Quercetin Improves the Histopathological Changes of Liver Caused by Interferon Beta 1b-induced Liver Injury

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Abstract:

Betaferon or interferon beta-1b (INFβ-1b) is a drug that used for multiple sclerosis (MS) treatment, this drug demonstrated an efficacy in relapse MS, but it can cause hepatotoxicity or liver injury, which present as brief elevations in liver aminotransferase enzymes. Quercetin which acts as antioxidant, anti-apoptotic and anti-inflammatory was used as a hepatoprotective in the current study.

This study aimed to investigate the histopathological changes of the liver tissue by betaferon and study the effect of different doses of quercetin on the liver pathological changes.

Methodology: (36) male rats were divided into 6 groups include the negative control group (given only normal saline i.p), betaferon or induction group (given only betaferon 250µg/kg i.p), treatment groups (quercetin 25,50,100 mg/kg + betaferon 250µg/kg) and silymarin group (silymarin 100 mg/kg i.p). In the current study, liver histopathological changes were examined by Hematoxylin and Eosin staining.

Results: In the control group the histological appearance of the liver showed normal tissues (no histopathological changes). Betaferon group the histopathological appearance of the liver showed sever hepatitis with multiple focal necrosis, sever dilation of central vein and marked cellular swelling (Ballooning cells) of hepatocytes. Histopathological examination of the groups pretreated with different doses of quercetin reflected dose-dependent hepatoprotective effects. Pretreatment with (25mg/kg) quercetin mildly improved the histopathological appearance of the liver. When the dose was increased to (50mg/kg), the score improved additionally, with the hepatic injury resolving at (100 mg/kg) of quercetin pretreatment as in silymarin group.

Conclusions: Quercetin can improve histopathological changes on the tissue of the liver of male rats indicates that it possesses advantageous properties in reducing the histopathological damage induced by betaferon.

Keywords: Quercetin, interferon beta 1b(betaferon), liver injury, multiple sclerosis (MS).

كيرسيتين يحسن التغيرات النسيجية المرضية للكبد الناتجة عن إصابة الكبد التي يسببها الإنترفيرون بيتا1-ب

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الخلاصة:
انترفيرون بيتا 1-ب هو دواء مستخدم في علاج تصلب الاعصاب المتعدد اظهر هذا الدواء كفاءة في علاج المرض.لكن هناك العديد من الآثار الجانبية من بيتافيرون مثل ضرر الكبد ، وتتمثل هذه الاثار بشكل ارتفاعات في مستويات انزيمات الكبد. كيرسيتين الذي يعمل كمضادا للأكسدة، مضاد للاستماتة ومضاد للالتهابات تم استخدامه في الدراسة الحالية لعلاج سمية الكبد. تهدف الدراسة الحالية لدراسة التغييرات النسيجية المرضة لانسجة الكبد بواسطة بيتافيرون ودراسة تاثير الجرعات المختلفة من الكيرسيتين على هذه التغيرات المرضية للكبد.تم تطبيق هذه الدراسة على36 جرذ وتم تقسيم الجرذان عشوائيا الى 6 مجموعات اعطيت هذه المجاميع الجرعات يوميا عن طريق الحقن داخل الصفاق على النحو التالي: **مجموعة السيطرة السالبة** (تم اعطاؤها الماء المقطر), **مجموعة البيتافيرون أو مجموعة الحث**(تم حقنها بدواء البيتافيرون بجرعة250مايكروغرام/كغم), **مجموعات العلاج وهي مجموعات بيتافيرون+كيرسيتين**. عولجت جرذان هذه المجموعة مسبقا بجرعات 25، 50، 100 ملغم/ كغم من معلق كيرسيتين المجموعة الأخيرة هي **مجموعة سيليمارين** (عولجت الجرذان مسبقا بـ 100 ملغم/ كغم من معلق سيليمارين). في الدراسة الحالية، تم فحص التغيرات النسيجية المرضية للكبد بواسطة (صبغة الهيماتوكسيلين والإيوزين). **النتائج**: تظهر أنسجة الكبد في مجموعة السيطرة السالبة أن المظهر النسيجي للكبد ظهر بشكل أنسجة طبيعية. مجموعة البيتافيرون التي تلقت الجرعة القصوى من الدواء اظهرت فيها الأنسجة المرضية للكبد: التهاب الكبد، قطع مع نخر متعدد، تمدد قطع الوريد المركزي وتورم الخلوية ملحوظ (تضخم الخلايا) من خلايا الكبد. يعكس الفحص النسيجي المرضي للمجموعات المعالجة بجرعات مختلفة من الكيرسيتين تأثيرات حماية الكبد المعتمدة على الجرعة. المجموعات المعالجة مسبقا باستخدام (25 مجم/كجم) كيرسيتين حسنت بشكل معتدل مظهر الكبد المرضي. عندما تم زيادة الجرعة إلى (50 مجم / كجم)، تحسنت النتيجة بشكل إضافي، مع معالجة إصابة الكبد عند (100 مجم/كجم) بالكيرسيتين كما هو الحال في مجموعة سيليمارين. الاستنتاجات: أظهر كيرسيتين تاثيرات التي يمكن من خلالها أن تحسن التغيرات النسيجية على أنسجة الكبد من الجرذان الذكور، مما يشير إلى أنه له آثار مفيدة على التقليل من الآثار النسيجية الناجمة عن بيتافيرون.

الكلمات المفتاحية: الادوية التي تسبب اصابة الكبد، انترفيرون بيتا 1-ب(بيتافيرون)، اصابة الكبد، تصلب الاعصاب المتعدد، كيرسيتين

Introduction

Betaferon (IFNβ-1b) is a prescription medication utilized for treating the signs and symptoms of multiple sclerosis (MS) with the aim of reducing the occurrence of clinical exacerbations [1]. IFNβ 1-b is a member of the immunomodulator drug class [2] that can induce liver injury (DILI) or hepatotoxicity [3]. Patients treated with betaferon have been shown to undergo significant liver damage, such as instances of liver failure, some of which have been related to autoimmune hepatitis [4]. The precise process by which betaferon induces hepatic damage remains unclear. However, it is hypothesized that betaferon either directly destroys hepatocytes or induces an immunological response targeting the liver [5].

The enhanced suppressor cell activation, suppression of cytotoxic T-cells, cytokine alterations, and effects on the blood- brain barrier may all contribute to IFNβ's immunomodulatory effects [6]. Research has shown that oxidative stress plays an important part in the liver damage produced by IFNβ-1b. Although most individuals with multiple sclerosis (MS) benefit from these immunomodulatory effects, this medicine can also exacerbate or worsen other autoimmune disorders, such as thyroid problems and hepatitis [7]. The immunostimulant activities of betaferon may potentially trigger autoimmune disorders [8]. Women generally have more severe hepatotoxicity compared to men [9]. The etiology of these gender-based differences remains uncertain, however, it has been postulated that susceptibility to hepatotoxicity may be influenced by a reduced body mass index and a greater level of treatment compliance [9]. Co-administration of this drug with other pharmaceuticals or herbal treatments elevates the likelihood of betaferon-induced liver injury [5]. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) a prevalent flavonoid

in the human diet [10], its commonly found in fruits and vegetables, including: onions, broccoli, blueberries, and leeks [11]. It has the potential to be utilized for protecting human health especially for limiting liver disorders. It is generally known that quercetin has a wide range of bioactivity, including antioxidative, lipid lowering, reactive oxygen species scavenging, anti-inflammatory, and anti- fibrotic properties [12].

Quercetin (Qt) demonstrates antioxidant protection by scavenging free radicals and inhibiting the oxidation of certain compounds [13]. Therefore, it serves as a beneficial agent in reducing oxidative stress in several brain cells [14]. The primary mechanism behind the protective effect of Qt is its antioxidant defense against oxidative damage [13]. When male albino rats were pre- treated with the highest dose of IFNβ, which caused liver damage, Qt was found to have a protective effect on the liver. This is because the flavonoids in Qt are known to protect against drug-induced adverse effects, particularly oxidative stress [13]. The aims of the current study were to evaluate the hepatoprotective role of quercetin against histopathological changes in the liver of male rats caused by betaferon.

Materials and Methods:

Animals:

Thirty-six healthy, male Wister rats of approximately (9-13weeks old) with weight of (160 -185 g) were used in this research. The Mustansiriyah University College of Pharmacy's Ethical Community granted its permission according to the Ethical Committee on Animal Care (File No.7 on 13 November 2022).

Animals were divided randomly into 6 groups each group contain six animals. the study continued for 17 days. Pilot study was carried out for 10 days to detect the hepatotoxicity caused by betaferon:

I) Control group (n=6); rats were administered distilled water I.P. for 16 days.
II) betaferon group or induction group (n=6); rats were administered betaferon in a dose of 250 µg/kg (maximum dose) I.P for 16 days.
III) Quercetin+ IFNβ-1b (betaferon) [Qt (25mg)] treated group (n=6); rats were pre-treated with a dose of 25 mg/kg/day of Qt solution I.P. for 6 days, then continued administering the same dose of Qt plus a maximum dose of IFNβ-1b (betaferon 250µg/kg) daily for 10 days.
IV) Quercetin+ IFNβ-1b (betaferon) [Qt (50mg)] treated group (n=6); rats were pre-treated with a dose of 50 mg/kg/day of Qt solution I.P. for 6 days then continued administering the same dose of Qt plus a maximum dose of IFNβ-1b (betaferon 250µg/kg) daily for 10 days.
V) Quercetin+ IFNβ-1b (betaferon) [Qt (100mg)] treated group (n=6); rats were pre-treated by a dose of 100 mg/kg/day of Qt solution I.P. for 6 days then continued administering the same dose of Qt plus a maximum dose of IFNβ-1b (betaferon 250µg/kg) daily for 10 days.
VI) Silymarin (S100) group (n = 6); rats were pre-treated with a dose of 100 mg/kg/day of silymarin suspension I.P. for 6 days, then continued administering the same dose of silymarin plus a maximum dose of IFNβ-1b (betaferon 250µg/kg) daily for 10 days. Silymarin is used as a reference drug because it has well-known hepatoprotective effect [15].

Histopathological study

Histopathological analysis after fixation involves the following steps [16]:

A-Embedding

Following fixation, the tissue samples have been immersed in higher concentrations of ethanol to prevent excessive tissue stiffening and prepare the sample for the subsequent steps.

B-Sectioning

Paraffin blocks were produced for sectioning into 4µm-thick pieces using a semiautomatic microtome. One section measuring 4 µm in thickness was taken from each block. This section was then affixed to a standard slide for histological investigation in order to identify any pathological alterations occurring in the liver tissues.

C-Hematoxylin and Eosin staining

After de-waxing step, tissue sections were passed through descending grades of ethanol (99.9%, 95%, 80%, and 70% for 2 minutes for each one), washed with tap water for fifteen minutes, stained with hematoxylin for 1½minutes, then washed again with tap water for fifteen minutes, and then plunged four to seven times in acid alcohol. After that, tissue sections were stained by eosin for 45 seconds, dehydrated in raised grades of ethyl alcohol (70%, 80%, 95%, and 99.9% for 2 minutes for each one), then cleaned in xylene. The slides rapidly covered with cover which fixed with disteren plasticizer xylene (DPX) (1-2 drops), then left the slide to dry overnight to be ready for light microscopical examination.

Results of histological assessment

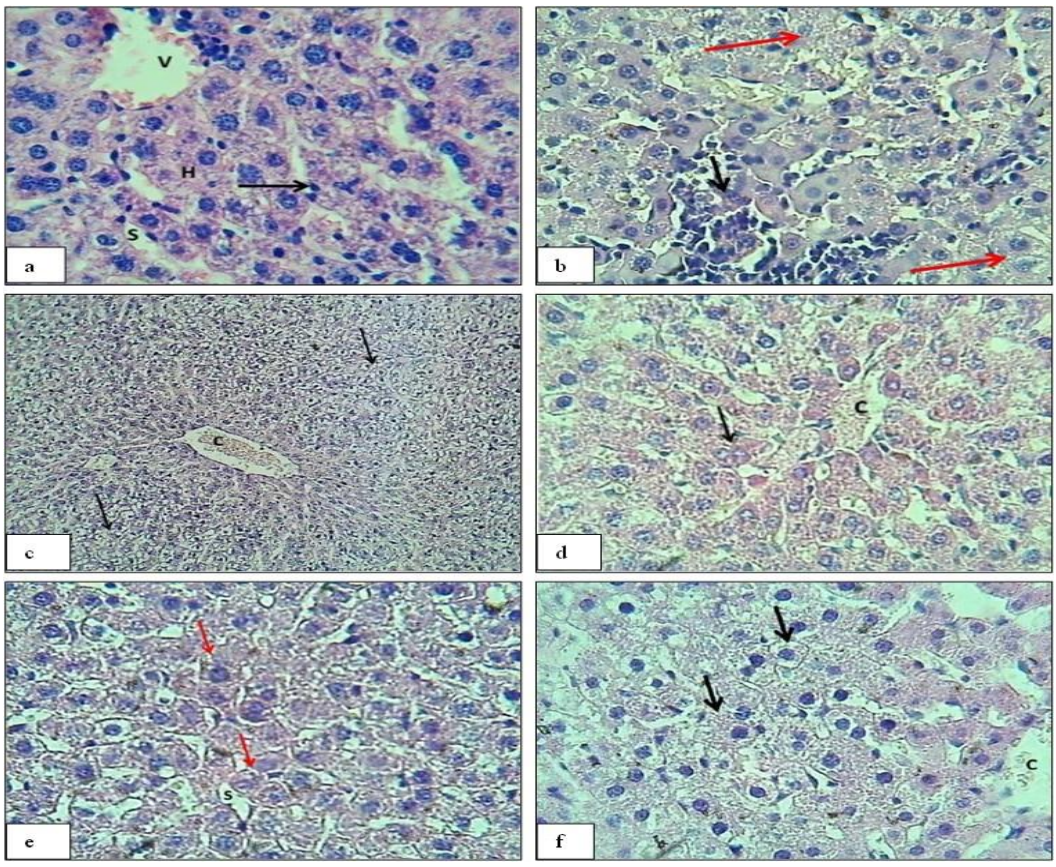

Figure 1. Histopathological changes in the liver sections. (a) Control group shows normal central vein (V), hepatocytes (H), sinusoid (S) and kupffer cells (arrow). H and E stain.400x. (b) betaferon group shows sever dilation and congestion of sinusoid with focal necrosis infiltration of mononuclear leukocytes (black arrow), degeneration and necrosis of hepatocytes (red arrows). H and E stain. 400x. (c) quercetin 25 mg+ betaferon group shows mild dilation with congestion of central vein (C) and mild zonal hepatic cellular swelling (ballooning cells) (arrows). Hand E stain.100x. (d) quercetin 50mg+ betaferon group shows normal central vein (C) and normal hepatocytes (arrow). Hand E

stain. 140x. (e) quercetin100mg+ betaferon group shows normal sinusoid (S) and normal hepatocytes (arrows). Hand E stain. 400x. (f) Silymarin100mg group shows normal central vein (C) and mild zonal normal hepatic degeneration (arrows). Hand E stain.400x.

Discussion

The current study design to assess Qt ability to protect the liver from damage brought on by IFNβ-1b. Betaferon plays a significant role in the treatment of MS [1], but its clinical use is severely restricted due to its tendency to cause liver damage [3,4]. IFNβ-1b is not a model for hepatotoxic drugs because the mechanism by which it causes hepatotoxicity or liver injury is not fully known. Drug-induced liver injury (DILI) is divided into either non-idiosyncratic (predictable) or idiosyncratic (unpredictable) types [17]. Idiosyncratic drug-induced liver injury (DILI) refers to a number of rare diseases that exhibit specific clinical, histologic, and analytical characteristics [18]. However, like other medications, IFNβ-1b has the potential to induce liver injury due to an idiosyncratic reaction, hypersensitivity, or by triggering an autoimmune response in those who are already susceptible [19]. These effects frequently occur within a few weeks after initial exposure to betaferon and can continue for a significant period of time even after discontinuing the drug [19]. Moreover, the use of concomitant medication, namely ibuprofen, increases the likelihood of betaferon-induced hepatotoxicity [20].

In addition, it was found that betaferon increased apoptosis, and inflammation in the liver tissue, which consider one of the possible mechanisms for this drug by which it causes hepatotoxicity or liver injury which in turn lead to histopathological changes [21]. Hepatocyte apoptosis and inflammation, as well as a rise in AST/ALT levels, are indicators of hepatocyte necrosis. Increased apoptosis destroys liver cells, releasing excessive levels of liver enzymes (ALT, AST) into the bloodstream, which can be evaluated as indications of liver injury [22].

The current study compares the histological features of the liver between the different groups. It was found that there are very high significant difference in the histological figures of the liver between control and induction group in that control group show normal central vein, normal sinusoid , normal hepatocytes , normal hepatic cord without marked cellular swelling (Ballooning cells) of hepatocytes. Conversely the histopathological study of the liver of the IFNβ induction group supported the biochemical results by revealing a marked hepatic injury by showing sever dilation of central vein , sever dilation and congestion of sinusoid , necrosis of hepatocytes with marked cellular swelling (Ballooning cells) of hepatocytes also there are infiltration of mononuclear leukocytes, sever hepatitis with multiple focal necrosis and intravascular hemolysis. Additionally, sections showed excessive necrosis, apoptosis that appeared within the cytoplasm of hepatocytes. apoptosis and inflammation (sever hepatitis) are the main factors contributing to the tissue damage brought on by IFNβ. By increasing the production of ROS and lowering the quantity of antioxidant enzymes, ROS interact with membrane lipids to cause lipid peroxidation and necrosis [23]. Previous study found that a reactive oxygen species generated in addition to their direct damaging effect on cell membranes, can act as chemotactic agents for leukocytes. mononuclear Leukocyte infiltration caused by IFNβ can by activated neutrophils further aggravate tissue damage [24]. results showed increased caspase 9 and caspase 3

levels coinciding with histopathological findings of increased apoptosis that's why IFNβ is regarded as a signaling molecule with significant therapeutic promise in cancer because IFNβ - induced gene transcription promotes antiproliferation and cell death [25]. Also, apoptosis could be the result of IFNβ upregulation of caspase 3 in liver tissues (IFN-β also increases apoptotic markers like Annexin-V and active caspase-3 via Fas-receptor/transmembrane activator and calcium modulator (CAML) interactor (cyclophilin ligand interactor TACI) signaling, resulting in decrease in the memory B cells and interleukin [26].

Pretreatment with Qt or silymarin had a hepatoprotective effects [13,14,15]. By examining the chemical structure of quercetin, it found that it has an OH group linked at positions 3, 5, 7, 30, and 40. Quercetin's antioxidant mechanism is mostly apparent in its effects on glutathione (GSH), signaling pathways, reactive oxygen species (ROS), and enzyme activity [27]. It is well known that quercetin act as antioxidants by reducing ROS; it can also scavenge ROS to protect against drug-induced oxidative injury and in the current study against betaferon-induced liver injury [28].

Histopathological examination of the groups treated with Qt reflected dose-dependent hepatoprotective effects, which supported the biochemical results. Pretreatment with (25mg/kg) Qt mildly improved the histopathological score. When the dose was increased to (50mg/kg), the score improved additionally, with the hepatic injury resolving at (100 mg/kg) of Qt pretreatment (histological figure of the liver show normal central vein, normal hepatic cords and normal hepatocytes) in contrast to induction group. The hepatoprotective effect of Qt could be attributed to its antioxidant, anti-apoptotic and anti-inflammatory properties [29]. The group treated with (200 mg/kg) of silymarin showed comparable results to the negative control group owing to silymarins known hepatoprotective effects including anti-inflammation and anti- oxidation [30].

Conclusions

The study revealed that Qt has a hepatoprotective effect against IFNβ - induced hepatotoxicity by improving liver histopathological changes. The highest doses of Qt (50,100 mg/kg) produced the best results.

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