groups: the first group was control (negative control) feeding silage and water, second Five rats were given 0.5 ml of oil every day for 30 days (positive control), the third group five rats were given 20 mg/ kg of the drug dissolved in 1 ml of oil given by oral gavage every 24 hours for 30 days, the fourth group five rats were given 40 mg/ kg of the drug dissolved in 1 ml of oil given by oral gavage every 24 hours for 30 days, fifth group Five rats were given 20 mg/ kg of the drug dissolved in 1 ml of oil given by oral gavage every 48 hours for 30 days, sixth group five rats were given 40 mg/ kg of the drug dissolved in 1 ml of oil given by oral gavage every 48 hours for 30 days, seventh group Five rats were given 20 mg/ kg of the drug dissolved in 1 ml of oil given by oral gavage every 72 hours for 30 days, eighth group Five rats were given 40 mg/ kg of the drug dissolved in 1 ml of oil given by oral gavage every 72 hours for 30 days. This study was conducted in the animal house in the College of Science / University of Babylon for the period from 22/11/2021 to 22/12/2021. Animals were maintained at a temperature of (25±3°C) and 12 h (light-dark cycle). At the end, the liver was removed from each group and dissected for histological analysis. Routine slices stained with hematoxylin and eosin were used for histological diagnosis (H&E).

## Statistical analysis

Graph Pad Prism was used to analyze the data of blood collected for liver functional enzyme evaluation. Liver functional enzymes include (AST, ALT, and ALP) and biomarkers of liver damage include (FABP1 and Kallistainen).

## Results

The liver functional values for each group were examined at the conclusion of the study, as shown in the Table (1) The results showed a significant increase ( $P < 0.05$ ) at the dose of 20 mg for a period of 24 hours, while in the case of a dose of 40 mg the significant increase ( $P < 0.05$ ) occurred in the time period of 24 and 72 hours when compared with the control groups, while it was noted that there were no significant ( $P > 0.05$ ) differences in the dose of 20 during the period of 48 and 72 hours as well. There are no significant differences in the case of a dose of 40 mg at the time of 48 hours when compared with the control groups relative to ALT. While the results of the table showed that there was a significant increase ( $P < 0.05$ ) when treated with a dose of 40 mg for the time periods 24 and 72 hours, there were no significant ( $P > 0.05$ ) differences in the treatment with 20 mg for all periods of time compared with the control groups for AST. Through the table of results, it was noted that there was a significant increase ( $P < 0.05$ ) at the dose of 20 mg for all periods of time, while there were no significant ( $P > 0.05$ ) differences at the dose of 40 mg for all periods when compared with the two control groups with respect to ALP

Table (1): Liver function level of some blood parameters in male rats treated with Isotretinoin (20 and 40) mg/kg, in different periods for 30 days

| Groups<br>Parameters | Control<br>(D.W)negative | Control<br>(Oil)positive | 20 (mg/Kg)        |                   |                   | 40 (mg/Kg)        |                   |                   | LSD (0.05) |
|----------------------|--------------------------|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------|
|                      | Mean $\pm$ S.D           |                          | 24h               | 48h               | 72h               | 24h               | 48h               | 72h               |            |
|                      |                          |                          |                   |                   |                   |                   |                   |                   |            |
| ALT (IU/L)           | 41.00 $\pm$ 1.6          | 43.40 $\pm$ 5.8          | 65.40 $\pm$ 10.2  | 47.00 $\pm$ 6.0   | 46.60 $\pm$ 16.5  | 71.25 $\pm$ 7.4   | 53.60 $\pm$ 3.7   | 57.80 $\pm$ 5.5   | 13.642     |
| AST (IU/L)           | 126.33 $\pm$ 11.1        | 126.20 $\pm$ 36.3        | 235.60 $\pm$ 11.4 | 114.20 $\pm$ 21.3 | 142.40 $\pm$ 16.8 | 386.25 $\pm$ 29.1 | 260.40 $\pm$ 17.6 | 308.00 $\pm$ 23.1 | 167.889    |
| ALP(IU/L)            | 415.00 $\pm$ 10.8        | 454.66 $\pm$ 24.1        | 810.66 $\pm$ 21.0 | 607.80 $\pm$ 14.4 | 609.20 $\pm$ 36.9 | 626.75 $\pm$ 17.3 | 599.20 $\pm$ 14.4 | 510.60 $\pm$ 17.6 | 247.871    |

## Biomarker damage

The results in the table (2) showed that there was a significant increase ( $P < 0.05$ ) in FABP 1 when treated with 20 mg at the time periods 48 and 72 hours compared to the two control groups, while no significant ( $P > 0.05$ ) differences appeared in the dose of 20 mg and for the period 24 hours, and there were no significant differences when treated with a concentration of 40 mg for the time period 48 hours. In addition, it was found that there were no significant differences when treated with a dose of 40 mg for the time periods 24 and 72 hours compared with the positive control group of oil with respect to the biomarker FABP1. While the results of the table showed that there was a significant decrease ( $P < 0.05$ ) when treated with 40 mg for a period of 24 hours, which was noted that there were no significant ( $P > 0.05$ ) differences when treated with both doses and for all periods compared to the positive control group except for treated with 40 mg for a period

of 24 hours showed a significant decrease ( $P<0.05$ ) compared to the two control groups with respect to kallistaien

Table (2): Biomarker damage level of some blood parameters in male rats treated whit isotretinoin and 40 mg/kg, in different periods for 30 days.

| Groups<br>Parameters | Control<br>(D.W) | Contr<br>ol<br>(Oil) | 20 (mg/Kg) |           |           | 40 (mg/Kg) |           |           | LSD<br>(0.05) |
|----------------------|------------------|----------------------|------------|-----------|-----------|------------|-----------|-----------|---------------|
|                      |                  |                      | 24h        | 48h       | 72h       | 24h        | 48h       | 72h       |               |
|                      | Mean±S.D         |                      |            |           |           |            |           |           |               |
| FAPB(ng/ml)          | 6.75±0.5         | 7.86±1.7             | 7.08±2.3   | 8.35±0.6  | 8.75±2.3  | 8.03±1.1   | 7.44±0.8  | 8.06 ±1.7 | 1.029         |
| Kalstainen(n g/ml)   | 71.78±4.0        | 64.19±5.8            | 58.44±3.9  | 64.19±7.4 | 64.08±8.1 | 55.93±9.7  | 61.26±7.3 | 59.52±8.4 | 6.483         |

### Liver histology

Microscopic examination of the rat's liver shows variable changes during a different meme of the study. isotretinoin affects the texture of liver tissue.

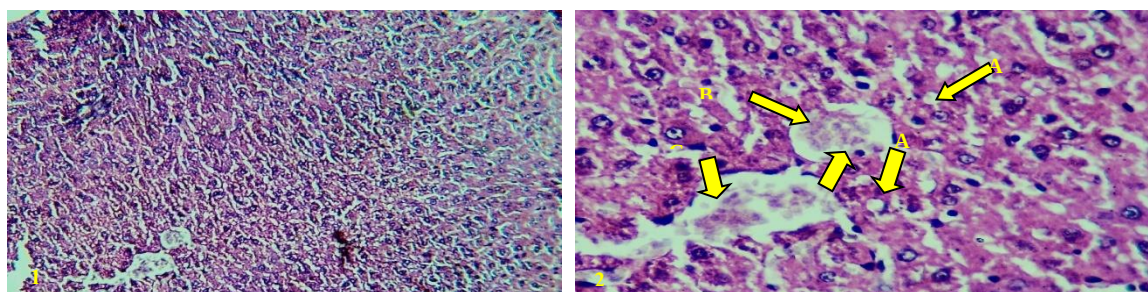


Figure (1) cross section of rat liver showed treated with isotretinoin group 20 mg ,48 hour for 30 days.(1) showed intact structure apart from some dilated characterized by sinusoids and the activation of kupffer cells (10X). (2) marked pathological change steatosis (A) mononuclear cell infiltration (B) ) central vein congestion with inflammatory cell infiltrate and angiectasis (C) (H and E ,40X).

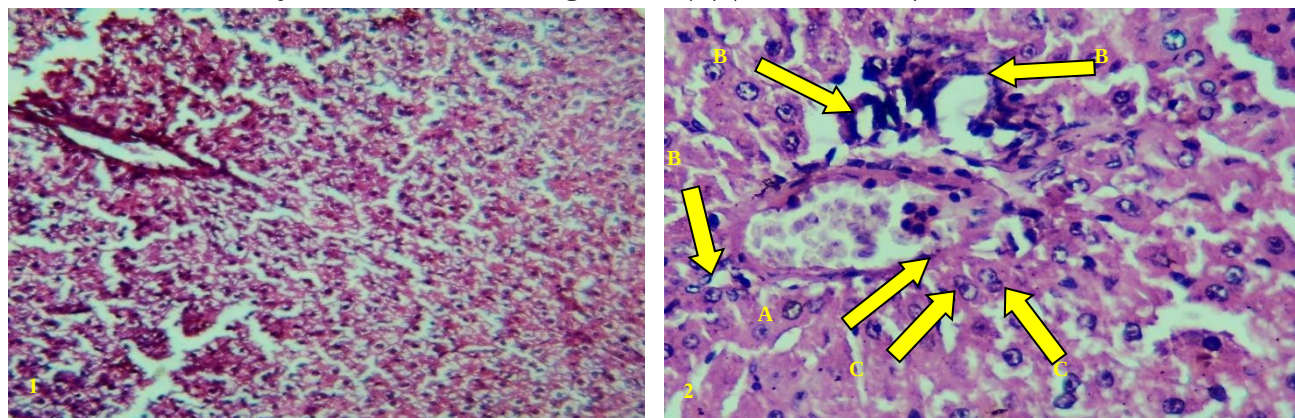
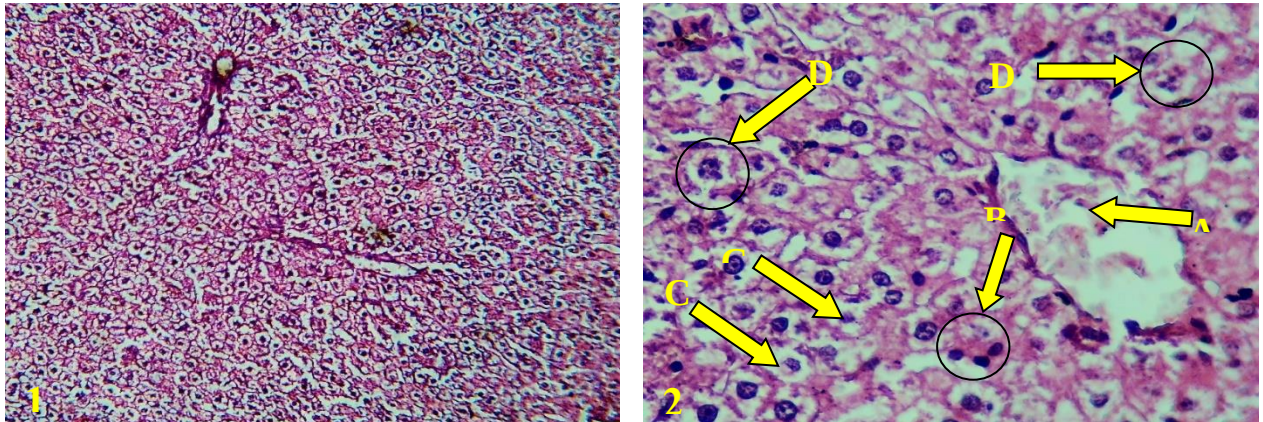
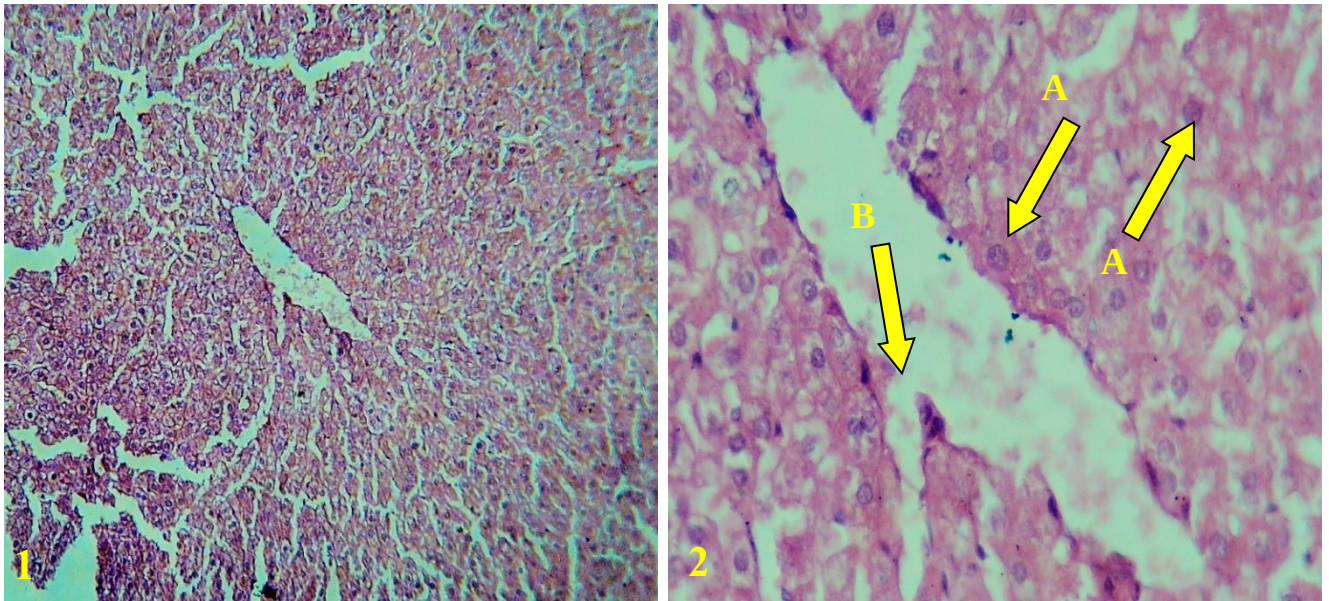


Figure ( 2) Cross section of the rat liver showed treated with isotretinoin group 20mg ,24 hour for 30 days (1) showed dilatation of hepatic sinusoids and portal vein (10X) (2) ) portal vein congestion with inflammatory cell infiltrate (A), bile duct proliferation (B) and with normal hepatocytes while some bi-nucleated cells refer to regeneration (C) .(H and E,40 X),



Figure(3) Cross section of the rat liver showed treated with isotretinoin group 20 mg, 72 hour for 30 days, (1) showed dilatation of hepatic sinusoids and central vein (10x) .(2) central vein congested with blood (A), Kupffer cell proliferation(B), cellular swelling associated with hydropic degeneration and cell necrosis(C) with karyolitic nucleus (D) (H & E, 40x



Figure(4) Cross section of the liver rat liver showed treated with isotretinoin group 40 mg, 72 hour for 30 days (1) showed an abnormal liver with high level of vacuolation and enlarged sinusoids (10x) .(2) marked pathological changes characterized by karyomegaly (A), herniated centralveins (B) (H & E, 40x).

## Discussion

The findings of this investigation, which are presented in Table, clearly demonstrate the impact of isotretinoin on the functioning enzymes in rats' livers (1). Numerous studies (9), some of which suggest that the level of serum AST, ALT and ALP remain within the normal range or only slightly increase during medication with low dose (10), and that was in agreement with the outcome of this study as it was clearly seen that there were no significant differences in the level of AST, ALT, and ALP of the low dose isotretinoin. The variations in the liver functional enzymes during different medicating duration and different dose of isotretinoin were confirmed by these (1). Although the exact mechanism by which isotretinoin alters serum aminotransferase is unknown, it may be characterized by a direct toxic effect that worsens with greater dose therapy. Hepatotoxicity and liver damage are brought on by isotretinoin (11). While the liver biomarker shows the extent to which the liver tissue is affected. When Kallistinen is low, tissue damage increases, while high FABP1 increases the possibility of damage to liver tissue. Both of these are considered an important biological marker for early detection of liver damage.

The histological alterations of isotretinoin at low and high doses were documented in this study by inflammatory cell infiltration, central vein enlargement, vacuolization, degeneration, sinusoid congestion, necrosis, and hepatocyte swelling. The study's findings revealed that, in addition to sinusoid congestion and vacuolar degeneration in hepatocytes in the high dose treatment group, infiltration of inflammatory cells increased in the low dose isotretinoin-treated group compared to the control group. The effects of isotretinoin increase over time, and the histological abnormalities become more pronounced. These changes mostly involved inflammatory cell infiltration, central vein enlargement, and hepatocyte vacuolar degeneration. Confirms the hepatotoxicity and strong effect of this medicine over time by demonstrating severe infiltration of inflammatory cells surrounding congested central vein and sinusoid with coagulated necrosis hepatocytes (12).

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