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Study of the effect of bezafibrate with ginkgo biloba extracts in an animal model of hepatotoxicity induced by doxorubicin

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ABSTRACT

This study aimed to evaluate the hepatoprotective effect of combining bezafibrate with ginkgo biloba in doxorubicin-induced hepatotoxicity in rats. Thirty Wister albino rats were allocated into five groups: The negative control group, the positive control group, both received 1 ml of D.W, bezafibrate group received (100 mg/kg), ginkgo biloba group received (60 mg/kg) and the fifth group received bezafibrate + ginkgo biloba. All the treatments were for 14 days along with doxorubicin on days 11–14 except for the negative control. Blood samples were used for the measurement of ALT, AST, ALP, total protein, total bilirubin, albumin, globulin, GSH, catalase, and IL-6. Liver tissue was sent for histopathological examination. The combination of ginkgo biloba and bezafibrate significantly decreased AST, ALP, AST/ALT ratio, albumin/globulin ratio, and IL-6 with significant elevations of catalase, and GSH. The combination group produced more hepatoprotection. This could be attributed to the additive anti-inflammatory and antioxidant effects of the combination.

1. Introduction

Cancer is a chronic destructive disease that arises from uncontrolled cell proliferation and division which cause organ death and is the main cause of mortality [1]. The majority of the currently available anticancer medications are characterized by immunosuppressive and cytotoxic side effects, despite advancements in cancer biology and cancer therapies [2]. doxorubicin (DOX) is an anthracycline antibiotic group and counts as an effective anticancer agent in the treatment of hematological and solid tumors like bladder cancer, lung, ovary, and others [3]. Despite the beneficial effects of DOX as an anti-cancer; it has many deleterious effects on vital organs such as the liver, heart, and kidney that limit its use in the treatment of many types of cancer [4]. Many mechanisms contribute to DOX-induced toxicity. It counteracts cell proliferation, produces oxidative stress, and attenuates the immune system. Furthermore, DOX suppress topoisomerase type II leading to cell death [5]. Additionally, DOX builds up in mitochondria, causing structural and functional changes as a result of the cell's elevated levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which ultimately lead to cell death by either apoptosis or necrosis depending on the availability of ATP [6].

The liver is a crucial organ for metabolism, it acts as a detoxifier for xenobiotics and has a role in homeostasis. Overproduction of reactive species may disturb its anti-oxidant function. The pivotal role played by the liver in the activation and detoxification of most of the xenobiotics, renders it to be more prone to the toxicities of the byproducts of enzymatic reactions [7]. Many medications are known as drug induced liver injury (DILI) that could take the form of acute or chronic injury and the most common type of damage is hepatocellular death. Other mechanisms of liver injury include oxidative stress, mitochondrial damage and inflammation [8].

Cancer patients treated with DOX may provoke various degrees of hepatocellular degeneration [9]. The damages occur through the induction of inflammatory response triggering the generation of ROS, which plays a crucial role in anti-cancer signaling events, including tumor suppressor p53 and cytochrome-c release, followed by activating caspase enzymes and causing apoptosis [10].

DOX is metabolized by CYP450 and carbonyl reductase in the liver and the hepatotoxicity is mediated by ROS that cause hepatocellular death [11]. Many biomarkers can be used for the diagnosis of hepatic damage, for instance, high serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). During liver injury; these

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enzymes leak into the circulation causing inflammation and mitochondrial dysfunction [12,13]. Another mechanism of DOX-induced hepatotoxicity is by increasing lipolysis in adipose tissues and decreasing fatty acid metabolism leading to steatohepatitis [14].

Ginkgo biloba is herbal extract made from the green leaves of the ginkgo tree. It contains a wide range of bioactive substances, such as proanthocyanidins, terpene trilactones, biflavones, alkylphenols, and phenolic acids [15]. Ginkgo biloba extract (GBE) has been studied for its ability to improve blood flow, decrease platelet aggregation, protect cell membranes from free radical damage, and provide protective benefits against cardiac and brain ischemia/reperfusion injury. The doses used for the mentioned studies were (10–100 mg/kg) in experimental rats [16]. Additionally, GBE (200 mg/kg) exhibited anti-inflammatory activity in animal models of inflammation [17], and improved the outcome of patients with T2DM [18,19], and metabolic syndrome using (120 mg/day) [20,21]. It also has been shown to have hepatoprotective effects against oxidative stress and fibrosis in animal studies using a dose range between (25–50 mg/kg) [22–24].

Bezafibrate is a ligand of the peroxisome proliferator-activated receptors (PPARs) used as a hypolipidemic agent [25]. It has been proven to have beneficial effects in attenuating the risk of cardiovascular events in patients with dyslipidemia [26], Furthermore, peroxisome proliferator-activated receptors (PPARs), which control the expression of genes involved in a variety of processes, including lipid homeostasis, cellular differentiation and proliferation, inflammation, and energy metabolism in peripheral and central nervous system tissues, mediate the effects of bezafibrate [27,28].

A study revealed the hepatoprotective activity of bezafibrate via attenuating MDA levels and preventing intrahepatic cholestasis [29]. Another study showed the protective effect of PPAR-α activators in doxorubicin-induced cardiotoxicity through ameliorating oxidative mitochondrial DNA damage [30]. Bezafibrate is also used in the management of primary biliary cholangitis [31], and is shown to decrease liver enzymes in patients with metabolic syndrome via increasing mitochondrial fatty acid oxidation [32]. The aforementioned information about the hepatoprotective effect of both ginkgo biloba and bezafibrate encourages the use of this combination. In order to reduce doxorubicin-induced hepatotoxicity, the current study was designed to explore the hepatoprotective effects results from combining bezafibrate with ginkgo biloba.

2. Methodology

2.1. Chemicals

Below are the chemicals used in the current study, which were all purchased from well-known drug companies:

- Adriamycin® doxorubicin hydrochloride intraperitoneal injection (50mg/25 ml) from Pfizer.
- Ginkgo biloba extract standard powder (EGb 761) from Apollo Healthcare Resources, Singapore.
- Bezalip® bezafibrate tablets (200 mg) from Actavis.

2.2. Experimental animals

Thirty male Wistar albino rats weighing (150–200 g) were obtained from the College of Medicine/Tikrit Medical University. The rats were housed in the animal house of the College of Pharmacy/University of Sulaimani animal house in well-ventilated plastic cages under regular conditions; temperature of 25 ± 2 °C and humidity of 55 ± 5 %, and 12 h of dark/light cycle. They were fed a conventional pellet diet and were provided unlimited access to water. The animals were kept for one week before the experiment for acclimatization. The experimental protocols met the Guidelines for Animal Experimentation and were approved by the Ethical Committee of the University of Sulaimani, College of Pharmacy (Certificate no. PH68-22 on October 18, 2022) following the Institutional Animal Ethics Committee. The study was performed following the U.K. Animals (Scientific Procedures) Act, 1986.

2.3. Study design

Thirty Wistar albino male rats were used in the current study. The rats were housed in pairs within individual 8 cubic feet cages and they were allocated randomly into five groups each consisting of 6 rats as described below:

1. G1: Negative control group (NCG): received 1 ml of D.W. for 14 days.
2. G2: Positive control group (PCG): received 1 ml of D.W. for 14 days. Starting from day 11, DOX was administered as I.P. injection - dosage of 3.7 gm/kg/day for 3 days.
3. G3: Bezafibrate group: receive BZF (100 mg/kg) for 14 days + DOX protocol
4. G4: Ginkgo biloba group: receive GKB (60 mg/kg 1 gm/100 ml) for 14 days + DOX protocol.
5. G5: BZF + GKB group: receive BZF 100 mg/kg + GKB 60 mg/kg for 14 days + DOX protocol.

The doses of DOX [33], Bezafibrate [34], and GKB [35] used in the current study have been chosen from knowledge derived from previous studies. The rats were euthanized on day 14 30 min after the last dose of DOX.

2.4. Biochemical tests

By performing a cardiac puncture, blood samples were obtained and utilized to assess the serum concentrations of ALT, AST, ALP, total protein, total bilirubin, albumin, and globulin, GSH, catalase, and IL-6 using ELISA kits (Bioassay technology laboratory, Shanghai, China).

2.5. Histotechnique

The histological protocol was performed at the endpoint of the experiment. At first, animals were fasted before sacrifice and then euthanized in human practice. Consecutively, after animal scarification necropsy findings were started by collecting tissue samples for histological preparation. Liver samples were fixed into tissue cassettes and then dipped into a 10 % buffered formaldehyde solution for about 48 h. After that, portions underwent a sequence of rising ethanol alcohols followed by three stages of xylene clearing to dehydrate. The treated portions were then infiltrated and heated to between 60 and 70 °C in an automated wax embedder before being embedded in molten paraffin blocks. Using a semi-automated rotary microtome, paraffinized blocked tissues were cut into 5 m sections. After that, tissue sections were placed on glass slides and dried using a hot plate tissue holder. Later on, tissue sections were deparaffinized and cleaned with xylene solution for 30 min then dried for 5 min. Tissue slices were then washed once again with xylene before being stained with Harris's hematoxylin and eosin solution.

2.6. Semi-quantitative lesion scoring

Tissue samples were examined using a light microscope (NOVEL XSZ-N107T, China) for lesion scoring utilizing an eye-piece camera for the microscope (MD500, 2019) and image analysis software (AmScope, 3.7). Hydropic and fatty degenerations within liver sections were estimated and measured in percentage of calculated cell numbers from randomly selected different fields, whereas vascular congestion was assessed in μm and statistically evaluated as an area of mean percentage. The calculated values were expressed as lesion scoring and scores were graded based on the mean percentage as follows: (score 0–10 % as no lesions; score 10–25 % as mild; score 25–50 % as moderate; score 50–75

% as severe; score 75–100 % as critical lesions).

2.7. Statistical analysis

Statistical analysis was conducted using GraphPad Prism 8. Standard deviation (SD) and the mean were used to represent the measured parameters. For multiple comparison between the groups, one-way analysis of variance (ANOVA) was used. Thereafter, Tukey's test was applied for comparing each group with the DOX-treated group. When (p-value< 0.05), statistical significance was determined.

3. Results

3.1. Effect of bezafibrate and GKB alone or in combination on serum levels of ALT, AST, and ALP

In the present study, there was a non-significant change in ALT level observed in the positive control group (PCG) in comparison with the negative control group (NCG) (p-value = 0.3; Fig. 1A), and with the other treatment group. Meanwhile, AST level was significantly elevated in the PCG when compared with the NCG, (p-value = 0.0006). The combination of GKB + BZF significantly decreased the level of AST when compared to the positive control group (PCG) (p-value = 0.018, Fig. 1B), whereas non-combination treatments only showed a slight decrease in AST levels. Regarding ALT/AST Ratio; a significant decrease has been detected in the PCG in comparison with the NCG (p-value = 0.0001), and the combination group significantly decreased the ratio in comparison with the positive control group, (p-value = 0.008; Fig. 1C). The level of ALP was slightly increased in the PCG and the combination treatment was the most effective at decreasing the level in comparison to the PCG, (p-value = 0.022; Fig. 1D).

3.2. Effect of bezafibrate and GKB alone or in combination on serum level of total protein, total bilirubin, albumin, and globulin

All the treatment groups showed no significant changes in the level of total protein when compared to the PCG (p-value >0.05, Fig. 1 A). Total bilirubin level significantly increased in the PCG when compared with the NCG, (p-value = 0.044; Fig. 2 B). Bezafibrate and GKB alone and in combination significantly decreased the level when compared to the PCG, (p-value = 0.0002, p-value = 0.0001 and p-value = 0.0001; Fig. 2 B) respectively. Albumin levels were only decreased significantly in the GKB treated group when compared to the PCG, (p-value = 0.001; Fig. 2C). No significant changes have been observed in the level of globulin, (p-value >0.05, Fig. 2 D). Meanwhile, a significant decrease in the albumin/globulin ratio has been observed in GKB and the combination treatments in comparison with the PCG, (p-value = 0.0005 and p-value = 0.03 respectively; Fig. 2 E).

3.3. Effect of bezafibrate and GKB alone or in combination on serum level of catalase, GSH and IL-6

In the present study, regarding serum catalase level, a non-significant decrease was observed in the PCG in comparison with the NCG, (p-value = 0.054). All the treatment groups increased the levels of serum catalase, however only the combination group was potent enough to reach significant levels when compared with the PCG (p-value = 0.01; Fig. 3A). Regarding serum GSH, levels decreased in the PCG when compared to the NCG, (p-value = 0.026). The bezafibrate group was able to elevate GSH level however, the elevation did not reach a

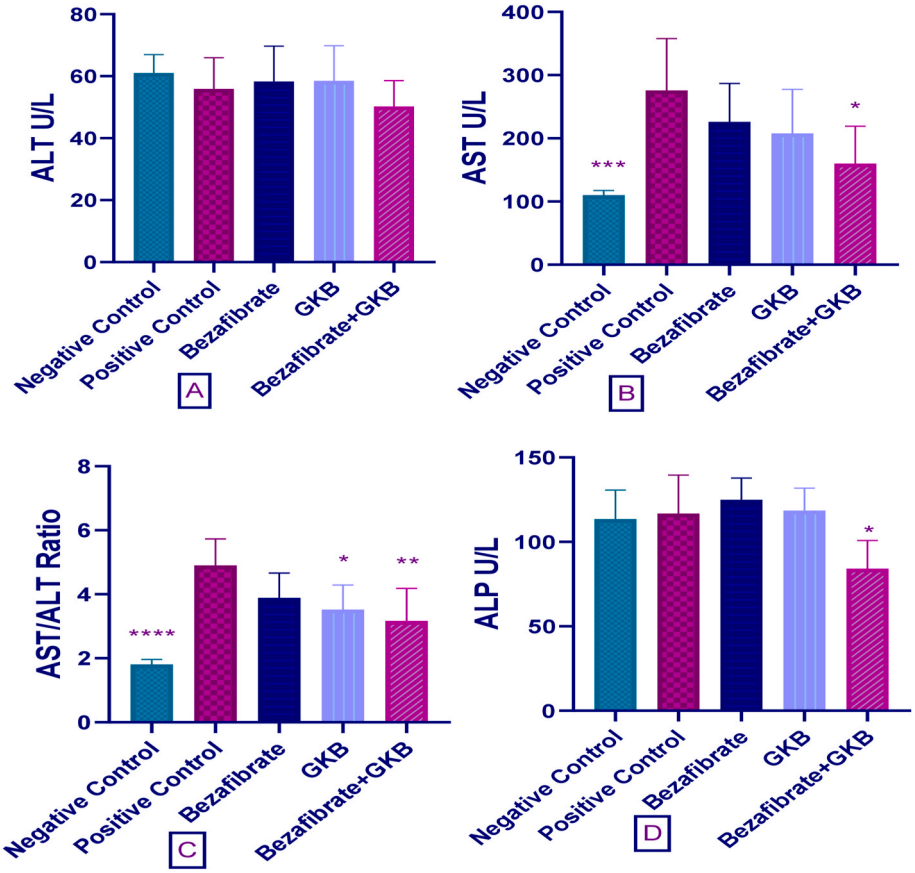

Fig. 1. Effect of Bezafibrate and GKB alone or in combination on A) ALT, B) AST, C) AST/ALT Ratio, and D) ALP. Values were presented as mean ± S.D (n = 6 animals in each group); values with (*) are significantly different from the positive control using ANOVA and post hoc test (*p < 0.05), (**p < 0.01), (***p < 0.001), and (****p < 0.0001).

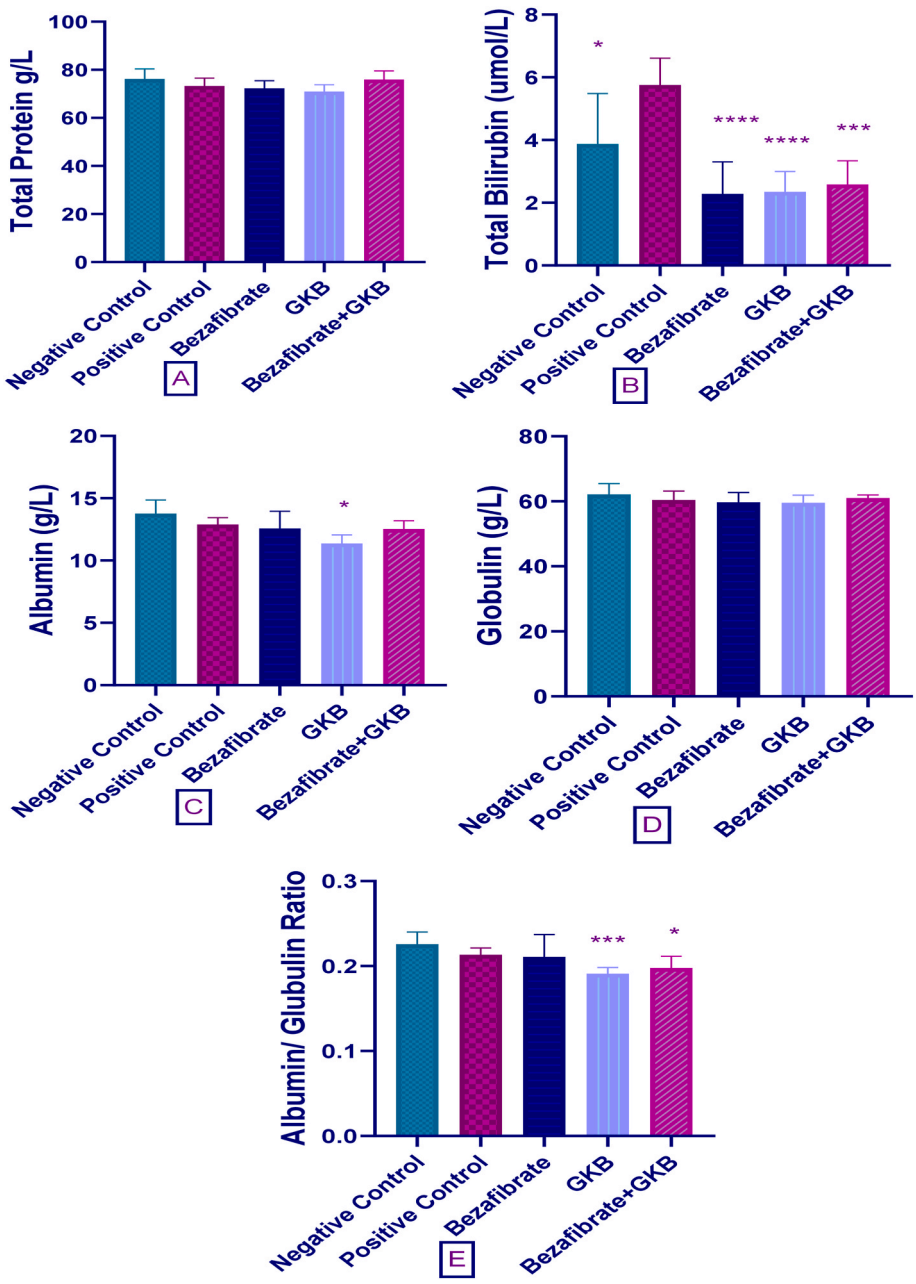

Fig. 2. Effect of Bezafibrate and GKB alone or in combination on A) Total protein, B) Total bilirubin, C) Albumin, D) Globulin, and E) Albumin/Globulin ratio. Values were presented as mean ± S.D (n = 6 animals in each group); values with (*) are significantly different from the positive control using ANOVA and post hoc test (*p < 0.05), (***p < 0.001), and (****p < 0.0001).

significant level, (p-value = 0.07). The GKB and the combination groups were able to increase GSH level significantly, (p-value = 0.0026 and p-value = 0.04 respectively; Fig. 3 B). The inflammatory marker IL-6 significantly increased in the PCG compared to NCG, (p-value = 0.0073). All the treatment groups including bezafibrate, GKB, and their combination significantly decreased the level of IL-6 with maximum effect resulted by the combination group, (p-value = 0.0032, p-value = 0.0037, and p-value = 0.0003 respectively; Fig. 3C).

3.4. Histopathology findings

Measuring hydropic degeneration depended on the increased granulite and obvious dilution of the cytoplasm which was evident by a decrease in the acidophilic pinkish coloration or dilution of the pinkish color of the cytoplasm and appearance of whitish vacuoles within the cytoplasm. As for fatty degeneration, the presence of clear, not granular, and well-demarcated variable-sized lipid vacuoles within the acidophilic cytoplasm which eventually pushed the cytoplasm to the periphery of the cell is a very apparent criterion for the fatty degeneration of the cell. Moreover, the criteria for vascular congestion were indicated by the obvious dilation and engorgement of blood vessels by the presence of deep red-colored RBCs. Fig. 4, demonstrates a microscopically examined liver section in the negative control group which showed typical histological architecture of the standard control liver which continued unchanged and exhibited a normal arrangement of hepatocytes around the central vein. In contrast, liver sections in animals that received Doxorubicin (DOX) 3.6 mg/kg for three days, reveal the presence of severe vacuolar degeneration as well as significant fatty degeneration, many sinusoidal capillaries in addition to central veins appear dilated and engorged with blood. On the other hand, animals

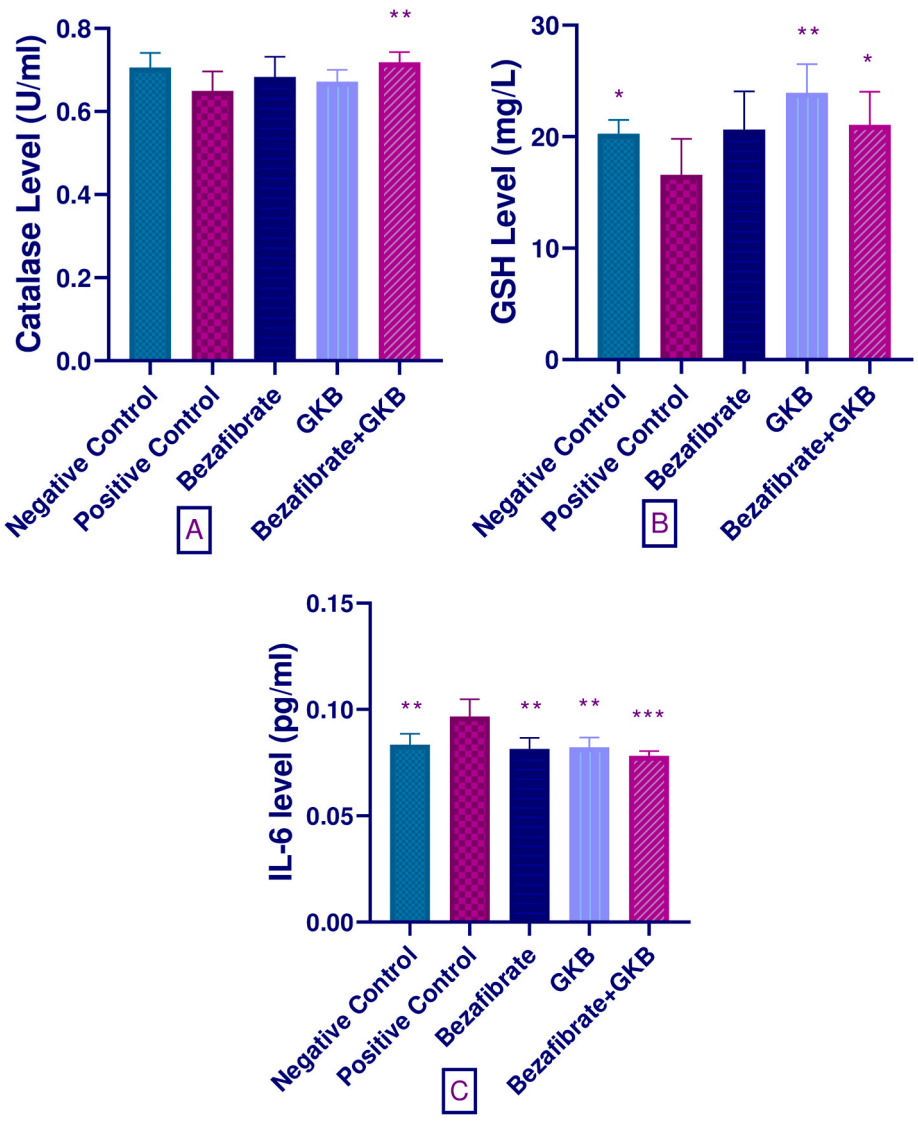

Fig. 3. Effect of Bezafibrate and GKB alone or in combination on A) Catalase, B) GSH and C) IL-6. Values were presented as mean ± S.D (n = 6 animals in each group); values with (*) are significantly different from the positive control using ANOVA and post hoc test (*p < 0.05), (**p < 0.01), and (***p < 0.001).

treated with Bezafibrate (BZF) 100 mg/kg in G3 and Ginkgo biloba (GKB) 60 mg/kg in G4 presented significant $P < 0.05$ reduction in lesion scoring as shown in Table 1, in addition to the mitigation of the lesion severity of fatty and hydropic degenerations in comparison to G2. Over and above that, animals remediated with both BZF and GKB in G5 for 14 days displayed significant alleviation in lesion severity evident by the moderate aspect of vacuolar degenerations.

For the scoring record, the major type of the inflammatory cells that have been infiltrated and proliferated within the given sections was mononuclear inflammatory phagocytic cells which are indicated by the presence of proliferated residence and native macrophages of the hepatic tissue represented by Kupffer's cells, that's found as black dotes attached to the wall of the hepatic sinusoids. Furthermore, these cells were counted from the different selected fields under high power microscopic lens, the macrophages were separated from the hepatocytes via their smaller size and location within the endothelial lining of hepatic sinusoids. Generally speaking, the severity score in Table 1 reveals significant improvement in all treatment groups in comparison with G2, which is much clear in group five.

4. Discussion

It is believed that several distinct mechanisms contribute to DILI. Several of these include direct impairment of the structural and functional integrity of the liver; production of a metabolite that modifies hepatocellular structure and function; generation of a reactive drug metabolite that binds to hepatic proteins to produce new antigenic drug-protein adducts, and the onset of a systemic hypersensitivity response [36,37]. Doxorubicin is metabolized in the liver to a toxic metabolite that induces direct damage to the liver [12]. In the current study, doxorubicin increased serum levels of liver enzymes, and total bilirubin in addition to attenuating catalase, GSH levels and increasing IL-6 level. Histopathological findings also supported the biochemical test showing the deleterious effects of doxorubicin on liver tissue. These effects could be attributed to the generation of reactive species that initiate oxidative stress, mitochondrial dysfunction, and inflammation [12]. All the treatment groups, most notably the combination group were able to decrease liver enzymes and proteins and restored the levels of catalase, and GSH in addition to ameliorating the level of IL-6. The hepatoprotective effects of GKB could be attributed to the modulatory effects on inflammatory pathways [23]. The anti-inflammatory effects of GKB are well documented; many studies showed the mitigating effect of

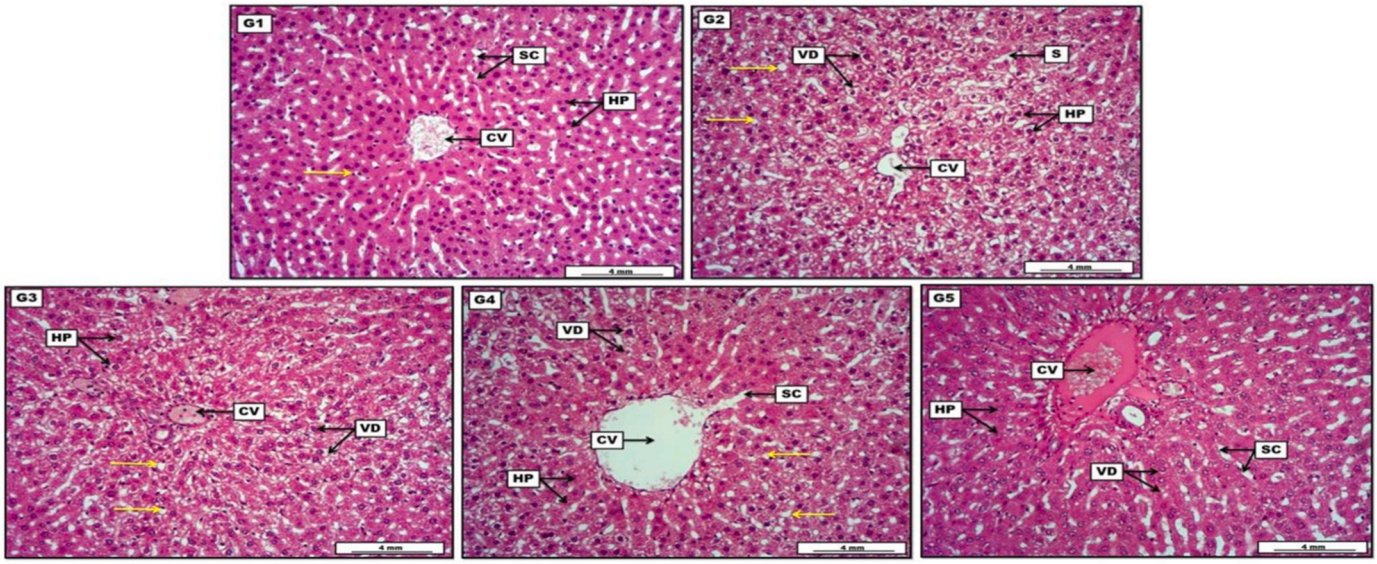

Fig. 4. Photomicrograph of Liver from groups; (G1): Received D.W, shows no apparent lesions within the liver sections, represented by typically arranged radiated hepatocytes (HP) around the central vein (CV) with typical sinusoidal capillaries (SC), together with mild infiltration of sinusoidal Kupffer cells (yellow arrow). (G2): Received 3.7 mg/kg Doxorubicin for three days, demonstrates severe vacuolar degeneration (VD), distributed diffusely within the hepatocytes (HP), along with significant fatty degeneration (yellow arrows). Sinusoidal capillaries (S) appear dilated in some areas, together with the presence of a standard central vein (CV). (G3): Received Doxorubicin and treated with 100 mg/kg of Bezafibrate, elucidate the presence of moderate fatty degeneration (yellow arrows), many other hepatocytes (HP) show obvious hydropic and vacuolar degeneration (VD), together with the presence of vascular congestion within the sinusoidal capillaries as well as the central vein (CV). (G4): Received Doxorubicin and treated with 60 mg/kg of Ginkgo biloba, showing sensible vacuolar degeneration (VD) and a moderate degree of fatty degeneration (yellow arrows). The section also reveals some normally appeared sinusoidal capillaries (SC), with centrilobular Hepatocytes (HP) showing standard morphological arrangement around the central vein (CV) which displays no apparent vascular congestion. (G5): Received Doxorubicin and treated with 100 mg/kg of Bezafibrate and 60 mg/kg of Ginkgo biloba, displaying slight moderate cloudy hydropic vacuolar degeneration (VD). Sinusoidal capillaries (SC) appear normally settled; however, the central vein (CV) shows a moderate grade of vascular congestion. Hepatocytes (HP) demonstrate centrilobular typical adjustment with no obvious morphological lesions. H&E. Scale bar: 4 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Micromorphological quantitative assay of liver sections.

Experimental Groups N = 6	Hydropic Degeneration[a] (Mean %)[b]	Fatty Degeneration[a] (Mean %)[b]	Vascular Congestion[a] (Mean %)[b]	Inflammatory Cells[a] (Mean %)[b]	Lesion Scoring (0–100 %)	Lesion Grading
(G1) NCG[c]	5.37 %^{A}#	3.21 %^{A}	6.42 %^{A}	2.81 %^{A}#	0–10 %	No lesion
(G2) PCG (DOX)	92.78 %^{E}	84.39 %^{E}	79.62 %^{E}	76.48 %^{E}	75–100 %	Critical
(G3) BZF + DOX	75.61 %^{D}	69.56 %^{D}	70.85 %^{D}	74.55 %^{D}	50–75 %	Severe
(G4) GKB + DOX	71.64 %^{D}	70.94 %^{D}	74.61 %^{D}	62.56 %^{D}	50–75 %	Severe
(G5) BZF + GKB + DOX	50.69 %^{C}	49.72 %^{C}	53.82 %^{C}	48.51 %^{C}	25–50 %	Moderate

Notes.
#Statistical comparison among groups: Mean values with different capital letters have significant differences at ($P < 0.05$).
[a] Hepatocytes hydropic degeneration, fatty degeneration and inflammatory cells were estimated in (%) of cell numbers. Area of vascular congestion estimated in mean percentage of (μm).
[b] Each value represents the mean percentage (n = 6).
[c] G1: Negative control group (NCG), Distilled water; G2: Positive control group (PCG), Doxorubicin (DOX) 3.6 mg/kg; G3: Bezafibrate (BZF) group 100 mg/kg with DOX; G4: Ginkgo biloba (GKB) group 60 mg/kg with DOX; G5: Bezafibrate 100 mg/kg and Ginkgo biloba 60 mg/kg with DOX.

GKB on NFκB and the proinflammatory cytokines such as TNF-α, IL-6, and IL-1α [38,39] Moreover, the antioxidant capacity of GKB also contribute in protecting the liver from the harmful effects of doxorubicin [24]. GKB exerts free radical scavenging activity; it can capture ROS and RNS such as superoxide anion, NO, hydroxyl, and peroxyl radicals. Additionally, it serves as a hydrogen atom donor to alleviate the pathological consequences of free radical chain reaction, and lipid peroxidation [40]. Numerous studies have demonstrated that GKB has hepatoprotective effects by lowering liver function enzymes, total protein, total bilirubin and free radical scavenging activity by increasing antioxidant enzyme levels and reducing lipid peroxidation [22,41–43]. Additionally, fibrates also demonstrated hepatoprotective effects by decreasing liver enzymes level [32] and total bilirubin. Bezafibrate showed a hepatoprotective effect when used in patients with primary biliary cholangitis by attenuating total bilirubin levels [44]. Additionally, bezafibrate has anticholestatic effect which also contributed to the liver protective effect [29]. In a study conducted on patients with rheumatoid arthritis taking methotrexate and developing acute liver damage, bezafibrate was shown to be effective in ameliorating hepatic toxicity induced by methotrexate [45]. Furthermore, many studies focused on the role of PPAR-α agonists in attenuating hepatic inflammation by downregulation of IL-6 [46], and IL-1β [47]. An additional mechanism through which fenofibrate derivatives induce liver protection may be attributed to the activation of PPAR-α, which enhances the transcription of anti-inflammatory genes that play a crucial role in inhibiting NFκB [48,49]. In addition to the role of inflammation in

hepatic damage; liver injury could be secondary to the generation of reactive species [50]. Moreover, hepatocyte peroxisomes serve as a source of antioxidant enzymes and may aid in preventing oxidative damage to hepatocytes [51]. Additionally, the antioxidant capacity of PPAR-α agonist is related to elevating the expression of antioxidant enzymes and molecules such as catalase, superoxide dismutase, and GSH which directly protect the liver from the deleterious effects of hepatotoxicants [52]. In an animal study investigating hepatic mitochondrial damage and oxidative stress induced by 3-methyl glutaric acid, bezafibrate demonstrated protective effects by mitigating lipid peroxidation and preserving mitochondrial function [53]. The histopathological findings aided the biochemical results with maximum protection achieved by the combination group. The aforementioned information may explain the effective role of combining bezafibrate with GKB at producing more hepatoprotective effects than the use of each individually.

5. Conclusion

Doxorubicin induced hepatotoxicity through oxidative stress and inflammation. The combination of Bezafibrate with GKB extracts has hepatoprotective effects. This combination ameliorated inflammation via suppressing IL-6 production, and attenuated oxidative stress by restoring the levels of catalase and GSH. Additionally, this combination resulted in the reduction of AST, AST/ALT ratio, ALP, albumin/globulin ratio, and bilirubin levels. This may be attributable to the additive antioxidant and anti-inflammatory actions of bezafibrate and GKB.

Author contribution

Zhwan Azad Abdalla: Methodology, Software, Validation, Investigation, Resources, Visualization, Writing original draft. Aso Nihad Abtar: Methodology, Data curation, Software, Validation, Investigation, Resources, Visualization. Ahmed Azad Kareem: Methodology, Software, Validation, Investigation, Data curation, Visualization. Zheen Aorahman Ahmed: Conceptualization, Investigation, Methodology, Validation, Visualization, Project administration, Supervision. Tavga Ahmed Aziz: Conceptualization, Investigation, Project administration, Writing – review & editing first and final draft, Formal analysis, Supervision.

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Declaration of competing interest

All the authors report no conflicts of interest in this work.

Data availability

Data will be made available on request.

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