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Possible Hepatoprotective Effect of Quercetin Against 2-Butoxyethanol Induced Hepatic Damage in Rats

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Abstract: The pathogenesis and progression of alcoholic liver disease (ALD) are associated with free radical injury and oxidative stress, which could be partially attenuated by antioxidants and free radical scavengers. Quercetin, one of the most widely distributed flavonoids in plants, is a natural antioxidant. The hypothesis that Quercetin could prevent the 2-Butoxyethanol (2BE) induced oxidative damage in hepatocytes was investigated. 2BE is an oxidative stress inducer in liver and Quercetin is a potential antioxidant and is known for its hepatoprotective properties. Oral administration of 2BE (225 mg/ kg b. wt./day) for 28 day resulted in a significant elevation of liver enzymes in serum such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase(ALP). Also, several changes reported in total protein (TP), albumin (Alb), total bilirubin (Tb) concentrations as well as histopathological changes of liver tissues when compared with control one. Oral administration of Quercetin (50mg/kg b. wt./day) along with 2BE significantly decreased the activities of liver enzymes as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin (Alb) and total bilirubin (Tb) in the liver of 2BE intoxicated rats. These results suggested that Quercetin exhibited decreased serum enzymes against 2BE induced oxidative stress in liver.

Key words: Quercetin · 2BE · Liver injury · Oxidative stress · Hepatocytes · Iron · Rats · Kupffer cells

INTRODUCTION

Liver is the largest internal organ in human body. It processes and stores many of the nutrients absorbed from the intestine that are necessary for body functions some of these major functions include protein, carbohydrate, fat metabolism [1]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise [2].

Glycol ethers are commonly used as solvents, detergents or emulsifiers in a huge number of products due to their excellent chemical and physical properties. Ethylene glycol monobutyl ether (or 2-butoxyethanol, 2-BE) is one of the most widely used glycol ethers [3].

2BE is a key ingredient in hundreds of products, including industrial and consumer hard surface cleaners and water- and solvent-based paints and coatings. Human health risk assessments and exposure guidelines developed for 2BE has generally focused on hemolysis as the most sensitive toxic endpoint [4, 5]. Numerous studies have shown that humans are significantly less sensitive to the hemolytic effects of 2BE major metabolite, but oxyacetic acid (BAA), than are rats or mice [6-8]. However, long-term exposure studies of rats and mice have reported both hemolytic and carcinogenic effects [9].

Three to 4-days of exposure to the xenobiotic chemicals, thrombosis becomes apparent in several organs in the female rats, including the cardiac atrium, lungs, brain, submucosa of the anterior nasal septum, pulp of the incisor teeth, liver, coccygeal vertebrae and femur [10-13]. Chronic exposure of rats and mice to 2BE resulted in an increase in the incidence of hepatocellular carcinomas and liver hemangiosarcomas selectively in the male mouse [9]. The mechanism by which these liver neoplasms are formed is unknown. Moreover 2BE was not mutagenic in bacterial mutagenesis assays and was negative in standard genotoxicity assays [14].

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Associated with 2BE-induced hemolysis in the rodents was an increase in hemosiderin (iron deposition) in Kupffer cells [9]. Presumably arising from red blood cell hemolysis [15]. The use of natural antioxidants, including flavonoids for curing pesticide induced liver toxicity or injury is being studied extensively [16]. Even though newly developed drugs have been used to treat chronic liver diseases, they have often side effects hence there is a need for hepatoprotective compounds [17].

Flavonoids are phenolic phytochemicals present in human diet and promote optimal health partly via their antioxidant effects [18]. Antioxidants have been reported to provide protection from the toxicity of pesticides [19].

Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a polyphenolic flavonol molecule that occurs in many fruits and vegetables such as onions, apples, berries, peanuts, soybeans, potatoes, broccoli, grapes, citrus fruits and tea [20, 21].

The antioxidant capacity of these molecules seems to be responsible for many of their beneficial effects and confers a therapeutic potential in diseases such as cardiovascular diseases, gastric or duodenal ulcers, cancer and hepatic pathologies [22]. In addition Quercetin is a more potent antioxidant than the other antioxidant nutrients such as vitamin C, vitamin E and b-carotene etc**.** [23].

It has been demonstrated that Quercetin exhibits its therapeutic potential against many diseases, including ischemic heart diseases, atherosclerosis, liver fibrosis, renal injury and chronic biliary obstruction [24-26]. Consequently, our aim is to clarify the possible hepatoprotective effect of Quercetin against 2BE-induced liver toxicity in rats.

MATERIALS AND METHODS

Chemicals: 2-butoxyethanol (2BE) and Quercetin were purchased from Alfa (MT, USA).

Tween 20 was obtained from El-Gomhorya Company, Cairo, Egypt.

Experimental Animals: This study was carried out using Sprague-Dawley male albino rats weighing (200±8 gm) were obtained from animal house colony of the Egyptian Organization for Biological Products and Vaccines (Hellwan Farm).. All animals were housed in animal house of School of Science at Al Azhar University under standard conditions (room at 25±3°C, 55± 5% humidity) and were provided with a standard laboratory diet and water. The animals were randomly divided into four groups of eight animals in each group.

Experimental Design: *1st group* served as control. *2nd group*: received 2BE dose of (225 mg/kg b.w) daily via gavage for 28 day (5 times per week). *3rd group*: received Quercetin dose of (50 mg/kg b.w.) daily via gavage for 21 day. *4th group*: received the same dose of Quercetin group (50 mg/kg b. w.) and 2BE (225 mg/kg b. w.) in combination for 28 day.

At the end of the experiment rats were euthanized under deep Ether anesthesia for collection of blood and liver tissue samples. Animals were dissected and liver tissues were quickly removed, divided two halves rinsed in ice cold saline, one half was fixed in 10% formalin and processed for histopathological examination. The other half was processed for examination by light microscope. Heart blood was drawn for each rat kept at 4°C and centrifuged at 1000 g for 30 min. Sera were collected for using in biochemical investigation of enzymatic hepatic markers.

Liver Function Tests: Aspartate Aminotransferase (AST)) and Alanine Aminotransferase (ALT) were assayed spectrophotometrically according to the method of Bergmeyer and Bernt [27]. Alkaline Phosphatase (ALP) activity was assayed according to the method of Andersch and Szczypinski [28]. and serum Albumin was determined following the method of Doumas *et al* [29]. While, total protein (TP) concentration was estimated according to the method of Tietz [30] and serum albumin (Alb) concentration was estimated according to the method of Doumas *et al*. [29].

Histopathological Investigation: The liver samples were fixed in 10% formal-saline and then dehydrated by passing successively in different mixtures of ethyl alcohol and water, cleaned in xylene and embedded in paraffin. Sections of liver (4-5 µm thick) were prepared and then stained with haematoxylin and eosin (H/E) and mounted in neutral DPX medium for microscopic observations according to method of [31].

Iron Staining: Sections of liver were stained for ferric iron using the Perl (Prussian blue) according to the method of Bugelski [32].

Statistical Analysis: The SPSS 20.0 statistical software package program for Windows was used for statistical calculations. Statistical significance among groups was analyzed by one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons post-test. Data were presented as mean ± standard error (SEM). Differences were considered significant at ($P < 0.05$).

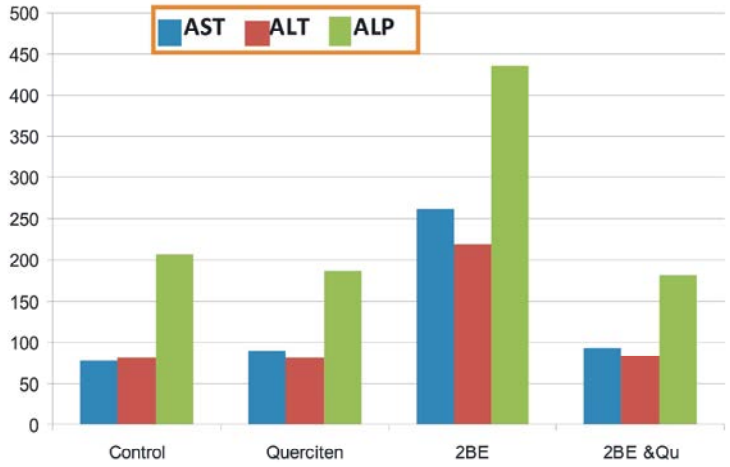

Fig. 1: Effect of Quercetin and BE administration on the concentration of liver enzymes

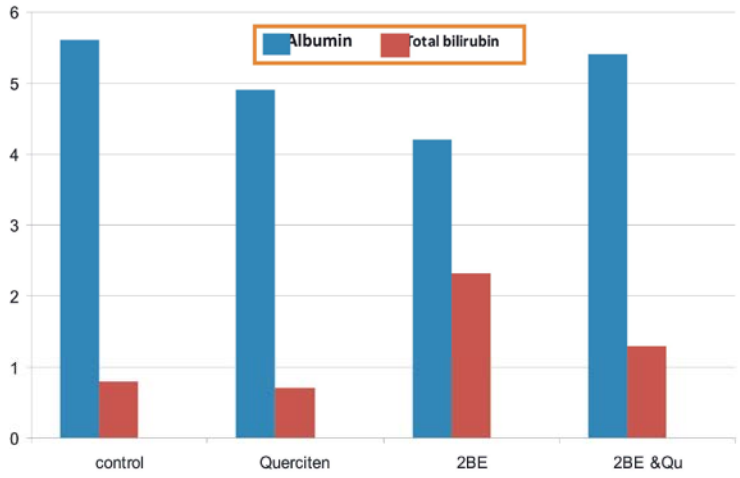

Fig. 2: Effect of Quercetin and BE administration on the concentration of Albumin and Total bilirubin.

RESULTS

Biochemical: The effect of 2BE on the liver enzymes, albumin and total bilirubin of treated rats represented in figures (1&2) the data showed that 2BE exert effect on liver resulted in induction of hepatotoxicity as manifested by elevation in the concentration liver enzymes alkaline aspartate transaminase (AST), alanine transaminase (ALT) and phosphatase (ALP). Also, 2BE induced significant decrease in albumin concentration levels and increase in total bilirubin levels when compared with control group. These changes were statistically significant when compared with the control group.

While, rats treated with Quercetin showed no significant differences in the concentrations of liver enzymes, albumin and total bilirubin when compared to the control group as shown in Figures (1&2).

Liver enzymes of rats treated with combination of 2BE and Quercetin showed highly significant decrease in ALT, AST and ALP when compared with 2BE alone group as shown in Figure (1&2). While, showed statistically non-significant difference when compared with both control and Quercetin groups. Combined administration of 2BE and Quercetin to rats resulted in increased concentration of albumin and total bilirubin which was statistically non-significant for albumin and significant for total bilirubin when compared with control group.

Histopathological Examination: Histologically, the liver of control rat group revealed normal characteristic architecture (Plate I A). Concerning liver sections from rats treated with 2-Butoxiethanol showing abnormal hepatic tissue represented by fatty liver, Congested blood vessels, multi necrotic cells with appearance of

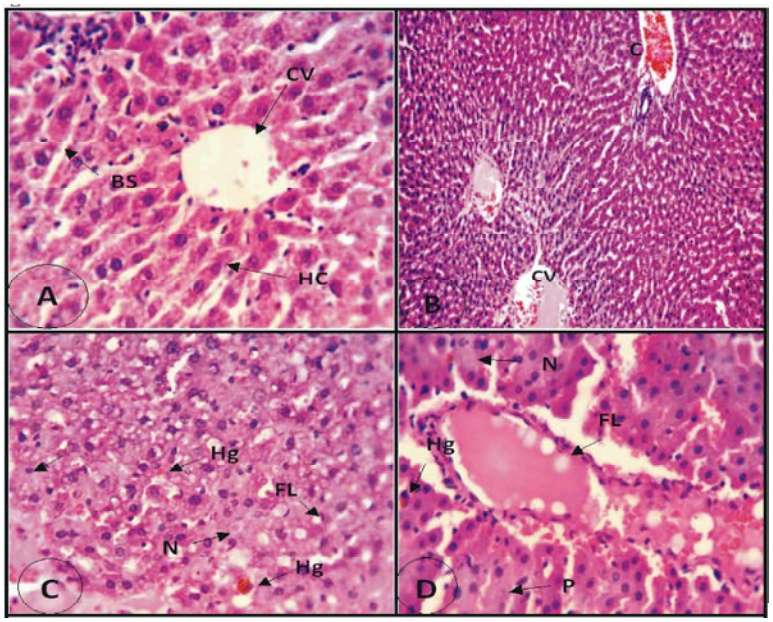

Plate I: (A): Enlarged section of normal liver structure in adult albino rat showing hepatic polygonal cells (HC), normal central vein (CV) and blood sinusoids (BS) (Hx. & E., x400).
(B, C, D): Sections of liver tissue from a rat treated 2-Butoxiethanol showing abnormal hepatic tissue represented by dilated central vein (CV), congested blood vessels (C), fatty liver (FL), multi necrotic cells (N), some piknotic hepatic cells (P) and haemosedrine granules (Hg) also were recorded (Hx&E., Mg 100,400).

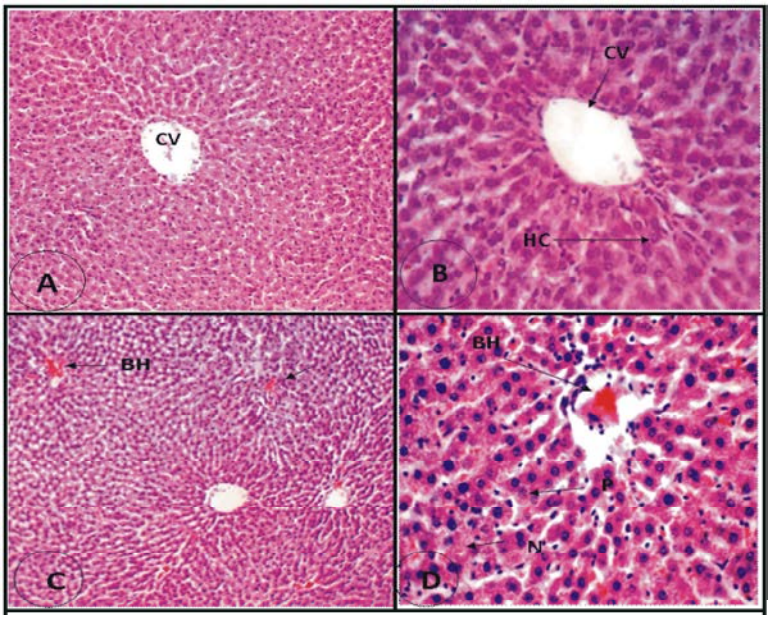

Plate II: (A, B) Liver Sections from rats treated with Quercetin showing, normal structure hepatocytes like as control, normal central vein (CV) and hepatocytes are arranged in cords (HC) (Hx&E., Mg 100,400 respectivelly).
(C): Liver Sections from rats treated with combination of 2BE plus Quercetin, showing, abnormal structure hepatocytes, represented by some blood haemolysis in central vein (BH) and hepatocytes moderately arranged in cords, (Hx. & E., x100)
(D): Enlargement Liver Sections from rats treated with combination of 2BE plus Quercetin, showing, abnormal structure hepatocytes, represented by some blood haemolysis in central vein (BH), piknotic cells (P) and necrotic hepatocytes (N) also appear (Hx. & E., x400)

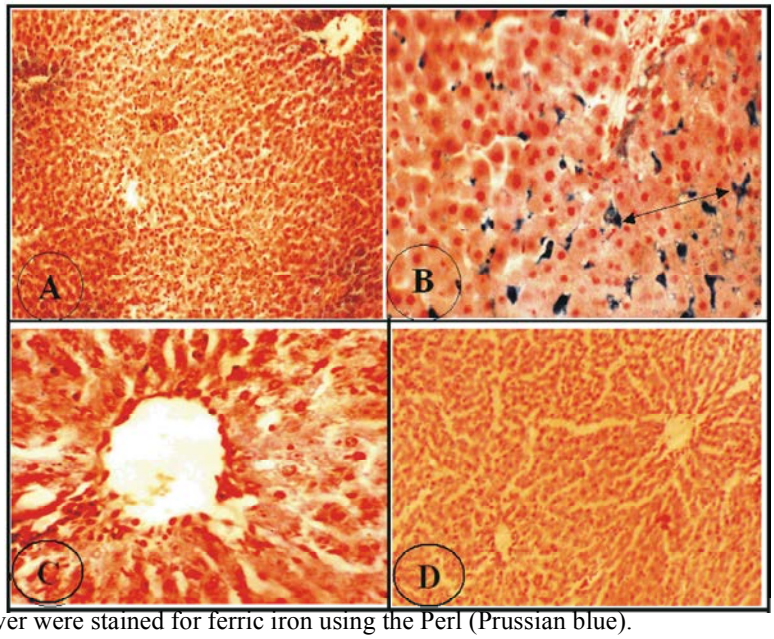

Plate III: Sections of liver were stained for ferric iron using the Perl (Prussian blue).
(A,C,D): sections from Control group, Quercetin treated group and 2BE plus Quercetin treated group showing negative Prussian blue stain.
While, (B) Section from liver section of rats treated with 2BE showing positive for Prussian blue stain.

haemosedrine granules as shown in (Plate I B,C and D). On the other hand, liver sections from rats treated with Quercetin only showing no pathological effect and the section appear like as control, as shown in (Plate II A&B). While, examining liver sections from rats treated with combination of 2BE and Quercetin together showed abnormal hepatic architecture, represented by some blood haemolysis in central vein and hepatocytes moderately arranged in cords, pyknotic cells and necrotic hepatocytes also appear, as shown in (Plate II C&D). Staining liver sections of rats from control group, Quercetin group and 2BE & Quercetin treated group showed negative for Prussian blue stain as shown in (Plate III A, C, D), While, liver sections from a rat treated to 2-Butoxiethanol showing positive for Prussian blue stain as shown in (Plate III B).

DISCUSSION

Liver diseases appeared nowadays to be more complicated. Part of this complication may be due to our frequent contact with chemicals and other environmental pollutants. The amount of medicine consumed has increased greatly which could be danger to the liver.

The hepatoprotective effect of Quercetin (50 mg/kg) used in the present study for 3 weeks were used to ameliorate the liver injury in rats intoxicated 2-butyoxyethanol (2_butyoxyethanol in chronic dose at 225 mg/kg b.w.). Where 2-butyoxyethanol, a well-known model compound for induction of hepatic injury. The induced liver toxicity manifested with elevation of serum levels of AST, ALT and ALP enzymes which are usually indicative of liver damage in animals [33, 34].

In this study after 28 days of (2BE) intoxication as a chronic high dose, rats showed highly significant increase ($P < 0.05$) in main liver enzymes (AST, ALT and ALP) in comparison with control group, significant decrease in albumin (Alb) when compared with control group and significant increase in Total bilirubin when compared with control group.

Clinical diagnosis of disease and damage to the structural integrity of the liver is commonly assessed by monitoring the status of serum ALT, ALP and γ-GT activities which are sensitive serological indicators of liver toxicity [35,36]. Higher activities of these enzymes in serum have been in response to oxidative stress induced by high fat diets [36].

These results were in agreement with Gualtieri *et al.* [37] who observed that, man was known to abuse alcohol and trichloroethylene, which probably contributed strongly to the abnormal liver function. In a man who ingested two doses of a concentrated glass cleaner containing 2-butoxyethanol 12 days apart, increased serum alanine aminotransferase, aspartate

aminotransferase and bilirubin. Hepatic failure was reported in an 87-year-old woman who died of cardiac arrest 3 days after ingesting an unknown volume of glass cleaner containing 6.5% 2-butoxyethanol [38].

NTP [39] reported that, statistically significant increases were found for serum alkaline phosphatase and for serum alanine aminotransferase when the rats exposed to 2- butoxyethanol in the drinking water. Studies with alcoholic patients and animal models showed that ethanol decreased the hepatic zinc levels and caused an elevation in lipid peroxidation products [40].

Cahill *et al* [41] showed that acute alcohol stress causes depletion of the protective effects of antioxidants. Acute alcohol stress inhibits its own metabolism by cytochrome P450 1E2 and, probably, metabolism of some lipophilic xenobiotics.

The present study approved that, after the rats received Quercetin at a daily dose of 50 mg/kg b.wt for 21 day, there was no statistically significant change in AST, ALT and ALP albumin (Alb), Total bilirubin but showed significant decrease ($P < 0.05$) in its level when compared with control group [42]. Groups treated with Quercetin showed no differences in hepatic markers compared to control group.

In this study, also after eight weeks of (2BE) injection in combination with (Qu) the rats which were treated with (Qu) after treatment with (2BE) showed highly significant decrease in ALT, AST and ALP in comparison with (2BE) group ($P < 0.05$) and insignificant difference in ALT, AST and ALP when compared with control group. Change in albumin (Alb) showed significant decrease ($P < 0.05$) but total bilirubin showed significant increase($P < 0.05$) in its level when compared with control group.

This in agree with the data published by Marcolin and his coworkers who stated that Quercetin showed a significant decrease in hepatic damage enzymes, lipoperoxidation, DNA damage and a lower degree of macrovesicular steatosis, ballooning and inflammatory process [43].

Quercetin showed beneficial effects on liver damage by enhancing antioxidant enzyme activity and decreasing prooxidant effect. This is due to the ability of Quercetin to interact with hydroxyl, superoxide, alkoxyl and peroxyl radicals subsequently scavenging them. Quercetin supplementation led to a slight decrease in antioxidant defense in controls. This may be due to pro-oxidant effect of Quercetin in normal cells [44].

Insignificant change in serum total lipids and total cholesterol of rats treated with an antioxidant (Quercetin) and rats treated with Quercetin combined with Polychlorined Biphenyls may be due to the protective role of Quercetin to prevent oxidation of the hormone-sensitive lipase which regulate lipid and cholesterol metabolism [45].

These results in agreement with Aydin [46] whereas, administration of Quercetin significantly reduced the levels of hepatic injury marker enzymes in the serum of Methotrexate treated mice. The antioxidant efficacy of Quercetin may be due to its higher diffusion into the membranes [47]. allowing it to scavenge oxyradicals at several sites through the lipid bilayer. It can be also resulting from its pentahydroxyflavone structure allowing it to chelate metal ions via the orthodihydroxy phenolic structure, thereby scavenging lipid alkoxyl and peroxyl radicals [48]. Quercetin also protected from liver damage as demonstrated by the decrease in plasma transaminases, AlP and LDH activities, with consequent restoration of plasma total protein, albumin, globulin and bilirubin [49].

Rats treated with Fenvalerate plus Quercetin showed significant reduction in the activities of AST, ALT, ALP, GGT and LDH. The enhanced activities of these enzymes may be due to lysis/damage of hepatocytes resulting in the permeation of these enzymes into serum. Enzymes like ALP, AST, ALT and GGT have been commonly associated with liver dysfunction/ damage and released into the circulation after cellular damage leading to their elevation in serum [50, 51].

El-Denshary *et al*. [52]. reported that Quercetin ameliorated the altered enzyme levels and protected rat liver against chemicals- or drugs-induced hepatotoxicity.

The hepatoprotective effect of Quercetin on liver injury is well evident, which significantly inhibits the elevation of these enzymes levels in rats with ethanol-induced hepatotoxicity treated with Quercetin, by keeping the structural integrity of the liver [53].

The histological abnormalities of the liver in patients with alcoholic liver disease or hepatitis C virus infection have been well described. However, liver histology in patients with dual pathology (alcoholic liver disease and HCV infection) is less clearly understood. In a previous study, Nakano and his coworkers assessed liver biopsy abnormalities in 17 alcoholics with anti-HCV antibodies [54].

Chronic exposure to 2-butoxyethanol also increased hemosiderin pigmentation in Kupffer cells in mouse and rat liver [9]. Evidence of liver damage, usually manifest as a result of architectural disarray, vascular congestion, hepatocytes necrosis, apoptosis, or inflammatory cell infiltration in either acute or chronic conditions. Some of these features were observed in the rats administered with

the highest dose of the extract. Generally, cells died as a result of necrosis or apoptosis when they are challenged with toxins, noxious agents or injuries [55].

The present study showed that 2-Butoxyethanol induced many histopathological changes in the liver of rats, showing, fatty liver (FL), multi necrotic cells (N) with appearance of haemosedrine granules and, congested blood vessels (C) leading to hemolysis (H).

Oxidative stress plays an important role in the formation of liver fibrosis via increasing the stellate cell activation and collagen synthesis. The hepatotoxins develop hypoxic conditions which can damage the perivenular zone of the hepatic acinus. The hepatocytes in the perivenular area contain less antioxidant factors and antioxidant enzymes [56]. and Histological examination of the liver treated with 2-Butoxyethanol revealed hepatocellular alterations (hepatocytes that stained more eosinophilic and lacked the amphophilic to basophilic granularity of the cytoplasm) centrilobular hepatocellular degeneration in males and brown to green granular pigment staining strongly positive for iron in Kupffer cell cytoplasm. NTP [39] and Ghanayem *et al.* [57] concluded that 2-butoxyethanol also caused histopathologic changes in the liver, consisting of focal coagulative necrosis of hepatocytes in one of six adult rats at 250 mg/kg. An association between Kupffer cell pigmentation (hemosiderin) caused by hemolysis and liver hemangiosarcomas has been reported in male mice for a limited group of chemicals including 2-butoxyethanol [9,58].

Histopathological examinations of animals that died prior to the end of the 15-day observation period showed congestion in the liver, with small necrotic foci with mesenchymatous reactions and inconstant (i.e., not always present) steatosis. A single 6-hour occluded dermal exposure of male and female New Zealand white rabbits to 451 and 902 mg/kg 2-butoxyethanol caused mottled livers with pocked surfaces in animals that died [59].

According to Mollendroff [60], reported that tissue toxicity usually manifests itself, especially in the histological preparation, in the form of cell degeneration accompanied by formation of large vacuoles, accumulation of fat and tissue necrosis.

The present study showed that the control animals which were given normal diet showed normal liver structure. Examination of liver sections obtained from rats treated with Quercetin (50 mg/kg) for 3 weeks showed normal structural organization of the hepatic lobules and normal central vein with normal portal vein and hepatocytes are arranged in cords. This result agree with Selvakumar *et al*. [61]. Liver sections of control and Rats treated with Quercetin rats showing normal histological appearance of the liver, including portal canal (PC), hepatocytes (H), centrally located nuclei (N) and Kupffer cell (K). and agree with Shizuma [62]. In the Quercetin-treated group, liver cell structure was maintained.

In this study the combination of 2-butoxyethanol in chronic dose and Quercetin prevented the antioxidant and antifibrotic effects of Quercetin represented by some blood hemolysis in central vein and hepatocytes moderately arranged in cords.

The flavonoid was given at a dose previously reported to have maximal beneficial effects in rats with biliary obstruction [24].

When liver injury is already present, animals were treated with Quercetin only after initiation of the cirrhogenic process. Results obtained indicate that Quercetin administration ameliorates the oxidative stress elicited by the hepatotoxins, results in a cytoprotective effect [63]. Liver sections of the rat exposed to Polychlorinated Biphenyls and simultaneously supplemented with Quercetin demonstrated restoration of normal arrangement and reduced apoptosis of hepatocytes [61].

It has been documented that the structure of Quercetin plays an important role in its antioxidant property. The O-dihydroxy structure of Quercetin has been recognized to accord higher stability to the radical form there by enabling it to participate in the delocalization of electrons [64]. The protective effect of Quercetin against lindane-induced hepatotoxicity was investigated by Abo-Salem *et al*. [65] who suggested that the protective effect of Quercetin on oxidative damage was attributable to its free radical scavenging action and antioxidant nature

Quercetin has beneficial effects on liver fibrosis in rats by enhancing antioxidant enzyme activity and decreasing the pro oxidant effect [63]. In conclusion, this study proved protective effect of Quercetin against chemicals-induced hepatotoxicity in rats.

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