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Investigating the neuroprotective effects of Resveratrol on encephalopathy induced by bile duct ligation in male rats

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ABSTRACT

Hepatic encephalopathy (HE) is a complex neuropsychiatric disorder without definitive treatment. The precise mechanism that leads to HE is not fully understood. Resveratrol (RES) is a polyphenol compound with antioxidant and anti-inflammatory properties that mitigates the progression of different illnesses such as inflammatory and ischemic diseases. This study reports the effects of RES on neuronal injures in bile duct-ligated rats. The rats were randomly distributed into three groups including sham, bile duct ligation (BDL), and BDL + RES. Behavioral and biochemical studies were performed to evaluate neuronal injuries. The obtained data indicate that BDL experienced a balanced impairment and an increase in the hepatic enzymes. RES had a preserving role in the treated animals. Moreover, RES treatment declined neuronal injuries induced by BDL. For the first time, the results of this study showed that RES has beneficial effects in the rat model of HE probably because of its antioxidant and anti-inflammatory properties.

Keywords: *Bile duct ligation; Hepatic encephalopathy; Rat; Resveratrol; Neuroprotection*

1. INTRODUCTION

Hepatic encephalopathy (HE) is a debilitating complication of chronic liver failure [1]. HE may lead to coma and death in patients with long term liver failure [2]. Bile duct ligation (BDL) has been used as an animal model of hepatic cirrhosis (HC) [3]. Rat models of BDL show neurological impairments [4] similar to that observed in humans. BDL causes learning, memory, and motor deficits in animals [5]. Different agents with antioxidant activity such as melatonin have beneficial effects against neurological impairments observed in BDL rats [6].

Resveratrol (RES) (trans-3, 5, 4' -trihydroxystilbene) is an abundant polyphenolic compound found in plants such as grapes, peanuts, and mulberries. The two isomers of RES are in the trans and cis forms [7]. Accumulating evidence indicates the useful effects of RES on neurological and hepatic systems [7]. This study was conducted to investigate the effects of RES treatment on behavioral deficits and neuronal injuries induced by BDL in male rats.

2. MATERIALS AND METHODS

Male Wistar rats (n = 30, 220–250 g) were used in the present work. The handling and care of the animals were approved by the Ethics Committee of the Kerman University of Medical Sciences (KMUS) (Ethics Code: IR.KMUS.REC.1396.682.). All rats were kept under standard conditions; a 12-h on-off light-dark cycle; free access to food and water, and the room temperature (20 ± 3 °C). The animals were randomly assigned into three groups consisting of 10 animals in each group (sham, BDL surgery, and BDL surgery + RES). RES was prepared from Nanjing Zelang medical technology Company (Jiangsu, China) and administered every day (100 mg/Kg) intraperitoneally (i.p.) for one week 14 days following the BDL. Sham and BDL surgery groups received saline+ethanol (i.p.) in the same volume and based on the time schedule considered for BDL surgery+RES group. All experimental procedures were performed in a blind manner.

2.1. BDL surgery.

Under deep anesthesia (ketamine 100 mg/kg, xylazine 20 mg/kg, i.p.) the bile duct was identified and ligated with 4-0 silk suture at two points. Then, the abdominal wall was closed in two layers. In sham animals, the common bile duct was identified and manipulated but not ligated. All animals were maintained for six weeks following the surgery and they had free access to water and food.

2.2. Biochemical assays.

At the end of the study, the animals were sacrificed under deep anesthesia and blood samples were collected by carotid bleeding. Using a commercially available kit, the following parameters were biochemically analyzed in the plasma separated from blood samples: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total bilirubin, and hepatic albumin.

2.3. Open field test.

An open field test was performed to evaluate the locomotor activity and exploratory in animals. The open field apparatus consisted of a Plexiglas box (80 cm × 80 cm × 50 cm). Measurements were performed for 5 min as the total distance

traveled and velocity by placing the rats in the center of the box for the evaluation of the locomotor activity. Animal behaviors were recorded using the system software (Noldus Ethovision® system, version7.1) [8].

2.4. Accelerated rotarod test.

The rotarod test was used to evaluate motor coordination and balance skills. The rotarod consists of a suspended rod rotating from 10 rounds per minute (RPM) to 60 RPM. After 3 adaptation trials in 24 h before the test, each rat was placed on the rotating rod for 3 trials by 30 min inter-trial interval. The cut-off for each trial was 300 s and the time that an animal was able to hold itself on the rod was recorded as the latency to fall [9].

2.5. Hanging wire grip test.

Grasping ability for forelimb strength and coordination of movement was assessed using the wire hang test. The rats were fixed between two platforms and their forelimbs were used to suspend their bodies from a wire 7 mm in diameter and 80 cm in length. The time spent holding the wire was recorded during 3 trials for each rat that inter-trial interval was 5 min [10].

2.6. Histological evaluation.

The animals were killed six weeks after BDL under deep anesthesia. Afterward, their brain was removed, fixed in 10% formalin for 48 h, and embedded in paraffin. To evaluate the morphology of cortical neurons, the hematoxylin and eosin (H&E) staining method were used. Coronal sections (5 µm) were cut from the cerebral cortex using a rotary microtome (Leika RM 2145). Concisely, sections were deparaffinized and stained with H&E. Cerebral cortex pyramidal neurons were manually counted in three microscopic fields

2.6. Statistical analyses.

All statistical analyses were performed using the SPSS software (ver. 22). The normality of data was checked using the Kolmogorov-Smirnov test. Results were presented as the mean ± SEM and analyzed using one-way ANOVA. Post-hoc analysis (Tukey's) was used for the analysis of data, and $p < 0.05$ was considered as the significance level.

3. RESULTS

Biochemical parameters levels were elevated as a result of BDL surgery after six weeks. Our data showed that BDL resulted in a significant increase in bilirubin, ALT, ALP, AST levels and the RES administration counteracted these effects. The albumin level markedly increased in the BDL rats with the RES treatment compared to BDL (Table 1).

3.1. Effect of BDL and RES on locomotor activity and exploratory in open field test.

Animals in the BDL group exhibited a decrease in the total distance ($p<0.05$) and velocity ($p<0.01$) compared to the sham group (Figs. 1A and 1B). Treatment with RES increased both total distance and velocity and had no effects compared to the sham group (Figs. 1A and 1B). Therefore, it is clear that RES treatment affects the surgery-induced changes in distance moved or velocity.

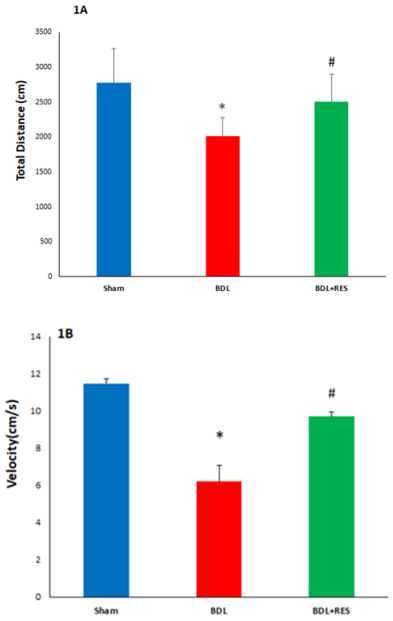

Figure 1. The effect of BDL on locomotion behavior in open field tests of rats; the mobility duration was decreased in the BDL group as compared to the sham group. There was a significant difference in both the total distance moved and velocity. *$p< 0.05$ compared to Sham; #$p< 0.05$ compared to BDL+RES, ANOVA, followed by Tukey's test. BDL: bile duct ligation RES: resveratrol

3.2. Effect of BDL and RES on motor coordination and balance skills in rotarod test.

In the rotarod test (Fig. 2), BDL caused a significant decrease in latency to fall in three trails ($p<0.05$, $p<0.01$, and $p<0.01$, respectively) compared to the sham group. Rats of the BDL+RES group spend more time on the rod ($p<0.01$). Our data showed that RES treatment had no significant effects compared to the sham group.

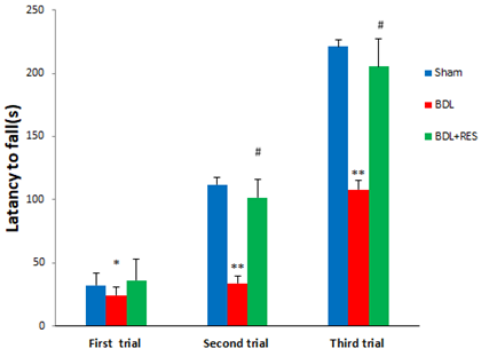

Figure 2. The effect of RES on the balance functions of rats; there were significant differences in latency to fall in a muscle strength test. Rats of the BDL model had a decreased time on the rod, which shows impairments of balance; administration of RES reversed this effect of BDL *$p<0.05$ compared to Sham; #$p<0.05$ compared to BDL+RES, ANOVA, followed by Tukey's test. BDL: bile duct ligation RES: resveratrol

3.3. Effect of BDL and RES on grasping ability in hanging wire grip test.

In three sequential trials, the BDL group showed a decrease in the time of falling as compared to the sham group in the wire grip test ($p<0.05$, Fig. 3). Rats of the RES treated showed a significant increase in the duration of staying on the wire grip and had no significant difference in comparison to the sham group.

3.4. Effect of BDL and RES on neuronal injury.

Results showed that BDL significantly increased the percent of degenerated neurons in the cortex (Fig. 4) compared to

the sham group. RES significantly decreased BDL-induced degenerated neurons in the treated rats.

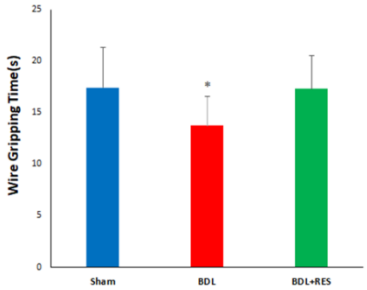

Figure 3. The effect of RES on muscle strength of rats; there were significant differences in latency to fall in a muscle strength test. Rats of the BDL model had a decreased time on the rod that shows impairments of balance; administration of RES reversed this effect of BDL *p<0.05 compared to Sham; #p<0.05 compared to BDL+RES, ANOVA, followed by Tukey's test. BDL: bile duct ligation RES: resveratrol

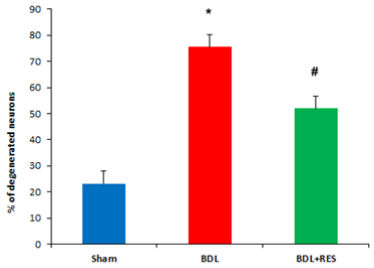

Figure 4. The effects of RES administration on neuronal damage induced by BDL in male rats; the figure demonstrates the quantitative analysis of cortical neurons in different groups. Data are the mean ± SEM. *p<0.001 compared with the sham group; #p<0.01 compared with BDL (BDL: bile duct ligation and RES: resveratrol

3.5. Discussion.

The results of the present study demonstrated that BDL leads to an increase in the levels of hepatic enzymes and degenerated pyramidal neurons in the rat cerebral cortex. In addition, BDL rats showed motor impairments that were reversed by RES administration.

Numerous earlier studies have demonstrated that BDL induces motor and cognitive impairments [11-13]. Although the mechanisms underlying these behavioral impairments are not completely understood, oxidative stress, neural apoptosis, and excitotoxicity have been suggested to underlie these deficits [14]. It has been reported that free radicals increased in the brains of BDL rats [2]. Thus, finding interventions to decrease these oxidative changes may help to decrease the behavioral deficits observed in BDL.

RES is a natural phytoalexin existing in the skin and seeds of grapes [15]. It has been shown that RES is a potent neuroprotective agent in different neurological disorders [16, 17]. BDL is known to induce neural damage through oxidative stress and neuro-inflammation [18].

Increasing evidence shows that motor dysfunction is related to neuronal oxidative injury in the brain [19]. In addition, exogenous antioxidant agents are able to inhibit ROS generation, inhibit oxidative injury, and may finally ameliorate motor dysfunction. It has been reported that RES has a specific neuroprotective effect on neurons. In the present study, RES improved behavioral impairments in BDL rats. RES is a highly potent antioxidant that could inhibit free radical generation in the brain [20] and the possible mechanism of action for RES may be through the reduction of free radicals in the environment.

In line with an earlier study by Huang et al. 2009 [6], BDL resulted in a significant increase in the bilirubin level of BDL rats (Huang et al. 2009). RES reversed the biochemical parameter alterations in the BDL rats that protect neurons from injury. Furthermore, as shown in this study, BDL induced a decrease in albumin (a marker of liver dysfunction) while RES administration reversed this effect of BDL and protected the neurons.

A decreased balance was seen in BDL rats compared to the other groups. RES could reverse this balance return it to the control value. Our data suggest the effect of BDL on balance function, which directly or indirectly is linked to the cerebellar function. We suggest conducting further studies in order to elucidate the underlying mechanisms.

Earlier studies have revealed that BDL can lead to neuronal degeneration in the hippocampus [18, 21]. Numerous studies have recommended that RES has potent protective effects in various disorders of the central nervous system (CNS) [17]. It possibly acts as a neuroprotective agent in the treatment of neurodegenerative diseases by modulating oxidants and inflammatory factors. A positive point for RES as a neuroprotective factor is its ability to cross the blood-brain barrier in animal models [22]. In the present research, BDL induced neuronal injury in rat cerebral cortex. This finding is in accordance with our previous study [18] .

Table 1. The effects of BDL and RES treatment on hepatic parameters in male rats

Groups (g/dl)	Bilirubin total (mg/dl)	ALT(U/l)	ALP(U/l)	AST(U/l)	Albumin
Sham	0.52±0.03	78.83±4.33	406±82	180±16.79	3.62±0.07
BDL	7.2±0.68*	187±21.05*	1217±106*	689±53.38*	3.15±0.06*
BDL+RES	0.71±0.07$^{@}$	101±6.52$^{@}$	744±94$^{@}$	289.5±32.19$^{@}$	3.47±0.08$^{@}$

BDL considerably increased the level of total bilirubin, ALT, ALP, and AST in male rats. Albumin level was decreased in BDL and BDL+RES animals. Moreover, RES treatment significantly reduced the total bilirubin, ALT, ALP, and AST levels in rats * p<0.05, compared with the sham; @ p<0.05, compared with the BDL

BDL: bile duct ligation RES: resveratrol ALT: alanine aminotransferase ALP: alkaline phosphatase AST: aspartate transaminase.

4. CONCLUSIONS

In conclusion, RES was found to be a promising neuroprotective agent because of its antioxidant properties. The current experimental study will shed more light on its true potential although it already contributes to our knowledge of neurodegeneration. Hence, we prove for the first time in our study that RES has positive effects on liver encephalopathy. Further

studies are warranted to explore the exact mechanism because the main mechanism of RES is still unknown. We reported this for the first time and our results confirm its novel role in liver encephalopathy.

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6. ACKNOWLEDGEMENTS

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