Journal of Applied Pharmaceutical Science Vol. 11(12), pp 158-168, December, 2021
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2021.1101215
ISSN 2231-3354

Progression of hepatic encephalopathy induced by bile duct ligation versus thioacetamide in rats: Regulatory role of apigenin

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ARTICLE INFO

Received on: 16/05/2021
Accepted on: 11/08/2021
Available online: 05/12/2021

Key words:
Hepatic encephalopathy, bile duct ligation, thioacetamide, apigenin, rats.

ABSTRACT

Hepatic encephalopathy (HE) is the decline in brain functions due to liver insufficiency. A high mortality rate was reported due to the rapid progression of HE from covert to overt, leading to detrimental consequences. This study aims to assess the progression of HE and the potential hepatoprotective and neuroprotective effect of apigenin (APG) in bile duct ligation (BDL) versus thioacetamide (TAA)-induced HE models in rats. Wistar albino rats were divided into eight groups; four groups for the BDL model and the other four groups for the TAA model (100 mg/kg, i.p., thrice weekly for five consecutive weeks). APG (20 mg/kg/day) or lactulose (LAC) (8 ml/kg/day), as the standard, was administered orally for three consecutive weeks starting from day 14 of the experiment. Liver enzymes, total bilirubin, serum ammonia, brain and liver glutathione and malondialdehyde, brain dopamine, hepatic interleukin-6, and nuclear factor kappa B were assessed, as well as the beam walking test and histopathological examinations were carried out. APG showed significant anti-hyperammonemic, anti-oxidant, and anti-inflammatory effects in HE groups. Additionally, improvement in behavioral test and histological image of livers and brains of HE rats treated with APG was observed. In conclusion, APG exerted a significant regulatory role compared to LAC in progression of HE in BDL and TAA models.

INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric disorder associated with liver disease after the exclusion of brain disorders (Butterworth, 2016a, 2016b), ranging from personality changes and intellectual impairment to a depressed level of consciousness (Ferenci, 2017). It is considered to be the second most common cause for hospitalization in cirrhotic patients after ascites, thus making HE an economic burden; although it is easily avoidable, it is the most common cause for readmission (Volk *et al*., 2012). High mortality rates were reported in patients with high grades of HE in the setting of end stage liver disease prior to coma (Wong *et al*., 2014).

HE is categorized to type A due to acute liver failure (ALF), type B due to portosystemic shunting without intrinsic liver disease (e.g., transjugular intrahepatic portosystemic shunting procedures), and type C results as a complication of liver cirrhosis (Allampati and Mullen, 2015). Although HE is a reversible syndrome of impaired brain functions accompanying advanced liver failure, it is not a single clinical entity and its diagnosis mainly depends on the exclusion and suspicion with no specific diagnostic test (Ferenci, 2017; Swaminathan *et al*., 2018). However, liver dysfunction can be treated successfully, HE is associated with poor survival and a high risk of recurrence and changes from covert HE to overt HE (Kaplan and Rossetti, 2011).

The broad spectrum of HE regarding the underlying cause, clinical manifestations, and risk factors making HE a complex condition and the exact cellular and molecular mechanisms are not yet fully elucidated (Liere *et al*., 2017). However, the hypotheses of hyperammonemia, neuro-inflammation, neurotransmitter system dysfunction and oxidative stress are among the main pathophysiological features of the

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available HE models (Butterworth, 2016c). A wide range of experimental animals have been used in HE research, including large animals, such as dogs, goats, pigs, and rabbits, and rodent, such as rats and mice, using either pharmacological models or surgical models to simulate HE in acute or chronic liver failure, respectively (Butterworth *et al*., 2009). Thioacetamide (TAA) is a hepatotoxicant that is extensively used either orally or intraperitoneally to induce ALF in mice and rats (Fontana *et al*., 1996). The TAA model shows good reproducibility and well-described hepatic and cerebral changes as type A HE, producing encephalopathy, metabolic acidosis, high transaminases, abnormal coagulation, and histological centrilobular necrosis (Peeling *et al*., 1993). Animal models of type C HE lead to decompensated liver cirrhosis and the bile duct ligation (BDL) model induces a reproducible model of biliary cirrhosis in rats, leading to liver failure, portal hypertension, translocation of bacteria, and immune system dysfunction, as well as hyperammonemia and decreased motor activities (Jover *et al*., 2005). Nevertheless, the BDL model simulates human neuropathology in type C HE, including Alzheimer type II astrocytosis, altered brain osmolytes, low-grade brain edema, inflammation, and motor activity deficits (Jover *et al*., 2006).

The addition of rifixamin, lactulose (LAC), l-ornithine-l-aspartate, and ammonia scavengers, such as glycerol phenylbutyrate and ornithine phenylacetate, to HE treatment guidelines are largely based on good quality clinical trials, although the current research and trials focus on liver support by removing circulating toxins that accumulate in the blood due to liver dysfunction, but more cost-effective studies are required (Hassanein *et al*., 2011). As HE severely impacts the quality of life of patients due to its unpredictable and complex nature, further treatment options are warranted to prevent the precipitants of HE and target the pathophysiological hallmarks of the disease, thus imposing actual benefit to HE patients. Therefore, cost-effective and less toxic treatment alternatives are highly needed. Phytochemicals and their derivatives are widely used in liver disease, among which flavonoids have drawn attention due to various pharmacological properties with no significant toxicity (Kumar and Pandey, 2013). Apigenin (APG) is a 4′,5,7 trihydroxy flavone that exists in a wide range of plants, and is found at significant levels in many fruits, vegetables, herbs, and spices (Lefort and Blay, 2013). The anti-carcinogenic, anti-metastatic, anti-angiogenic (Shukla and Gupta, 2010), antioxidant properties, hepatoprotective, and anti-inflammatory activities of APG have been reported earlier (Ali *et al*., 2014).

Hence, the current study aims to evaluate the progression of HE in both acute and chronic liver injury using TAA and BDL models, respectively, and the related neurological deficits as a consequence to liver dysfunction along with the identification of the potentials of APG as neuroprotective and hepatoprotective agents in both models of HE.

MATERIAL AND METHODS

Animals

Adult male Wistar albino rats weighing 150–250 g were purchased from the Egyptian Company for Production of Vaccines, Sera, and Drugs (EGYVAC; Cairo, Egypt). The animals were housed in plastic cages in the animal house, at the Faculty of Pharmacy, October University for Modern Science and Arts (MSA University). They were kept under appropriate conditions (temperature: 25°C ± 3°C; humidity: 50%; and 12/12-hour light/dark cycles) with sufficient food and water. This study was approved by the Research Ethics Committee of Faculty of Pharmacy, October University for Modern Science and Arts (MSA University) (approval number; PH 2/EC2/2018F).

Drugs and chemicals

APG and TAA were purchased from Amazon (England, UK), whereas LAC was obtained kindly from Abbott (Egypt). The reagent kits, chemicals, and reagents used in the present study were of analytical grade.

BDL rat model

Common BDL was carried out on Wistar rats to induce cirrhosis type C HE. The rats were randomly divided into four groups consisting of eight animals in each group (sham surgery, BDL surgery, BDL surgery + APG, and BDL surgery + LAC). APG and LAC were administered after 2 weeks of BDL and daily for three consecutive weeks. Sham and BDL surgery groups received saline intraperitoneally in the same volume, based on the time schedule considered for APG and LAC groups.

The rats were anaesthetized (ketamine 90 mg/kg and xylazine 12 mg/kg, i.p.). A middle abdominal incision was carried out. Then, after cutting the fascia and muscles, the common bile duct was ligated with a 4-0 silk suture at two points posterior to the hilum of the liver and anterior to the pancreas. The abdominal incision was closed in two layers. In sham animals, the common bile duct was manipulated but not ligated. All animals were maintained for 5 weeks following the surgery (Tag *et al*., 2015). The principles of laboratory animal care from the Guide for the Care and Use of Laboratory Animals [DHEW publication no. (NIH) 85–23, rev. 985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD, USA] were followed (National Research Council Committee for the Update of the Guide for the C, Use of Laboratory A).

TAA-induced HE rat model

Type A HE was induced in rats by the administration of TAA at a dose of 100 mg/kg, i.p., thrice weekly for five consecutive weeks (Mansour *et al*., 2015). To avoid hypoglycemia and electrolyte imbalance, 10% glucose water mixed with lactate ringer (25 ml/kg, i.p.) was injected every 12 hours since TAA injections. All rats were caged at 24°C with a 12-hour light/dark cycle and allowed free access to food.

Experimental protocol

Rats weighing 150–250 g were randomly divided into eight groups; each group consisted of eight rats. The groups were allocated as normal control groups; Sham group and normal saline group, respectively; HE groups; BDL group (Tag *et al*., 2015), and TAA group, respectively. APG groups received oral APG treatment (20 mg/kg/day starting from day 14) for three consecutive weeks in BDL rats and TAA-treated rats (Chen and Zhao, 2016; Sadraei *et al*., 2017). Finally, LAC groups as standard treatment for HE

(8 ml/kg/day, oral (p.o.), starting from day 14) for three consecutive weeks in BDL rats and TAA-treated rats (Jia and Zhang, 2005).

Behavioral test

Beam walking test is a test showing the capability of the rats to pass through a narrow beam to reach a dark box (2 × 100 cm), which is elevated 1 m above the floor. To force the rats to pass through the beam, a white light was used at the beginning of the beam. Four trails were made on the day of the test with 5-minute intervals to ensure that the rats were familiar with the beam and knew the existence of the box at the end of the beam. The time needed to cross the beam was recorded and expressed in seconds (Jover *et al*., 2006).

After 5 weeks, blood was withdrawn from the retro-orbital plexus. Then, rats were sacrificed. Liver and brain from each animal were rapidly dissected out, washed, and homogenized using phosphate-buffered saline (50 mM potassium phosphate, pH 7.5) at 4°C, producing a 20% homogenate. Liver and brain homogenates were kept at −80°C till time of analyses. Liver and brain tissues were kept in 10% formalin saline for histopathological examination.

Assessment of liver function

Activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and serum level of alkaline phosphatase (ALP), total bilirubin (TB), and ammonia were determined colorimetrically using commercially available kits (Biodiagnostic, Cairo, Egypt).

Assessment of liver and brain oxidative stress markers

Malondialdehyde (MDA) was determined using enzyme-linked immunosorbent assay (ELISA) kit provided by LifeSpan Biosciences, Inc. (Catalog No. LS-F4236). Reduced glutathione (GSH) was determined using the ELISA kit provided by LifeSpan Biosciences, Inc. (Catalog No. LS-F4612).

Assessment of inflammatory markers

Interleukin-6 (IL-6) was determined using the ELISA kit provided by LifeSpan Biosciences, Inc. (Catalog No. LS-F25921). Nuclear factor kappa-light-chain-enhancers of activated B cells [nuclear factor kappa B (NF-κB)] were determined using the ELISA kit provided by LifeSpan Biosciences, Inc. (Catalog No. LS-F21577).

Assessment of dopamine content

Dopamine was determined using the ELISA kit provided by LifeSpan Biosciences, Inc. (Catalog No. LS-F5364).

Histopathological assessment of rat liver and brain in TAA- and BDL-induced HE

Autopsy samples were taken from the liver and brain of rats in different groups and fixed in 10% formalin saline for 24 hours. Washing was carried out in saline and then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56° in a hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin stain, and then examined through the electric light microscope (Bancroft and Gamble, 2008).

Statistical analysis

All values are presented as means ± standard error of the means of eight experiments. Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison *post-hoc* test. The difference was considered significant when $p<0.05$. GraphPad prism®software version 6 for Windows (USA) was used to carry out these statistical tests.

RESULTS

Behavioral assessment

Effect of APG on beam walking test in BDL versus TTA-induced HE in rats

BDL resulted in a significant increase in time taken by rats to cross the beam as compared to that of sham rats. Treatment with APG (20 ml/kg, p.o.) for three consecutive weeks significantly decreased the time to cross, while treatment with standard LAC (8 ml/kg, p.o.) decreased the time, but it was at a higher time value than APG (Fig. 1).

Administration of TAA (100 mg/kg, i.p.) thrice weekly for five consecutive weeks increased in time to cross the beam when compared to that of normal control rats. Treatment with APG (20 mg/kg, p.o.) for three consecutive weeks significantly decreased the time taken to cross the beam. While treatment with standard LAC (8 ml/kg, p.o.) also decreased the time compared to that of normal control (Fig. 1).

Biochemical assessment

Effect of APG on hepatic function levels and serum ammonia in BDL versus TTA-induced HE in rats

BDL resulted in an increase in AST, ALT, and ALP when compared to that of sham group. Treatment with APG (20 mg/kg, p.o.) for three consecutive weeks significantly suppressed the elevated AST, ALT, and ALP. Treatment with standard LAC (8 ml/kg, p.o.) counteracted the elevations in AST, ALT, and ALP, but at a lower value than APG when compared to that of the BDL group (Table 1).

BDL resulted in an increase in TB levels in rats compared to that of sham group. Treatment with APG (20 mg/kg, p.o.) for three consecutive weeks significantly suppressed the elevated TB. Treatment with standard LAC (8 ml/kg, p.o.) decreased the levels as well, but at a slight higher value than APG when compared to that of the BDL group (Table 1).

BDL resulted in a significant increase in serum ammonia when compared to that of sham group. Treatment with APG (20 mg/kg, p.o.) for three consecutive weeks significantly inhibited the elevated ammonia levels. Treatment with standard LAC (8 ml/kg, p.o.) counteracted the elevations in ammonia levels, and the antihyperammonemic effect of LAC remained significant from that of APG as well as the BDL group (Table 1).

Administration of TAA (100 mg/kg, i.p.) thrice weekly for 5 weeks resulted in an increase in AST, ALT, and ALP levels

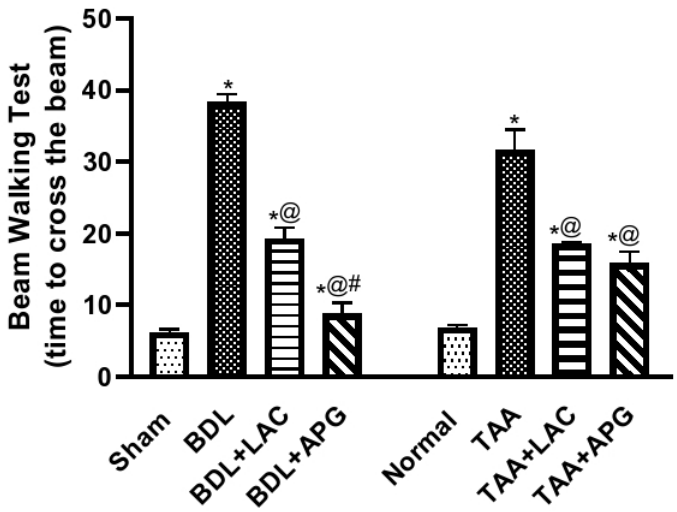

Figure 1. Effect of apigenin on beam walking test in BDL versus TTA induced-HE in rats, respectively. Data is represented as the mean ± SEM of eight animals. Statistical analysis was completed by one-way ANOVA proceeded by Tukey–Kramer test for multiple comparisons at $p \leq 0.05$. *Significant difference from corresponding control. @Significant difference from HE-induced control. #Significant different from reference drug.

Table 1. Effect of apigenin on serum hepatic function parameters in BDL versus TTA induced-HE in rats.

	Parameters / Groups	Aspartate aminotransferase (AST) U/l	Alanine aminotransferase (ALT) U/l	Alkaline phosphatase (ALP) U/l	TB (mg/dL)	Ammonia (mmol/ml)
TAA-induced HE BDL-induced HE	Sham control	9.627 ± 0.834	100.2 ± 4.644	113.7 ± 7.124	0.815 ± 0.038	52.11 ± 2.13
	BDL control	47.00 ± 4.177[a]	190.2 ± 6.890[a]	199.6 ± 6.603[a]	2.468 ± 0.0342[a]	131.00 ± 4.48[a]
	BDL + LAC	24.18 + 1.051[ab]	150.5 ± 7.286[ab]	152.1 ± 5.998[ab]	1.037 ± 0.084[b]	63.50b ± 2.39[ab]
	BDL + APG	16.12 ± 0.921[ab]	100.7 ± 10.14[bc]	114.4 ± 7.457[bc]	1.614 ± 0.093[abc]	95.36 ± 4.1[1abc]
	Normal control	12.37 ± 1.192	106.2 ± 9.809	108.5 ± 8.390	1.068 ± 0.093	45.14 ± 2.13
	TAA	73.36 ± 2.209[a]	200.1 ± 10.51[a]	215.7 ± 7.447[a]	8.550 ± 0.489[a]	134.80 ± 4.48[a]
	TAA + LAC	50.89 + 3.725[ab]	175.8 ± 6.478[ab]	172.4 ± 5.546[ab]	4.411 ± 0.206[ab]	93.80 ± 2.39[ab]
	TAA + APG	27.05 ± 1.843[abc]	131.2 ± 9.502[abc]	114.1 ± 7.896[bc]	2.094 ± 0.102[abc]	78.18 ± 4.18[abc]

Data is represented as the mean ± SEM of eight animals. Statistical analysis was completed by one-way ANOVA proceeded by Tukey–Kramer test for multiple comparisons at $p \leq 0.05$.

[a]Significant difference from corresponding control.

[b]Significant difference from HE-induced control.

[c]Significant different from reference drug.

when compared to that of normal control. Treatment with APG (20 mg/kg, p.o.) for 21 days significantly suppressed the elevated AST, ALT, and ALP. Treatment with standard LAC (8 ml/kg, p.o.) decreased the raised AST, ALT, and ALP, but at a lower value than APG when compared to that of the TAA control group (Table 1).

Induction of rats using TAA (100 mg/kg, i.p.) thrice weekly for three consecutive weeks resulted in an increase in TB levels when compared to that of normal control. Treatment with APG (20 ml/kg, p.o.) for 3 consecutive weeks significantly reversed the elevated TB. Treatment with standard LAC (8 ml/kg, p.o.) decreased the level of TB, but at a lower value than APG when compared to that of the TAA control group (Table 1).

Administration of TAA (100 mg/kg, i.p.) thrice weekly for 5 weeks resulted in an increase in ammonia levels when compared to that of normal control. Treatment with APG (20 mg/kg, p.o.) for 3 weeks significantly suppressed the elevated ammonia compared to TAA group as well as standard LAC. Treatment with standard LAC (8 ml/kg, p.o.) significantly decreased the raised ammonia levels when compared only to that of the TAA control group (Table 1).

Effect of APG on hepatic IL-6 and NF-κb levels in BDL versus TTA-induced HE in rats

BDL surgery resulted in a significant increase in IL-6 (Fig. 2A) and NF-κB (Fig. 2B) as compared to sham rats. Treatment with APG (20 ml/kg, p.o.) for three consecutive weeks significantly suppressed the elevated IL-6 (Fig. 2A) and NF-κB (Fig. 2B). While treatment with standard LAC (8 ml/kg, p.o.) decreased the levels of IL-6 (Fig. 2A) and NF-κB (Fig. 2B), but at a lower value than APG when compared to that of the BDL control group.

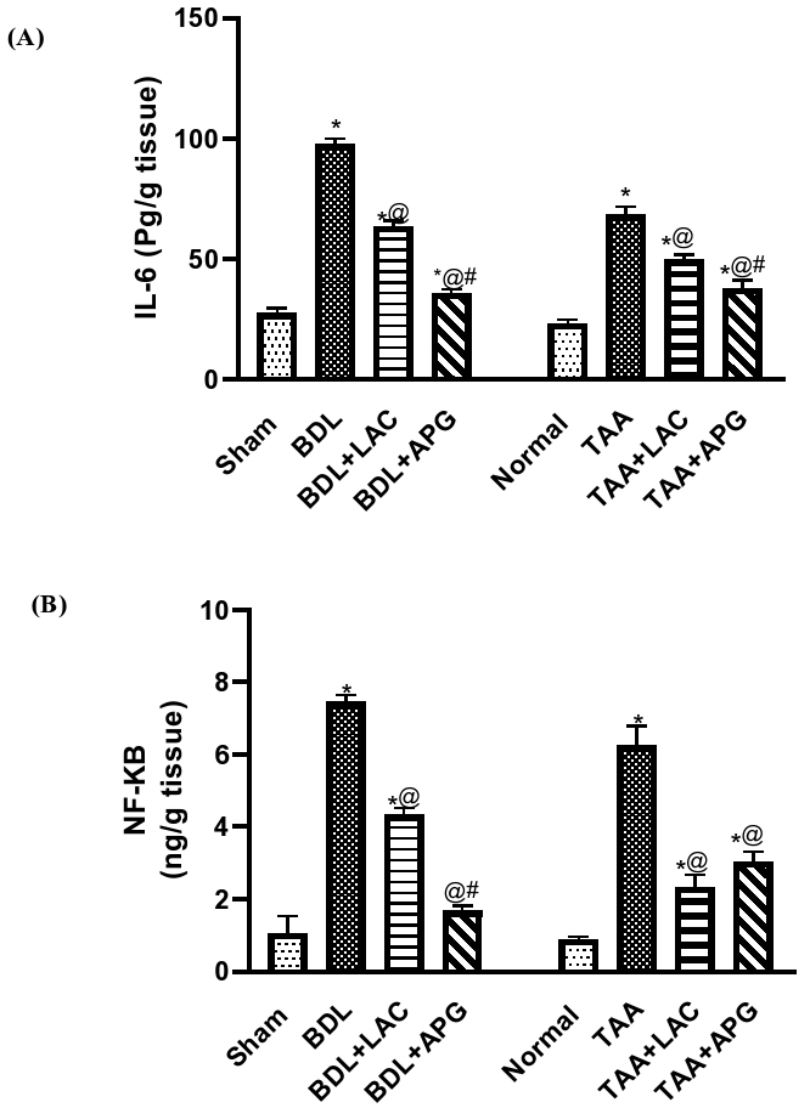

Figure 2. Effect of apigenin on interleukin-6 (A) and NF-kb (B) in BDL versus TTA induced-HE in rats, respectively. Data is represented as the mean ± SEM of eight animals. Statistical analysis was completed by one-way ANOVA proceeded by Tukey–Kramer test for multiple comparisons at $p \leq 0.05$. *Significant difference from corresponding control. @Significant difference from HE-induced control. #Significant different from reference drug.

Induction of HE using TAA (100 mg/kg, i.p.) thrice weekly for five consecutive weeks resulted in a significant increase in IL-6 (Fig. 2A) and NF-κB (Fig. 2B) levels when compared to that of normal control. Treatment with APG (20 ml/kg/day, p.o.) for three consecutive weeks significantly suppressed the elevations in IL-6 (Fig. 2A) and NF-κB (Fig. 2B), while treatment with standard LAC (8 ml/kg/day, p.o.) decreased the raised levels of IL-6 (Fig. 2A) and NF-κB (Fig. 2B), but at a lower value than APG when compared to that of the TAA control group**.**

Effect of APG on redox status biomarker in liver and brain tissues in BDL versus TTA-induced HE in rats

BDL resulted in a significant increase in liver and brain MDA while exerting a significant decrease in liver and brain GSH compared to sham rats. Treatment with APG (20 ml/kg, p.o.) for three consecutive weeks significantly suppressed the elevated MDA, but further increased the GSH levels. However, treatment with standard LAC (8 ml/kg, p.o.) decreased the elevated MDA, but further increased GSH levels in brain and liver tissues at an even lower value than APG as it should, when compared to that of the BDL control group (Table 2).

Induction of HE by TAA (100 mg/kg, i.p.) thrice weekly for five consecutive weeks resulted in an increase in liver and brain MDA and decrease in liver and brain GSH compared to that of normal control. Treatment with APG (20 ml/kg, p.o) for three consecutive weeks significantly suppressed the elevated MDA, but further increased GSH in liver and brain tissues. However, treatment with standard LAC (8 ml/kg, p.o.) decreased MDA, while it increased GSH levels in brain and liver at an even lower

Table 2. Effect of apigenin on liver and brain oxidative stress biomarkers in BDL versus TTA induced-HE in rats.

	Parameters / Groups	Hepatic MDA (ng/g tissue)	Hepatic GSH (ug/g tissue)	Brain MDA (ng/g tissue)	Brain GSH (ug/g tissue)
BDL-induced HE	Sham Control	71.03 ± 5.772	104.09 ± 2.334	0.6665 ± 0.052	3.062 ± 0.1104
	BDL Control	188.3 ± 11.47[a]	60.58 ± 4.733[a]	2.538 ± 0.057[a]	1.748 ± 0.166[a]
	BDL + LAC	142.8 + 7.200[ab]	72.64 ± 6.261[ab]	1.753 ± 0.157[ab]	2.086 ± 0.091[b]
	BDL + APG	106.4 ± 5.450[abc]	91.24 ± 2.658[abc]	0.9694 ± 0.057[abc]	2.891 ± 0.096[abc]
TAA-induced HE	Normal control	42.43 ± 2.860	24.73 ± 2.079	0.384 ± 0.037	2.899 ± 0.620
	TAA	132.2 ± 8.060[a]	7.487 ± 0.752[a]	1.702 ± 0.073[a]	0.620 ± 0.069[a]
	TAA + LAC	91.90 ± 2.454[ab]	19.66 ± 1.015[ab]	1.246 ± 0.072[b]	1.12 ± 0.060[b]
	TAA + APG	69.45 ± 3.116[abc]	18.71 ± 1.606[ab]	0.867 ± 0.057[abc]	2.195 ± 0.067[abc]

Data is represented as the mean ± SEM of eight animals. Statistical analysis was completed by one-way ANOVA proceeded by Tukey–Kramer test for multiple comparisons at $p \leq 0.05$.

[a]Significant difference from corresponding control.

[b]Significant difference from HE-induced control.

[c]Significant different from reference drug.

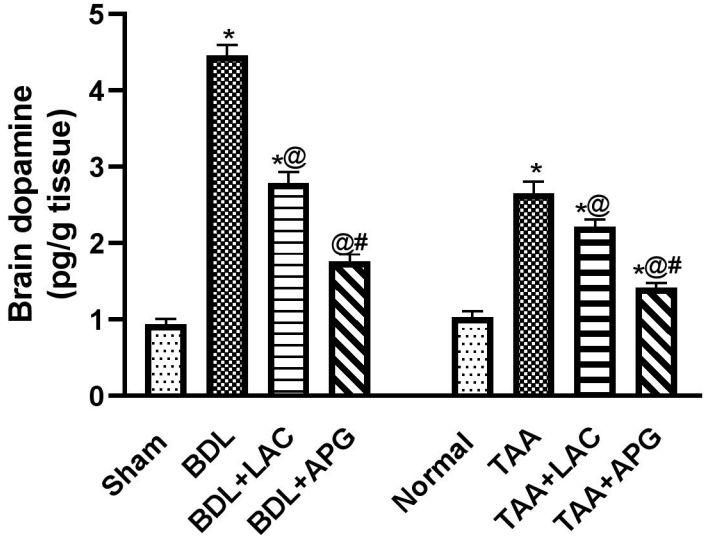

Figure 3. Effect of apigenin on brain dopamine levels in BDL versus TTA induced-HE in rats, respectively. Data is represented as the mean ± SEM of eight animals. Statistical analysis was completed by one-way ANOVA proceeded by Tukey–Kramer test for multiple comparisons at $p \leq 0.05$. *Significant difference from corresponding control. @Significant difference from HE-induced control. #Significant different from reference drug.

value specifically in the brain tissue for GSH and liver tissue for MDA than APG when compared to that of the TAA control group (Table 2).

Effect of APG on dopamine in brain tissue in BDL versus TTA-induced HE in rats

BDL in rats resulted in an increase in dopamine when compared to that of sham rats. Treatment with APG (20 ml/kg, p.o.) for three consecutive weeks significantly suppressed the elevated dopamine. However, treatment with standard LAC (8 ml/kg, p.o.) decreased the raised dopamine but at a lower value than APG compared to that of the BDL control group (Fig. 3).

TAA-induced HE (100 mg/kg, i.p.) thrice weekly for five consecutive weeks resulted in an increase in dopamine when compared to that of normal control. Treatment with APG (20 ml/kg, p.o.) for three consecutive weeks significantly suppressed the elevated dopamine. However, treatment with standard LAC (8 ml/kg; p.o.) decreased dopamine, but at a higher value than APG compared to that of the TAA control group (Fig. 3).

Histopathological examination

Effect of APG on liver histopathological alterations in BDL versus TTA-induced HE in rats

The photomicrographs of the liver of BDL control showed congestion in the central vein, edema, multiple newly formed bile ducts, and fibrosis in association with thickening and fibrosis in the hepatic capsule (Fig. 4IB). The TAA-treated rats showed centrilobular necrosis in the hepatocytes surrounding the central vein associated with fine fibrosis and

inflammatory cells infiltration with degenerated hepatocytes (Fig. 4IIB).

The liver isolated from BDL rats treated with LAC showed that focal hemorrhage was detected in the parenchyma associated with fine strands of collagen proliferation between the hepatocytes in the parenchyma (Fig. 4IC). On the other hand, liver isolated from TAA-injected rats treated with LAC showed multiple oval cells proliferation was detected surrounding the central vein with degeneration in the hepatocytes (Fig. 4IIC).

After APG treatment to BDL rats, histopathologic pictures showed collagen proliferation with few inflammatory cells infiltration in association with diffuse Kupffer cells proliferation. There was edema and thickening in the capsule but with no hemorrhage (Fig. 4ID). After administration of APG to TAA-injected rats, there were few centrilobular degenerations and sinusoidal dilatations as well as Kupffer cells proliferation and there were few dilatations in the central vein with degeneration in the surrounding adjacent hepatocytes (Fig. 4IID).

Effect of APG on brain histopathological alterations in BDL versus TTA-induced HE in rats

Mild congestion was observed in the blood capillaries in the brain tissue isolated from BDL rats; in subiculum, nuclear pyknosis and degeneration were observed. In the cerebellum, it was shown that mild congestion was detected in the meningeal blood vessels (Fig. 5IB). In the striatum, nuclear pyknosis and degeneration were detected in the neurons of the brain tissue isolated from TAA-injected rats associated with intracellular edema (Fig. 5IIB**)**. BDL rats treated with APG have shown no histopathological alteration, in the subiculum also mild congestion in the blood vessels of the meninges covering the cerebellum (Fig. 5ID). Mild intracellular edema was detected in the neurons of rats treated with APG in TAA-injected rats, which is lower than LAC (Fig. 5IID).

DISCUSSION

The broad range of neuropsychiatric manifestations in HE reflects complex pathophysiological mechanisms that interrelate to one another. The challenge remains to outline these mechanisms to improve treatment options. In the present study, special attention was paid to type A and type C HE. As both type A- and C HEs are associated with liver insufficiencies either acute or chronic, respectively. However, type B is associated with portal-systemic bypass and no intrinsic hepatocellular disease (Ferenci *et al*., 1998).

Induction of HE by BDL model in rats showed significant elevations in liver enzyme activities, hyperbilirubinemia, and hyperammonemia in 5 weeks, reflecting haptic injury along with impairment in hepatic excretory functions (Yang *et al*., 2015). BDL can be used to induce type C HE in animal models; BDL is a reproducible model in liver damage causing liver failure, jaundice, cirrhosis, portal hypertension, and hyperammonemia; the resultant hyperammonemia is the major etiologic factor in HE (Kwon *et al*., 2019). BDL rats showed significant decrease in both liver and brain reduced GSH with increased levels of lipid peroxidation biomarker MDA. Liver damage-induced by BDL is due to accumulation of toxic bile acids that trigger oxidative stress and hepatocellular damage, inflammation, and fibrosis (Padillo *et al*., 2004). The increased oxidative stress also affects the brain antioxidant homeostasis via systemic circulation in adult

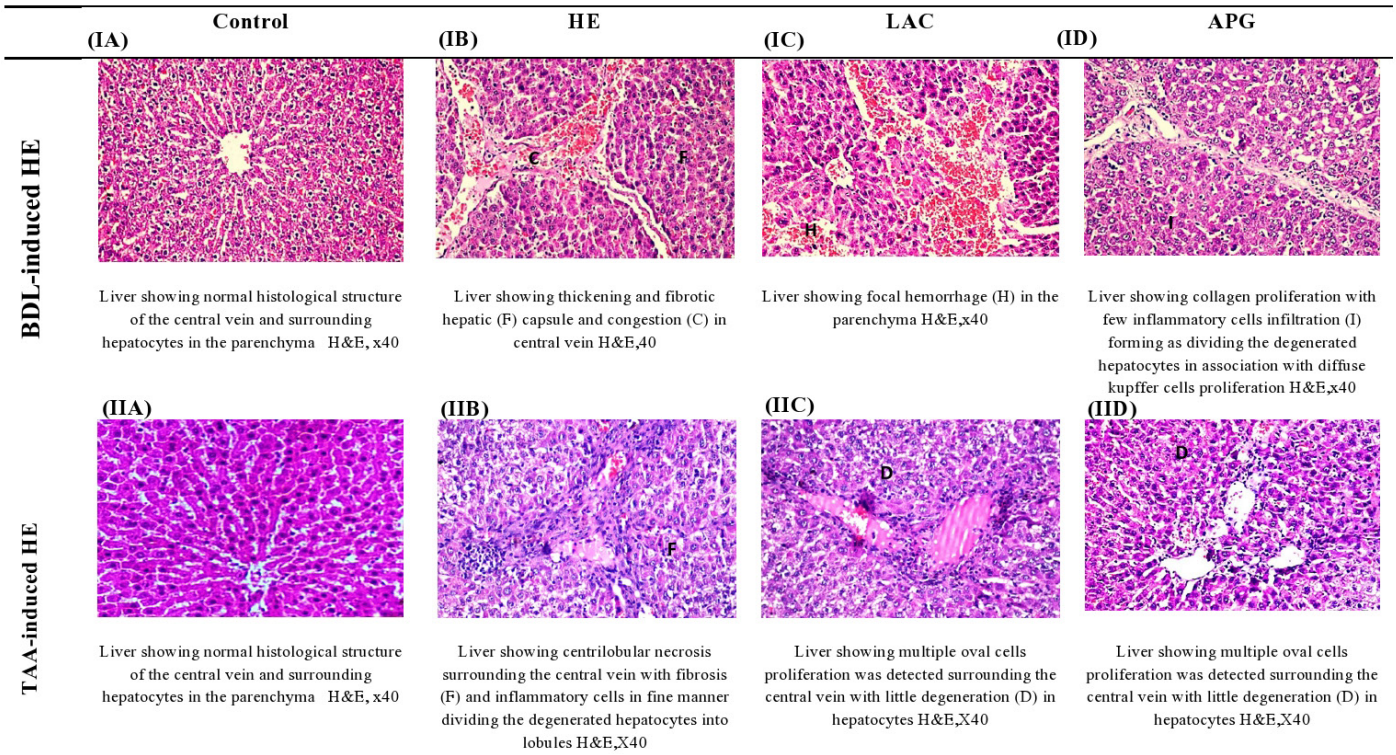

Figure 4. Effect of apigenin on liver histopathological changes in BDL versus TTA induced-HE in rats.

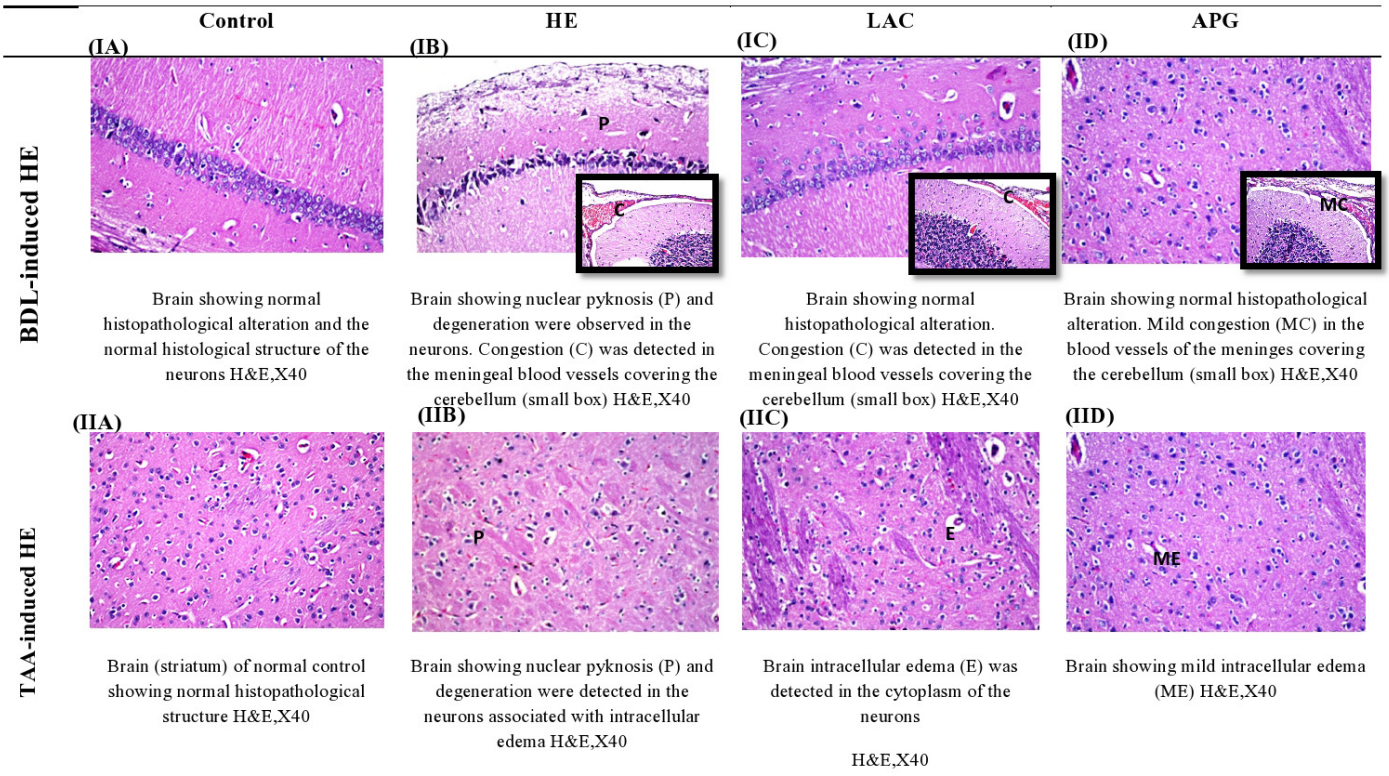

Figure 5. Effect of apigenin on brain histopathological changes in BDL versus TTA induced-HE in rats.

BDL-induced cholestatic rat (Huang *et al*., 2009). The altered equilibrium between antioxidant and pro-oxidant activities during cholestasis provoke an inflammatory response (Vazquez-Gil *et al*., 2004), significant elevations in hepatic NF-κB, and IL-6 in BDL animals was reported in the current study. It is well documented that cholestatic liver injury from biliary obstruction is accentuated by activation of TLR4/NF-κB signaling pathway and pro-inflammatory cytokines, such as TNF-α and IL-6, in both human and animal models (Fujiwara *et al*., 2001; Lechner *et al*., 1998).

Together, oxidative stress and inflammation during BDL impose significant injury to liver tissues evidenced by accumulation of ammonia and distorted hepatic architecture as supported by the histopathological examinations, such as cholestatic liver congestion of the central vein, multiple newly formed bile ductules, fibrosis, and thickening hepatic capsule. Obstruction of the biliary system causes retention of bile and it has shown that the acute infusion of toxic bile salts is responsible for cholestasis induces hepatocellular necrosis (Woolbright *et al*., 2015). Chronic bile retention leads to bile duct enlargement and proliferation, it activates hepatic stellate cells to produce collagen, and finally results in periportal and perineoductular fibrosis (Eshraghi *et al*., 2015).

Unlike cirrhotic patients who can develop slowly from minimal to persistent HE, patients suffering from acute liver insufficiency or ALF progress from altered mental status to coma within days, reporting a high rate of mortality (Misel *et al*., 2013). In the current study, induction of type A HE by TAA which mimics the ALF along with the neurological dysfunction (Weissenborn *et al*., 2001) is manifested by elevated levels of ammonia, neuro-inflammation, neurotransmitter system dysfunction, and oxidative stress which in turn leads to behavioral alterations due to HE confirmed by the histopathological evaluation. Unlike BDL model, TAA causes hepatocellular damage without any cholestasis. This model has been used to clarify changes in the functions of the CNS in HE (Butterworth, 2011). The current data display a significant elevation in liver enzymes, ALT, AST, ALP, bilirubin, and ammonia in TAA-treated rats as reported earlier (Afifi *et al*., 2020; Mostafa *et al*., 2017). Furthermore, TAA induced oxidative stress via depleting GSH and elevating MDA in liver and brain tissues of rats, as well as, provoking an inflammatory response evidenced by increased hepatic NF-κB and IL-6 contents (El-Marasy *et al*., 2019). Confirming liver injury, the histologic picture of TAA-treated rats showed centrilobular necrosis in the hepatocytes surrounding the central vein associated with fine fibrosis and inflammatory cells infiltration with degenerated hepatocytes. Several studies reported loss of hepatic normal histology with centrilobular necrosis, inflammation, edema, and collagen proliferation and hemorrhage (El-Marasy *et al*., 2019; Mansour *et al*., 2015; Mostafa *et al*., 2017).

The current study has outlined the relationship between hyperammonemia and neurological defects presented by dopamine level, beam walking test, and brain structure abnormalities in both BDL and TAA HE models. As accumulation of glutamine due to hyperammonemia leads to astrocyte swelling (Willard-Mack *et al*., 1996), the event is observed earlier in cirrhotic patients with minimal HE (Haussinger, 2006) and may contribute to elevated intracranial pressure in patients with fulminant hepatic failure (Blei and Larsen, 1999). Many factors interplay leading to

morphological abnormalities without diagnostic tool starting from oxidative stress due to hyperammonemia or glutamate leading to astrocyte swelling and excessive dopamine from damaged liver that passes brain blood barrier (BBB) aggravating oxidative stress and astrocyte swelling besides impairment in motor functions (Ding *et al*., 2014). Our results revealed a marked increase in brain dopamine levels in HE rats of BDL and TAA models, supporting previous data that elevated dopamine is a contributing factor in development of minimal HE in both chronic (Ding *et al*., 2013) and ALF. Moreover, elevated dopamine levels were accompanied by increase in time of the beam walking test reflecting motor defect in both BDL (Jover *et al*., 2006; Leke *et al*., 2012) and TAA-induced HE (Mendez *et al*., 2009). Morphological changes in brain tissues were confirmed by histopathological examinations revealing neuronal degeneration in cerebellum of BDL animals, while TAA induced evident neuronal degeneration with brain edema as reported earlier (Afifi *et al*., 2020; El-Marasy *et al*., 2019; Farjam *et al*., 2012).

LAC was used as standard treatment for being first-line therapy in management of HE (Pereira *et al*., 2016). APG is one of the most studied flavonoids, present primarily in significant amount as its glycosylated form in vegetables, fruits, herbs, and plant-based beverages (Hostetler *et al*., 2017). Recently, various animal models and human clinical trials evidenced the potential neuroprotective effect of APG in depression, Alzheimer's disease, and Parkinson's disease through its antioxidant activity and modulatory effect on β-amyloid peptide-induced amnesia (Liu *et al*., 2011; Nabavi *et al*., 2018). The antioxidant activity of APG was further reported in spinal cord injury (Zhang *et al*., 2014) along with an anti-inflammatory effect in human AD upon long-term administration of a formulation containing APG (de Font-Reaulx Rojas and Dorazco-Barragan, 2010). APG exerted a hepatoprotective activity in the setting of liver disease via anti-inflammatory and antioxidant effects (Saha *et al*., 2019; Sen and Chakraborty, 2017; Wan and Jiang, 2018). The hepatoprotective and neuroprotective effects of APG in BDL and TAA-induced HE were investigated against LAC in the current study. Data disclosed that administration of APG, at a dose of 20 mg/kg/day for 3 weeks, exerted significant hepatoprotective and neuroprotective activities compared to LAC at a dose of 8ml/kg/day for 3 weeks in BDL and TAA models of HE in rats. APG significantly ameliorated liver enzymes, bilirubin, and ammonia levels when compared to LAC and corresponding HE control groups.

Nevertheless, APG exerted powerful antioxidant and anti-inflammatory activity when compared to LAC in both liver and brain of HE rats via replenishing GSH, modulating MDA levels, and regulating NF-κB and IL-6, respectively, while improving the histologic picture of both liver and brain in both models of HE, thus confirming its hepatoprotective (Yue *et al*., 2020) and neuroprotective (Dourado *et al*., 2020) activities. The potent hepatoprotective activity of APG abrogated hyperammonemia and its neurological consequences in both chronic and ALF as evident by decreased brain oxidative stress and brain dopamine content, while improving motor activity in HE animals during the beam walking test.

CONCLUSION

The study concluded that the progression of HE in both chronic and ALF compromise several interrelated factors that contribute to irreversible neurological insults, although diagnosed early, and proper treatments can offend fatal consequences. Hyperammonemia due to liver insufficiency in both BDL and TAA-induced HE could trigger oxidative stress, inflammation, dopamine brain overload, motor impairment, and morphological abnormalities in both models. APG, proven antioxidant, and anti-inflammatory activities exerted hepatoprotective and neuroprotective potentials in type C and type A HE compared to the standard, LAC, in BDL and TAA-induced HE, respectively, in rats. With long-term and cost-effective clinical trials, APG may imply a possible treatment option to HE in patients with chronic and acute liver disease.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FUNDING

There is no funding to report.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Fayez AM, Mansour DF, Saleh DO. Progression of hepatic encephalopathy induced by bile duct ligation versus thioacetamide in rats: Regulatory role of apigenin. J Appl Pharm Sci, 2021; 11(12):158–168.