Arabian Journal of Chemistry (2022) **15**, 103599

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Potential cardioprotective influence of bupropion against CCl4-triggered cirrhotic cardiomyopathy

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Received 25 June 2021; accepted 25 November 2021
Available online 1 December 2021

KEYWORDS

Cardiomyopathy;
Cirrhosis;
Carbon tetrachloride;
Bupropion;
Inflammation;
Oxidative stress;
Nitric oxide

Abstract Bupropion, an atypical anti-depressant and smoking cessation aid, attenuates complications arising from the activation of inflammatory and oxidative pathways. In this study, the effect of bupropion on an inflammatory and oxidative condition induced by carbon tetrachloride (CCl_4) namely cirrhotic cardiomyopathy (CCM) was investigated in rats. CCM was induced by intraperitoneal injection of CCl_4 (0.4 g/kg, i.p.). Bupropion was treated orally at doses 30 and 60 (mg/kg, p.o.) for 8 weeks. CCl_4 treatment significantly lowered hepatic antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) while enhanced Malondialdehyde (MDA). Elevations in serum nitric oxide (NO) metabolites nitrite/nitrate, and cardiac tumor necrosis factor alpha (TNF-α) and interleukin 1-beta (IL-1β) levels were observed. Cirrhosis also decreased contractility in response to isoproterenol (10^{-10} to 10^{-5} M). The spleen weight and intrasplenic pressure increased and QTc, QRS and RR intervals prolonged. Pathological damages in the liver for example focal necrosis, fibrosis and the hepatic blocking increased. On the other hand, bupropion increased GSH, CAT and SOD and lowered MDA. Bupropion reduced NO metabolites and TNF-α levels and decreased IL-1β. The cardiac contractile force improved at maximal effect (Rmax) 10^{-5} M by bupropion. The intrasplenic pressure was reduced by bupropion. Bupropion reduces QTc, QRS and RR intervals and the liver tissue damages. Bupropion played a cardioprotective role reducing inflammatory and oxidative factors. It may recover the impairment of cardiac

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2021.103599

contractility and hyperdynamic condition in CCM, and this effect could be mediated at least in part by a NO-dependent mechanism.

1. Introduction

Cirrhosis is the damage of the liver cells and their continuing replacement with scar tissue which impairs blood flow leading to the hepatocyte death and loss of liver function (Wang and Nagrath 2011). Hepatic fibrosis arises as a result of liver damage and regenerates apoptotic cells subsequent to repeated injuries. It is usually initiated by hepatocyte damage, resulting in recruitment of pro-inflammatory cells and platelets, stimulation of Kupffer cells and preceding cytokines and growth factors releases. These factors possibly associate the inflammatory and reparative phases of liver cirrhosis, by activating the hepatic stellate cells (HSC). When activated, HSC proliferates and transforms into myofibroblast-like cells which deposit huge amounts of connective tissue constituents (Brenner, Westwick et al. 1993). Besides, in cirrhosis, vascular abnormalities are abundant and it may be stated as a vascular disease of the liver, because of the noticeable anatomic alterations that arise at the intrahepatic circulation (Bosch 2007). Abnormalities of humoral factors are said to play essential roles in cirrhosis, which lead to diminished cardiac responses or cardiac dysfunction (Yang and Lin 2012).

Notably, the estimated incidence rate of cirrhotic cardiomyopathy (CCM) is approximately 56% of patients waiting for an orthotopic liver transplantation (Myers and Lee 2000, Møller and Henriksen 2002). It is related to abnormalities in structure and function of the heart (SHORR, ZWEIFACH et al. 1951, Zardi, Abbate et al. 2010). The mechanism underlying CCM has been discussed in relation to hyperdynamic circulation; however, after the liver transplantation, 7–15% of deaths are still associated with cardiac-related dysfunctions (Therapondos, Flapan et al. 2004). The main clinical outcome of CCM are a decline in systolic contractility, changed diastolic relaxation responding to physiologic and pharmacological factors, abnormally prolonged-QT intervals and hyperdynamic state (Gould, Shariff et al. 1969, Møller and Henriksen 2002). Moreover, evidence has revealed a correlation between CCM and the portal hypertension (Zardi, Abbate et al. 2010). Numerous key mechanisms underlie the development of CCM, for instance stimulation of the nitric oxide (NO) signaling pathway (Kumar, Paladugu et al. 2007). Moreover, it has been shown that the NO pathway is involved in unusual cardiac contractility in response to agonists of β-adrenergic receptors (Ebrahimi, Tavakoli et al. 2006, Kumar, Paladugu et al. 2007). Evidence has revealed that the systemic NO overproduction stimulated by inducible nitric oxide synthase (iNOS) leads to a negative inotropic outcome. This process seems to be modulated by cytokines like tumor necrosis factor alpha (TNF-α) and interleukin 1-beta (IL-1β), leading to cardiovascular dysfunction (Battarbee, Zavecz et al. 1999; Liu, Ma et al. 2000). Accordingly, the development of cardiomyocyte hypertrophy has been stated to diminish using NOS inhibitors (Liu, Ma et al. 2000, Nahavandi, Dehpour et al. 2001).

Carbon tetrachloride (CCl_4), a clear, colorless, instable, heavy and non-flammable liquid, is considered a famous compound causing chemical toxicity by generation of free radicals in tissues (Adaramoye 2009) such as the liver, the kidneys, the heart, the lung, the testis, the brain and the blood (Ahmad, Cowan et al. 1987, Ozturk, Ucar et al. 2003). Long-term administration of CCl_4 is a known experimental model for hepatic cirrhosis. The creation of free radicals as a rate limiting phenomenon in peroxidative damage is the most extensively recognized molecular mechanism of CCl_4 -induced cardiotoxicity (Hernandez-Munoz, Diaz-Munoz et al. 1997; Pereira-Filho, Ferreira et al. 2008). Reactive free radicals cause the cell damage through two distinct mechanisms, they bind to the membrane proteins and lead to lipid peroxidation. Consequently, researchers have utilized CCl_4 to produce liver cirrhosis in experimental animal models (Parola, Leonarduzzi et al. 1992). Antioxidants and radical scavengers are being employed to assess CCl_4 toxicity mechanisms along with protecting cells by disrupting lipid peroxidation chains (Weber, Boll et al. 2003). Three vital antioxidants enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are among the top on the list. These enzymes in turn dismutase superoxide radicals, interrupt hydrogen peroxides and hydroperoxides to non-hurtful molecules (H_2O_2, O_2 and alcohol) (Ighodaro and Akinloye 2018).

Bupropion, also well-known as amfebutamone, is considered an atypical anti-depressant. The major mechanism bupropion acts through is blocking of neuronal reuptake of norepinephrine (NE), and serotonin (5-HT) and dopamine (DA) (Eisenberg, Grandi et al. 2013). Bupropion was primarily sold as an oral anti-depressant (Wellbutrin; London, UK) and afterward industrialized as a non-nicotine aid for smoking cessation (Hurt, Sachs et al. 1997). It diminished inflammation in major depressive disorder (MDD) patients (Hung, Huang et al. 2016). Furthermore, bupropion lowers the level of pro-inflammatory cytokines for example TNF-α and interferon-gamma (INF-γ) in a mouse lipopolysaccharide (LPS)-induced inflammation model (Brustolim, Ribeiro-dos-Santos et al. 2006).

The basis for choosing bupropion in this model of cardiomyopathy was its anti-inflammatory and antioxidant effects in several animal models which inflammation and oxidation underlie their pathologies such as psoriasis , atopic dermatitis (Altschuler and Kast 2003), hepatitis B (Kast and Altschuler 2003), inflammatory bowel disease (IBD) in rats (Rashidian, Dejban et al. 2020), some malignancies (Kast and Altschuler 2005) and multiple myeloma (Kast 2005). In the current investigation, we intended to assess the probable anti-inflammatory and antioxidative influences of bupropion in CCl_4-induced CCM in rats. In addition, possible protective effects of bupropion in cardiac inotropic disorder responding to stimulation by β-adrenoceptor, and abnormal ECG were demonstrated. SOD, GSH, CAT as the antioxidative enzymes and MDA as a lipid peroxidation and oxidative stress enzyme

are assessed. Furthermore, pro-inflammatory cytokines such as TNF-α and IL-1β and anti-inflammatory cytokines like IL-10, and the serum NO metabolites were measured.

2. Materials and methods

2.1. Chemicals

Bupropion hydrochloride, carbon tetrachloride (CCl_4), isoproterenol hydrochloride ([1-(3,4-dihydroxyphenyl)-2-isopropyla mino ethanol hydrochloride]) were prepared from Sigma com.

2.2. Animals

Male adult albino rats (Wistar strain) 200–250 g weights were kept in an air-conditioned room with standard laboratory environment and natural a light–dark cycle (12 h light/dark) under temperature 25 ± 2 ºC. They were fed by standard pellet and water. Notably, all animal use procedures were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, with the approval of the Ethics Committee in our university.

2.3. Grouping

Sixteen rats were randomly divided into six groups of ten as follows: 1- Control/NS group which received saline; 2- Cirrhotic group which received intraperitoneally (i.p.) injections of CCl_4 (0.4 g/kg), a solution of 1:6 of CCl_4 in mineral oil, 3 times a week for 8 weeks (Pérez-Vargas, Zarco et al. 2016); 3- and 4- Bupropion groups received bupropion orally at doses 30 and 60 mg/kg/day, p.o., 8 weeks, every day (Rashidian, Dejban et al. 2020); 5 and 6- Two treatment groups which received CCl_4 + bupropion (30 and 60 mg/kg/day, p.o.). Bupropion dissolved in saline solution (0.9%) which served as the vehicle control.

2.4. Electrocardiography (ECG)

Subsequent to the 8-week period prior to the excision of the hearts, ECG (QTc, RR and QRS intervals) was recorded for fifteen minutes from rats receiving under anesthesia of ketamine and diazepam (75 and 5 mg/kg, i.p.). ECG was recorded by three stainless steel subcutaneous electrodes linked to a bioamplifier (ADInstrument, Bella Vista, Australia). At that point, the augmented signals were digitized (rate 10 kHz) employing a Powerlab analogue/digital converter and illustrated by Chart7 software (ADInstrument). Heart rate, RR and QT intervals (the interval between the start of Q and end of T waves) were calculated for 5 min ECG. The QT intervals were shown as corrected QT (QT_c) using the Bazett's formula ($QT_c = QT/\sqrt{R\text{-}R}$). After that, the hearts were excised under deep anesthesia and used for the *in vitro* evaluations (Jazaeri, Tavangar et al. 2013).

2.5. Measuring the intrasplenic palp pressure (ISPP)

At the end of the study, the intrasplenic pulp pressure was measured using ADInstruments PowerLab (Australia, Chart 7), as an index for the portal pressure. Under anesthesia by ketamine (90 mg/kg i.m.), the abdominal cavity was exposed by a mid-line incision and the spleen was uncovered by retractions on the *peri*-splenic fat, and a one & gauge needle inserted into the splenic parenchyma. The needle was attached to a transducer which was calibrated for the venous pressure. The external reference point (zero) was mounted at the mid-section of rats. The pressure was recognized once a steady recording was obtained (Ackerman, Karmeli et al. 1996). Furthermore, the spleen of each rat was dissected and weighed after washing with saline, as the spleen weight is correlated with the liver fibrosis and cirrhosis (Li, Duan et al. 2017).

2.6. Sample collection

Twenty-four hours subsequent to the end of the treatment course, the rats were anesthetized. Subsequent to recording ECG and taking the blood, the liver and the ventricular muscles were isolated. The liver samples were prepared and stained with hematoxylin and eosin (H & E) reagent. Microscopic evaluation of the stained liver sections was performed for ensuring the induction of cirrhosis in rats (Bortoluzzi, Ceolotto et al. 2013). A small portion of the left ventricular muscle was snap frizzed in liquid nitrogen and kept at −70 °C for the biochemical assays.

2.7. Isolation of the papillary muscle from the left ventricle: Evaluation of the papillary muscle contractile force

After ECG recording, the animals were anesthetized via the intraperitoneal administration of ketamine. The hearts were rapidly separated and washed with saline solution. The left ventricular papillary muscles were exposed and isolated to be placed in a physiological salt solution in an organ bath as described by Yarmohammadi et al. (Yarmohmmadi, Rahimi et al. 2017, Sheibani, Nezamoleslami et al. 2020). The cold oxygenated physiological salt solution (PSS) was aerated with O_2 (95%) and CO_2 (5%) and includes the followings in mmol/l: NaCl, 112; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 1; NaH_2PO_4, 0.5; KH_2PO_4, 0.5; $NaHCO_3$, 25; glucose, 10; and EDTA, 0.004 (pH = 7.4) (Ma, Miyamoto et al. 1996). To obtain the maximum contractile force, the papillary muscles were connected to an isometric force transducer vertically under a resting tension of 500 mg. The papillary muscles were equilibrated in glass chambers (25 ml) of an organ bath ADInstruments PowerLab (Australia, Chart 7), 90 min before the assessments. The temperature of the bathing was retained at 33 °C. The basal contractility is described as the steady baseline contractile force of the muscles prior to adding the stimulating factors. After the equilibration, the responsiveness to isoproterenol, a β-adrenoceptor stimulation was assessed and the papillary muscles were excited by cumulative concentrations from isoproterenol (10^{-10} to 10^{-5} M) to attain a concentration–response curve. The maximal effect (Rmax) is described as the contractile force subsequent to adding the highest dose (10^{-5} M). For each concentration, the growth in recorded contractile force which was recorded by an isometric transducer is shown as percentage of the basal contraction (Liu, Ma et al. 2000, Mousavi, Rezayat et al. 2016).

2.8. Measurements of the pro- and anti-inflammatory cytokines

TNF-α and IL-1β were assessed in the cardiac samples as the key pro-inflammatory cytokines and interleukin-10 (IL-10) as an anti-inflammatory cytokine using rat TNF-α Enzyme linked immunosorbent assay (ELISA kit (RAB0479, Sigma-Aldrich, USA), the rat IL-1 β ELISA kit (RAB0277, Sigma-Aldrich, USA), and the rat IL-10 ELISA kit, respectively. In addition, the absorbance of the samples was measured at 450 nm using an ELISA reader (Bio-Tek Synergy HT, USA). The cytokine levels were expressed as pg/tissue.

2.9. Measurement of the serum NO metabolites: The Griess reaction method

The levels of nitrite/nitrate were measured in serum samples as indicators of NO presence (Miranda, Espey et al. 2001). Briefly, the blood samples collected from cirrhotic rats were centrifuged for 1.5–3 h at 4 °C, using a molecular weight filter, 30 kDa. After removing the substances sheet, the specimens were mounted in a 96-well microliter plate (100 µl) and concentrated solution of 100 µl of vanadium (III) chloride (VCl3) was supplemented to the wells in which the Griess reagent (100 µl each) poured. The plates then were assessed using an ELISA plate reader (540 nm), and 30 min were incubated at 37 °C. The rate obtained showed the quantity of plasma nitrite/nitrate and is expressed as µmol/l.

2.10. Evaluation of lipid peroxidation and oxidative stress: Malondialdehyde (MDA)

MDA produces as a marker to measure the level of oxidative stress in various organisms (Moore and Roberts 1998). The left ventricle was preserved at temperature of −80° C to assess the tissue levels of MDA based on the described protocol in the literatures (Ohkawa, Ohishi et al. 1979). The activity then was expressed as U/mg protein.

2.11. Glutathione (GSH): Tietze assay

The contents of total protein and reduced GSH were measured in the heart tissues. In addition, the heart sections were homogenized and centrifuged at 100,000 × grams and temperature of 4 °C for 30 min in a phosphate-EDTA buffer solution containing 25% HPO3. Afterwards, 4.5 ml of the phosphate-EDTA buffer was added to the collected supernatant. Two ml of the final reaction mixture (containing the phosphate-EDTA buffer, diluted tissue supernatant, and o-phthalaldehyde solution) were mixed and incubated at room temperature for 15 min. Finally, the absorbance of the samples was detected at 350 nm via fluorescence (Fakhraei, Hashemibakhsh et al. 2019). The activity was expressed as U/mg protein.

2.12. Superoxide dismutase (SOD): Winterbourn method

SOD is an antioxidant molecule, which plays a key role in the reduction of oxidative stress and inflammatory markers (Ighodaro and Akinloye 2018). According to the *Winterbourn method* (Winterbourn, Hawkins et al. 1975), the collected tissues were centrifuged at 15,000× grams and temperature of 4 °C for 20 min after using a mechanical homogenizer. Following that, the supernatant was separated and used for the analysis of the total cardiac tissue SOD activity using the activity assay kit ab65354 (colorimetric). The activity was expressed as U/mg protein.

2.13. Catalase (CAT)

Cardiac tissue specimens were homogenized in1% triton X-100, and the homogenated samples were diluted by a buffer of potassium phosphate. The reaction was stimulated adding H_2O_2 to the reaction mixture, and regarding the ability of tissue CAT to decompensate H_2O_2, the extent of enzyme action was quantitated observing the reduction at 240 nm of absorbance. CAT activity was calculated via measuring of the absorbance alterations after one min and expressed as U/mg protein (Shafaroodi, Hashemi et al. 2017).

2.14. Histopathology analysis

Liver tissue samples were taken immediately after sacrificing rats and fixed overnight in 10 % formaldehyde solution, followed by 70% alcohol, 100% alcohol and xylene. After embedding in paraffin, the samples were sectioned and stained with hematoxylin-eosin (H & E) reagent. Finally, an expert pathologist blinded to the study evaluated the H & E-stained slides and scored them according to previous animal studies Table 1 (Kim, Cho et al. 2011, Doustimotlagh, Dehpour et al. 2014).

2.15. Statistical analysis

Statistical analyses were done by GraphPad Prism software (version 7.0, Inc., San Diego, CA, USA) and the outcome expressed as mean ± SEM. Statistical evaluation was carried out by analysis of variance (one-way ANOVA), followed by *Tukey*'s post-test when three or more experimental groups were included. While examining the assessment between two groups, Student's *t*-test was performed. For estimation of two variables (cirrhosis vs. control and other treatment groups), the analysis was done by two-way ANOVA followed by Bonferroni post-test. Finally, a *P*-value less than 0.05 was considered a statistically significant difference.

3. Results

3.1. The spleen weights and the pulp pressure

The changes in the spleen weights and the intrasplenic pulp pressure are illustrated in Fig. 1. As can be observed, the spleen weight increased significantly in CCl_4-treated group compared to the saline-treated group ($P < 0.01$), Fig. 1a. In addition, the pulp pressure was enhanced in CCl_4-treated group compared to the saline-treated group ($P < 0.001$), Fig. 1b. Conversely, bupropion at dose 60 mg/kg lowered the pressure in CCl_4-treated rats in a significant manner ($P < 0.05$) Fig. 1b.

Table 1 The Laennec scoring system for staging fibrosis in the liver.

Stage	Name	Septa (thickness and number)	Criteria	Score
0	No definite fibrosis			0
1	Minimal fibrosis	+/-	No septa or rare thin septum	1
2	Mild fibrosis	+	Occasional thin septa	2
3	Moderate fibrosis	+ +	Moderate thin septa	3
4A	Cirrhosis, mild, definite or probable	+ + +	Marked septation with rounded contours or visible nodules	4
4B	Moderate cirrhosis	+ + + +	At least two broad septa, but no very broad septa	5
4C	Severe cirrhosis	+ + + + +	At least one very broad septum	6

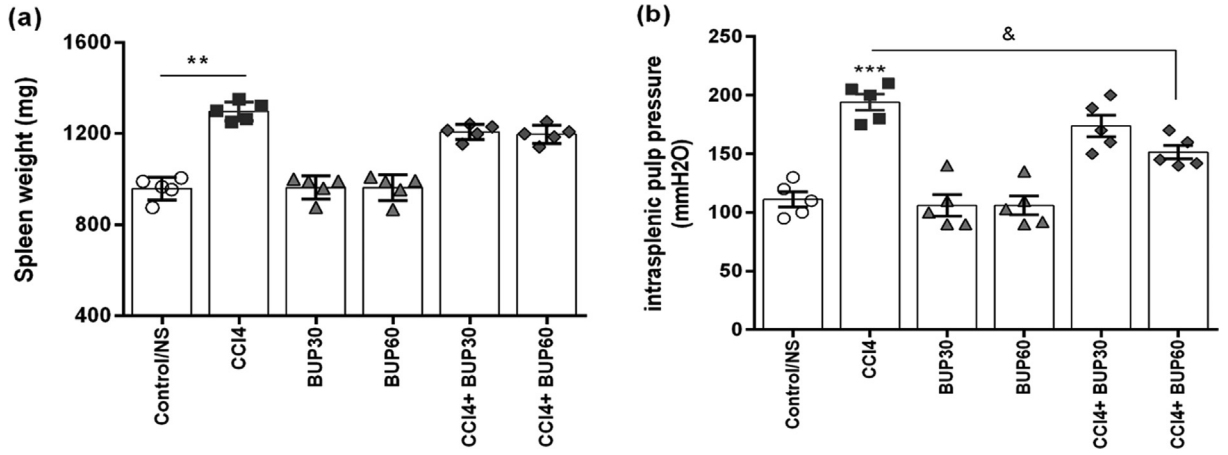

Fig. 1 The changes in the spleen weight (Fig. 1a) and the splenic pulp pressure of rats (Fig. 1b) in the study groups (results are expressed as Mean ± SEM; n = 10). ** ($P < 0.01$) and *** ($P < 0.001$) compared to the control group; & ($P < 0.05$) compared to CCl_4 group. Control/NS group: which received saline; CCl_4 group: the cirrhotic group which received CCl_4 injections (0.4 g/kg, i.p.), a solution of 1:6 of CCl_4 in mineral oil; Bu 30 and Bu 60 groups: which received bupropion orally at doses 30 and 60 mg/kg/day, p.o; CCl_4 + Bu 30 and CCl_4 + Bu 60: treatment groups which received CCl_4 + bupropion (30 and 60 mg/kg/day, p.o.).

3.2. ECG parameters

The changes in ECG parameters including QTc, QRS and RR intervals are given in Table 2. As can be understood, in the CCl_4 group, QTc, QRS and RR intervals were abnormally prolonged in a significant manner $P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively when compared to the saline-treated (control) group. Conversely, in comparison to the CCl_4 group, bupropion at dose 30 mg/kg significantly reduced QTc, QRS and RR intervals in cirrhotic rats ($P < 0.05$). Likewise, bupropion at dose 60 mg/kg significantly reduced QTc, QRS and RR intervals in cirrhotic rats $P < 0.01$, $P < 0.05$ and $P < 0.01$, respectively.

3.3. The isoproterenol-induced contractile forces

The cumulative concentration–response curve for isoproterenol in the ventricular papillary muscle at log concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M is shown in Fig. 2. As can be seen, the isoproterenol-induced contractile

Table 2 Effect of bupropion (30 and 60 mg/kg) on ECG parameters (QTc, RR, and QRS intervals) in CCl_4-treated rats (n = 10). Data are represented as Mean ± SEM. (QTc: QT-corrected). ** ($P < 0.01$) and *** ($P < 0.001$) compared to the control group; & ($P < 0.05$), and && ($P < 0.01$) compared to CCl_4 group.

	Control/NS	CCl_4	BUP 30	BUP 60	CCl_4 + BUP 30	CCl_4 + BUP
QTc interval	111.6 ± 3.26	232.5 ± 4.72***	110.6 ± 4.65	109.6 ± 4.32	198 ± 3.84&	17 3 ± 5.72&&
QRS	16.85 ± 0.54	26 ± 0.81**	17.25 ± 0.94	15.6 ± 0.65	21.10 ± 0.50&	19.4 ± 1.1&
RR interval	160.9.5 ± 2.37	259.8 ± 5.70***	161.3 ± 3.77	169.6 ± 4.65	230.5 ± 4.63&	217 ± 4.09 &&

Control/NS group: which received saline; CCl_4 group: the cirrhotic group which received CCl_4 injections (0.4 g/kg, i.p.), a solution of 1:6 of CCl_4 in mineral oil; Bu 30 and Bu 60 groups: which received bupropion orally at doses 30 and 60 mg/kg/day, p.o; CCl_4 + Bu 30 and CCl_4 + Bu 60: treatment groups which received CCl_4 + bupropion (30 and 60 mg/kg/day, p.o.).

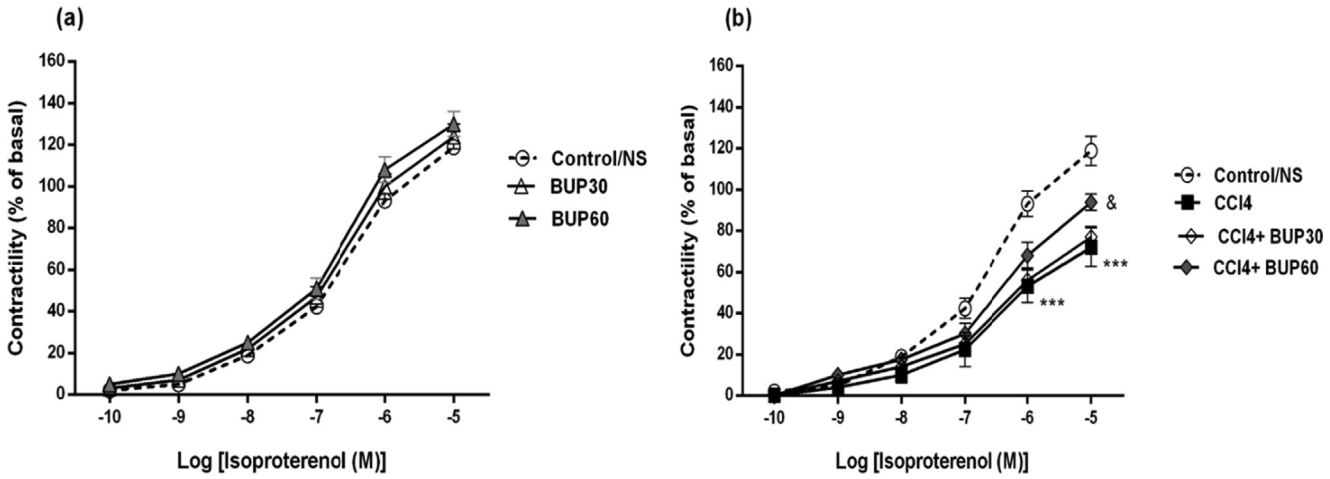

Fig. 2 Cumulative concentration–response curve (log concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) for isoproterenol in left ventricular papillary muscles isolated from control, CCl_4-induced cirrhotic and bupropion-treated rats (results expressed as Mean ± SEM; n = 10). *** ($P < 0.001$) compared to the control group; & ($P < 0.05$) compared to CCl_4 group.Control/NS group: which received saline; Bu 30 and Bu 60 groups: which received bupropion orally at doses 30 and 60 mg/kg/day, p.o. (Fig. 2a). CCl_4 group: the cirrhotic group which received CCl4 injections (0.4 g/kg, i.p.), a solution of 1:6 of CCl_4 in mineral oil; CCl_4 + Bu 30 and CCl_4 + Bu 60: treatment groups which received CCl_4 + bupropion (30 and 60 mg/kg/day, p.o.) (Fig. 2b).

forces (percentage of the basal contraction) were not significantly different between saline-treated group and bupropion -treated groups (30 and 60 mg/kg), Fig. 2a. On the other hand, CCl_4-treated group had significant reductions in contractility at log concentrations of 10^{-6} and 10^{-5} (M) ($P < 0.001$) in comparison to the saline-treated group, Fig. 2b. On the contrary, bupropion -treatment at dose 60 mg/kg markedly raised the contractility resulting from isoproterenol at *Rmax* (10^{-5} M) in CCl_4-treated group ($P < 0.05$), Fig. 2b.

3.4. The tissue levels of cytokines and NO

The tissue levels of TNF-α, IL-1β and IL-10, and the serum level of NO are given in Fig. 3. As can be observed, the levels of pro-inflammatory cytokines TNF-α and IL-1β significantly increased in CCl_4-treated groups compared to saline-treated groups, $P < 0.001$ (Fig. 3a and 3b). Conversely, bupropion at dose 60 mg/kg significantly lowered TNF-α level, $P < 0.05$ (Fig. 3a). Notably, in CCl_4-treated groups, bupropion at doses 30 and 60 mg/kg caused marked decreases in TNF-α level, $P < 0.01$ and at dose 60 mg/kg significantly lowered IL-1β level compared to CCl_4-treated group, $P < 0.05$. In addition, the serum NO level was significantly higher in CCl_4-treated group ($P < 0.001$) and lower in bupropion -treated group 60 mg/kg, ($P < 0.05$). On the contrary, bupropion at doses 30 and 60 mg/kg lowered NO metabolites nitrite/nitrate levels in cirrhotic rats compared to CCl_4-treated groups, $P < 0.05$ and $P < 0.001$, respectively (Fig. 3c). Particularly, bupropion at doses 60 mg/kg in CCl_4-treated group showed significantly more decrease compared to bupropion at dose 30 mg/kg in CCl_4-treated group $P < 0.05$ (Fig. 3c). Regarding the anti-inflammatory cytokine IL-10, in CCl_4-treated groups, bupropion at doses 60 mg/kg markedly enhanced IL-10 level in comparison to saline-treated group ($P < 0.05$) while other groups showed no significant changes (Fig. 3d).

3.5. The tissue levels of SOD, GSH, CAT and MDA

The tissue levels of SOD, GSH, CAT and MDA are illustrated in Fig. 4. As can be observed, the levels of antioxidant enzymes SOD, GSH and CAT significantly reduced in CCl_4-treated groups compared to saline-treated group, $P < 0.001$ (Fig. 4a, 4c, 4d). Conversely, the oxidative enzyme MDA markedly elevated in CCl_4-treated groups compared to saline-treated group, $P < 0.001$ (Fig. 4b). On the other hand, bupropion at dose 60 mg/kg significantly raised SOD and GSH levels ($P < 0.05$) (Fig. 4a, 4c) while lowered MDA level in normal rats compared to saline-treated group $P < 0.05$ (Fig. 4b). Notably, in CCl_4-treated groups, bupropion at dose 60 mg/kg caused marked increases in SOD and CAT levels compared to CCl_4-treated group, $P < 0.001$ and $P < 0.05$, respectively (Fig. 4a, 4d). Similarly, bupropion at doses 30 and 60 mg/kg enhanced GSH levels in cirrhotic rats, $P < 0.01$ and $P < 0.001$, respectively (Fig. 4c). Further, bupropion at doses 30 and 60 mg/kg lowered MDA levels in cirrhotic rats compared to CCl_4-treated group ($P < 0.01$) (Fig. 4b).

3.6. Microscopic outcomes

The histopathologic evaluations (H & E staining) are illustrated in Fig. 5. In the control group (saline), a mild infiltration of predominantly lymphocytes in some of the portal tracts is seen. Focal interface hepatitis is present in a minority of the portal tracts. No bile duct damage, paucity or proliferation are identified. Rats with normal liver architecture have no fibrosis and no confluent necrosis and steatosis are identified. Moreover, a marked congestion involving the central veins, the portal veins and the sinusoids is evident (Fig. 5A).

In CCl_4 group, severe focal necrosis is present within the lobules. Rats showing fibrosis development of the portal areas

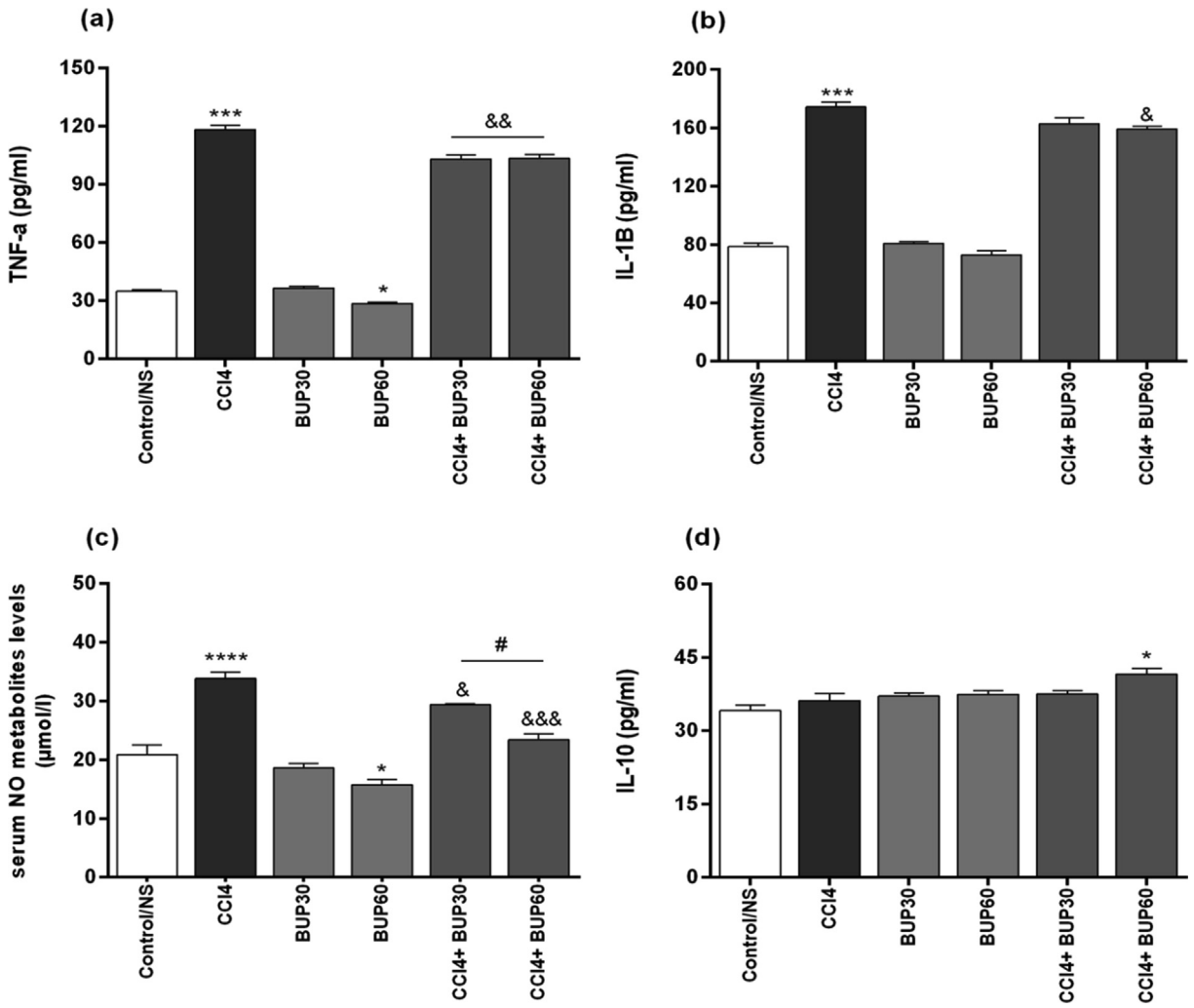

Fig. 3 Effects of bupropion (30 and 60 mg/kg) on the cardiac tissue levels of tumor necrosis factor alpha (TNF-α), interleukin 1-beta (IL-1β) and interleukin-10 (IL-10) (Fig. 3a, 3b and 3d), and the serum level of nitric oxide (NO) are given in study groups (Fig. 3c), results expressed as Mean ± SEM; n = 10. * ($P < 0.05$) and *** ($P < 0.001$) compared to the control group; & ($P < 0.05$), && ($P < 0.01$) and &&& ($P < 0.001$) compared to CCl_4 group; # ($P < 0.05$) comparison between bupropion treatments at doses 30 and 60 mg/kg in CCl_4-treated groups.Control/NS group: which received saline; CCl_4 group: the cirrhotic group which received CCl_4 injections (0.4 g/kg, i.p.), a solution of 1:6 of CCl_4 in mineral oil; Bu 30 and Bu 60 groups: which received bupropion orally at doses 30 and 60 mg/kg/day, p.o; CCl_4 + Bu 30 and CCl_4 + Bu 60: treatment groups which received CCl_4 + bupropion (30 and 60 mg/kg/day, p.o.).

with a marked portal-to-portal and also a portal-to-central bridging. There were solid connective tissue bands which appeared all over the sinusoids and the portal area. Further, proliferation of the bile ducts was observed, and there was hepatic blocking. Focal necrotic and apoptotic hepatic cells are evidently realized. Fatty degeneration and infiltration of patchy inflammatory cells are recognized (Fig. 5B).

In normal rats which received bupropion (30 and 60 mg/kg), no bile duct damage, paucity or proliferation are identified. The rats have a normal liver architecture and no fibrosis and no confluent necrosis and steatosis are identified (Fig. 5C, 5D).

In CCl_4 + bupropion (60 mg/kg) group, a mild to moderate focal necrosis is present within the lobules. Further, multifocal necrotic changes in the hepatocytes around the central vein are observed and mild hepatocyte necrosis in the portal area are evident (Fig. 5E).

In CCl_4 + bupropion (30 mg/kg) group, a moderate focal necrosis is present within the lobules. Besides, multifocal necrotic changes in the hepatocytes around the central vein are observed. Moreover, fibrosis expansion of the portal areas and proliferation of the bile ducts were detected. Further, the hepatic blocking was present (Fig. 5F).

4. Discussion

The present study investigated effects of bupropion oral treatments in a rat model of CCM induced by CCl_4. Our findings addressed that bupropion exerted cardioprotective effect in cirrhotic rats and this effect may be mediated at least in part

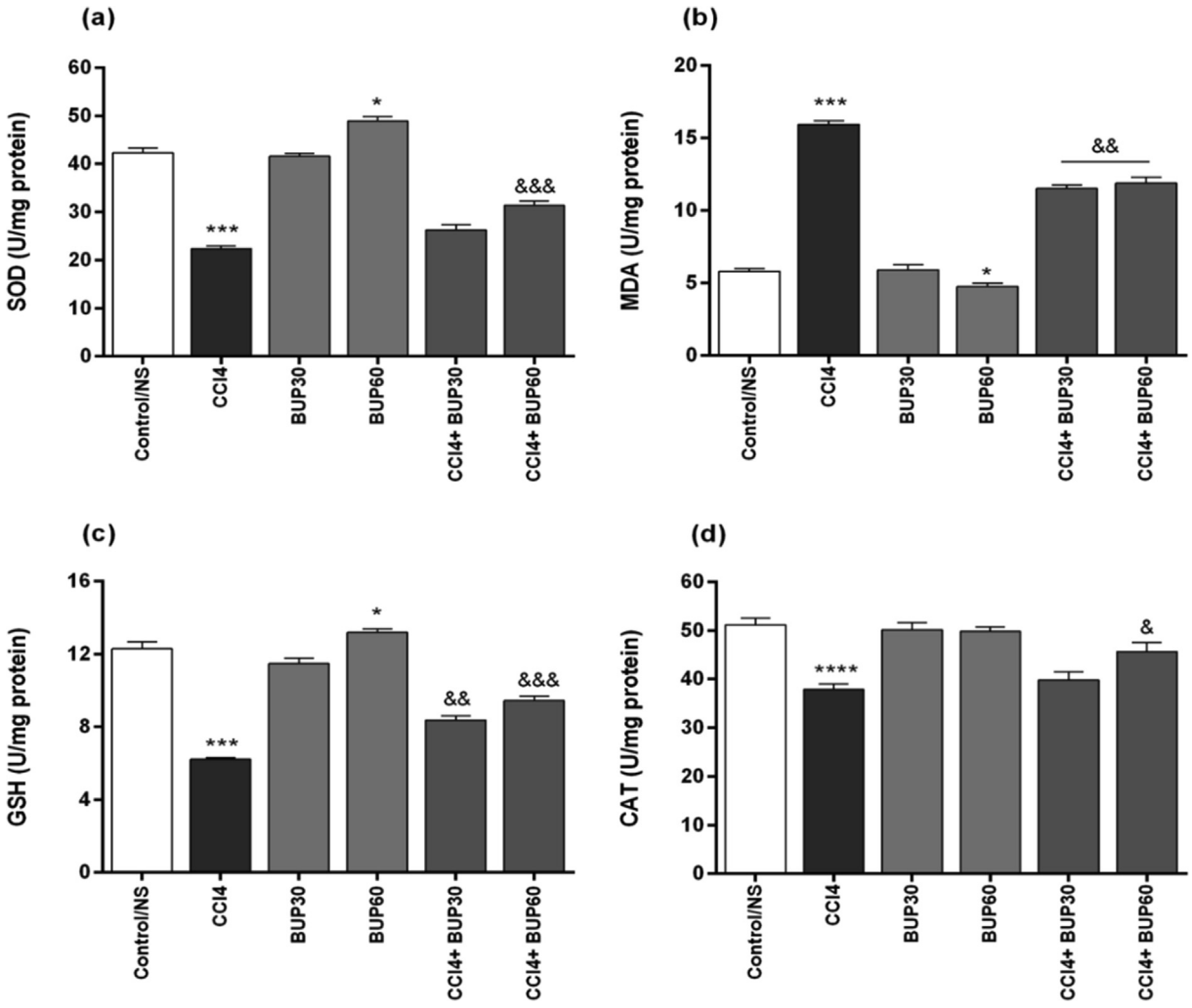

Fig. 4 Effects of bupropion (30 and 60 mg/kg) on the cardiac tissue levels of antioxidant and oxidant enzymes in CCl_4-induced oxidative stress in rat hearts. Superoxide dismutase (SOD) (Fig. 4a), malonyldialdehyde (MDA) (Fig. 4b); glutathione (GSH) (Fig. 4c) and catalase (CAT) (Fig. 4d) are given in study groups (results expressed as Mean ± SEM; n = 10). * ($P < 0.05$) and *** ($P < 0.001$) compared to the control group; & ($P < 0.05$), && ($P < 0.01$) and &&& ($P < 0.001$) compared to CCl_4 group.Control/NS group: which received saline; CCl_4 group: the cirrhotic group which received CCl_4 injections (0.4 g/kg, i.p.), a solution of 1:6 of CCl_4 in mineral oil; Bu 30 and Bu 60 groups: which received bupropion orally at doses 30 and 60 mg/kg/day, p.o; CCl_4 + Bu 30 and CCl_4 + Bu 60: treatment groups which received CCl_4 + bupropion (30 and 60 mg/kg/day, p.o.).

through its anti-inflammatory and antioxidant properties and also a down-regulation of NO.

In our study, rats treated with CCl_4 showed increases in the portal pressure and the spleen weight. The liver damage was also approved by microscopic evaluation. Consistent with our study, the cirrhosis resulted in hemodynamic disturbances like augmented resistance to the portal blood flow. The increased resistance is caused from the myofibroblasts contractions in the portal hypertensive circumstances. This endothelial malfunction can be attributed in part to modulating NO bioavailability in the intrahepatic microcirculation. Besides, the lower sensitivity of HSC_S may lead to the stimulation of HSC, the liver sinusoidal vasoconstriction, and consequently raise the portal hypertension (Perri, Langer et al. 2006, Kim and Baik 2010). Our records regarding the role of NO in the portal pressure have revealed that blocking NO production by bupropion may have a vital part in lowering the portal hypertension.

Notably, prolongation of QT or diastolic dysfunction, one feature of cirrhotic cardiomyopathy, at least appears in the majority of patients (Baik, Fouad et al. 2007). These observations are in line with our findings in which QTc, QRS and RR intervals are abnormally prolonged. Conversely, bupropion (30 and 60 mg/kg) reduced prolonged QTc, QRS and RR intervals. Moreover, in our experiment, the contractility of isolated papillary muscle reacting to isoproterenol stimulation was considerably impaired, which was in line with preceding results from bile duct-ligated (BDL) rats (van Obbergh, Vallieres et al. 1996, Liu, Ma et al. 2000). In cirrhotic rats, isoproterenol Rmax (10^{-5} M) on chronotropic and inotropic responses were markedly dropped in the atria and papillary muscles, and the expression and sensitivity of β-adrenergic

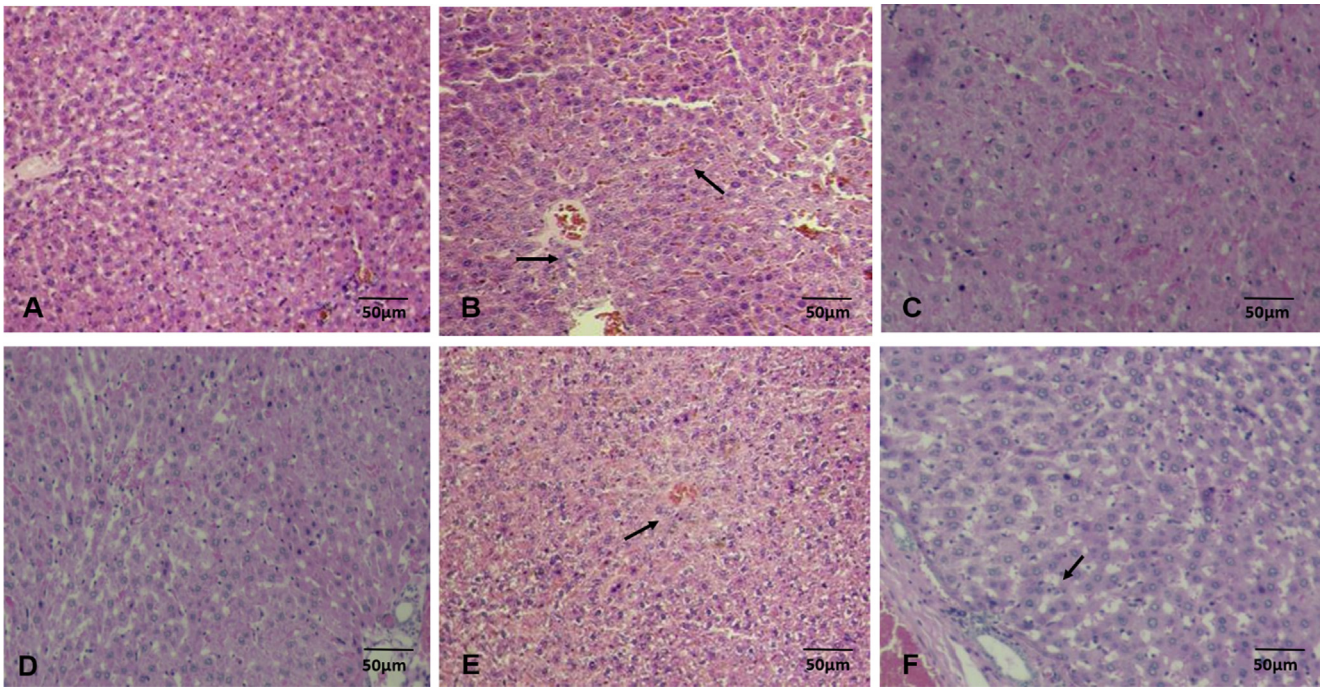

Fig. 5 The H & E stained histopathology photos of the liver. Control/NS group: which received saline (Fig. 5A); CCl_4 group: the cirrhotic group which received CCl_4 injections (0.4 g/kg, i.p.), a solution of 1:6 of CCl_4 in mineral oil (Fig. 5B); Bu 30 and Bu 60 groups: which received bupropion orally at doses 30 and 60 mg/kg/day, p.o (Fig. 5C, 5D); CCl_4 + Bu 30 and CCl_4 + Bu 60: treatment groups which received CCl_4 + bupropion (30 and 60 mg/kg/day, p.o.) (Fig. 5E, 5F).

receptors and their post-receptor signaling path in the heart are blunted (Lee, Marty et al. 1990, Ebrahimi, Tavakoli et al. 2006). Which is consistent with our finding in which CCl_4-treated groups had reductions in contractility at log concentrations of 10^{-6} and 10^{-5} (M).

Overproduction of NO shows a significant role in pathophysiology of cardiovascular complications in cirrhosis (Smith, Balligand et al. 1996, Liu, Ma et al. 2000) and the patients showing raised plasma levels of nitrites and nitrates, products of NO degradation (Guarner, Soriano et al. 1993). It has been stated that the diminished isoproterenol-stimulated inotropic effect may be related to cytokine-induced induction of iNOS activity in the cirrhotic heart (Liu, Ma et al. 2000). Consistent with our study, it is probable that the elevations in pro-inflammatory cytokines may cause the overproduction of NO metabolites in our measurement. Furthermore, the raised tissue levels of the cytokines like TNF-α and IL-1β together with cardiac contractility in cirrhosis linked to overproduction of NO, a negative inotropic agent, through stimulation of iNOS (Napoli, Bishop et al. 1994, Kumar, Paladugu et al. 2007) and enhanced NO synthesis in the cardiac tissue is related to the increased TNF-α level (Finkel, Oddis et al. 1992, Liu, Ma et al. 2000). We showed that bupropion decreased the elevated levels of TNF-α and IL-1β, leading to a normal inotropic response. This effect is thought to be mediated through a NO-dependent process. Our results presented evidence for bupropion in reduction of serum levels of NO metabolites and probably ensuing increase in the responsiveness of papillary muscles to β-adrenoceptor activation.

In addition, bupropion lowered inflammation in some diseases in which pathogenesis is associated to the inflammation and excess of TNF-α for example psoriasis, atopic dermatitis (Altschuler and Kast 2003), hepatitis B (Kast and Altschuler 2003), and some malignancies such as chronic lymphocytic leukemia (Kast and Altschuler 2005) and multiple myeloma (Kast 2005). Recently, the anti-inflammatory effect of bupropion on colonic injuries induced by acetic acid has been shown in rats. Bupropion (160 mg/kg, p.o.), for six consecutive days, ameliorated the colitis decreasing TNF-α and myeloperoxidase (MPO) activities (Rashidian, Dejban et al. 2020). These results are in line with our findings in which serum NO metabolites and cardiac pro-inflammatory cytokines TNF-α and IL-1β elevated.

In CCl_4-treated rats, the activities of the hepatic antioxidant enzymes SOD and CAT and GPx were decreased (Tsai, Hsu et al. 2009, Sarhan, Farid et al. 2019). In a study, the increase of MDA in rats attributed to the trichloromethyl radicals that caused from CCl_4 metabolism. Those radicals stimulate lipid peroxidation process with the formation of byproducts such as MDA (Madubuike, Onoja et al. 2015). In line with the aforementioned literature, CCl_4 treatment in our experiment lowered CAT, SOD and GSH levels while enhanced MDA. On the other hand, bupropion at doses 30 and 60 mg/kg increased GSH and at dose 60 mg/kg raised CAT and SOD levels and lowered MDA level.

On the whole, an elevation in serum NO metabolites as well as increases in cardiac TNF-α and IL-1β levels were observed in the current experiment. However, IL-10 remained unchanged. Cirrhosis also decreased contractility in response to isoproterenol stimulation. Additionally, the spleen weight and intrasplenic pressure increased. Furthermore, ECG parameters are abnormally prolonged. It also increased pathological damage in the liver tissue. On the other hand,

bupropion increased GSH and raised CAT and SOD levels while lowering MDA level. Besides, bupropion reduced serum NO metabolites and cardiac tissue TNF-α and IL-1β levels, and increased IL-10. In addition, the cardiac contractile force improved by bupropion-treatment (60 mg/kg) at Rmax. Additionally, the intrasplenic pressure was reduced. Moreover, bupropion significantly reduced QTc, QRS and RR intervals. Finally, the liver tissue damages decreased. According to our obtained results, bupropion especially at the dose of 60 mg/kg exerted

Evidence of other compounds that regulate oxidative and inflammatory responses were reported. For example, the protective effect of infliximab, a potent TNF-α inhibitor, against CCl4–induced hepatotoxicity has shown in rats. It suppressed transforming growth factor beta 1 (TGF-β1) and IL-1β levels (Sehitoglu, Tumkaya et al. 2015). In addition, herbal constituents such as *Piper cubeba* extract ameliorated CCl_4-induced liver injury and oxidative damage in a rodent model. The extract prevented increases in serum levels of hepatic enzymes and reduced the lipid peroxidation and restored activities of antioxidant enzymes. It down-regulated TNF-α and IL-6 mRNA expression, while upregulated IL-10 (AlSaid, Mothana et al. 2015). Similarly, thymoquinone and curcumin prevented gentamicin-induced liver injury in rats by enhancing antioxidant defense system, suppression of oxidative stress and attenuation of inflammation and apoptosis. They prevented elevations in serum AST, ALT and LDH as well as TNF-α and total bilirubin levels (Galaly, Ahmed et al. 2014).

It should be noted that the bupropion effects contribute not only to the mechanisms reported in this experiment and different inhibitors should be used in combination with bupropion. For example, it is suggested to address the NO pathway employing specific and non-specific NOS agonist and antagonist to clear which NOS is involved. In addition, immunohistochemistry (IHC) would strengthen histopathological evaluations. Furthermore, it is recommended to assess bupropion influences in other experimental models of cardiomyopathy and cardiotoxicity in rodents.

5. Conclusion

The present experiment provides evidence that bupropion played a cardioprotective role in CCM probably by reducing inflammatory and oxidative factors. Further, lowering NO level may diminish cardiac contractile dysfunction. Our outcomes may develop a new avenue in finding novel clues for bupropion as a promising candidate medication in CCM management.

Acknowledgements

This study was supported by National Natural Science of China (NO. , NO. , NO. , and NO.).

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