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BIOCHEMICAL AND HISTOLOGICAL STUDY OF RAT LIVER TOXICITY INDUCED BY GENTAMICIN AND PROTECTIVE ACTION OF BERBERINE

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Abstract

This study was conducted to find out hepatoprotective activity of berberine (BRB) 50, 100mg/kg body weight (b.w.) against gentamicin (GNT) 80 mg/kg induced hepatic toxicity in rats. Twenty eighty male rats were divided into four groups, group1: control, (1 ml/kg Saline orally) group 2: GNT (80 mg/kg), intraperitoneally (i.p.) for (7) consecutive days , group 3: GNT (80 mg/kg), i.p. plus BRB (50 mg//kg) orally for (7) consecutive days , group 4: GNT (80 mg/kg), i.p. plus BRB (100 mg//kg) orally for (7) consecutive days. All the rats were killed on the (8) day of the experiment, and then the blood, and livers samples were taken. GNT induced hepatic damage was proved by a significant ($p \leq 0.01$) reduction in the body weight ,and a significant ($p \leq 0.01$) increased serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), Malonaldehyde enzyme (MAD) and histopathological , changes. Protective hepatic toxicity effect and oxidative damage caused by GNT significantly ($p \leq 0.01$) increasing in body weight and significantly ($p \leq 0.01$) decreasing AST, ALT, ALP, MAD and improving tissue morphology in BRB (50 mg//kg) while, BRB (100 mg//kg) has more effects. These results assure that BRB (100 mg//kg) antioxidant effects can protect GNT -induced hepatotoxicity in rats.

Key words : Berberine, Gentamicin, Anti-oxidants, Hepatic toxicity

Introduction

One of the most widely used class of drugs are antibiotics. These drugs prevent many problems caused by infections. However, antibiotics have side effects and can damage various body organs including liver, kidney, brain, blood, skin, eyes, mouth, etc**.** (Ayatollahi, 2006). Aminoglycoside, a class of antibiotics, has been used as antibacterial therapy for a long time. It produces toxicity at slightly high doses. Following aminoglycosides treatment approximately 5-10% patients have to face adverse effects like hepatotoxicity, nephrotoxicity and ototoxicity . Gentamicin produces free oxygen radicals by acting on mitochondria of hepatocytes and accelerates the lipid peroxidation process (Alarifi *et al.*, 2012). Clinical use of gentamicin despite clinical benefits has been limited due to its side effects. The main side effects include liver damage that is one of the major factors of liver inefficiency in a significant number of people taking this medication. Therefore taking these medications face limitations due to the fact that one of the major side effects of Gentamicin is creating hepaotoxicity. (Masakazu *et al*., 2014; S t o j I *et al*., 2006). Berberine, an isoquinoline alkaloid, is a member of naturally occurring protoberberines class. This alkaloid is present in plants of Berberidaceae, Papaveraceae and Ranunculaceae families. Berberine widely consumed in Ayurvedic and Chinese medicine. (Imanshahidi *et al*., 2008). Berberine has been isolated from various parts of these species such as root, stem, bark, fruit and rhizome (Singh - Mahajan *et al*., 2013). Traditional use of root, bark, leaf and fruits of barberry as an immunemodulator and anti-microbial agent as well as a treatment for central nervous system, cardiovascular, gastrointestinal, endocrine and renal problems have been proved with so many pharmacological studies (Imenshahidi - Hosseinzadeh *et al*., 2017). Recently published articles demonstrated that barberry and berberine (its main constituents) have anti-oxidant (Abd El-Wahab *et al*., 2017), antiinflammatory (Lin *et al.*, 2013), anti-tumor (Yu *et al.*, 2007), anti-mutagenic (Cernakova

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et al., 2002) and anti-diabetic effects. Their hypoglycemic and cholesterol lowering properties (Jiang *et al.*, 20012), neuroprotective (Kulkarni *et al.*, 2010), and hepatoprotective (Feng *et al.*, 2010; Hermenean *et al.*, 2012).

Materials and Methods

This study was performed at the period November 2019 in physiology department of veterinary medicine of AL-Qassim green university.

Drugs

Berberine HCL (BRB) 100% Natural was purchased from BULK SUPPLEMENT

.com USA. GNT, Gentaject 10% was purchased from KEPRO- HOLLAND. Ketamine 10% inj. from KEPRO- HOLLAND. Xylazine, XYL-M2, VMD-Belgium.

Experimental rats

The number of laboratory animals used in the experiment are twenty eight healthy male rats at aged (190) days and weighted (190-200) grams, obtained from the animal house of the College of Veterinary Medicine, AL-Qadisiya University, were kept for (10) days as acclimatization period before the starting of the experiment, all rats were feed on concentrated food (pellets) and were given plain water, the animals room temperature was (19-23)°C and the humidity was (45-55), that room was washing and sterilization once a week.

Experimental design

After a quarantine period of (10) days, twenty eight rats were randomly divided into four equal groups, each group consist of (7) rats and they received the treatment as

follows :

Group I: Control (1 ml/kg Saline orally) for (7) days.

Group II: GNT (80 mg/kg, i/p) for (7) days (Ali noorani *et al*., 2011; Mohamed *et al*., 2011).

Group III: GNT (80 mg/kg, i/p) + BRB (50 mg/kg orally by stomach tube) for (7) days. (Lotfi *et al.*, 2018).

Group IV: GNT (80 mg/kg i/p) + BRB (100 mg/kg orally by stomach tube) for (7) days. (Saeed *et al.*, 2018).

Body weight

All animals were weighed before and after treatment with using digital electronic balance.

Serum Preparation

At the end of experimental period, rats were fasted for (10) hrs, anaesthetized with ketamine (75 mg/kg) combined with xylazine (2.5 mg/kg). (Molina *et al.*, 2015). Blood samples were collected by heart puncture in non-heparinized tubes, centrifuged at (4000) rpm for (10) minutes (Laessig *et al.*, 1976). After separation the serum from the clot, using a sampler, the samples were used to measurement of AST, ALT, ALP and MDA level concentration. The rats were sacrificed by cervical dislocation and the abdominal cavity was immediately opened, livers were removed and processed for histopathological studies.

Histopathological techniques

Sections were taken from livers tissues from different animals in each group immediately after sacrificed. These tissues were washed with the normal saline solution to remove blood, then fixed in 10% neutral formalin for (24) hrs, dehydrated in different concentration of alcohol, and processed for paraffin embedding. Sections of (5) µm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series stained with Haematoxylin and Eosin, cleared in xylene and examined microscopically according to (Bancroft *et al.*, 1996).

Table 1: Effect of BRB on GNT-induced change of the body weight /gram of rats.

GNT+BRB 100Mean ± SE	GNT+BRB 50Mean ±SE	GNTMean ± SE	ControlMean ± SE	Traits
7	7	7	7	No. of rats
195.380± 0.92 A	195.042 ± 1.28 A	195.158± 0.96 A	195.194 ± 0.87 A	Weight at 1 day (g/animal) NS
183.848 ± 1.48 B	176.584 ± 1.65 C	162.450± 1.21 D	205.144. ± 1.20 A	Weight at 8 day (g/animal) **

NS: Non significant. *significant difference at 0.05. ** high significant difference at 0.01.

Table 2: Effect of BRB on GNT-induced change in liver function of rats.

GNT+BRB 100Mean ± SE	GNT+BRB 50Mean ±SE	GNTMean ± SE	ControlMean ± SE	Traits
44.25 ± 1.27 C	57.88 ± 1.62 B	93.71 ± 1.03 A	36.49± 0.71 D	ALT(U/L) **
76.26 ± 0.63 C	90.17 ± 1.01 B	131.03 ± 1.10 A	65.63 ± 0.59 D	AST(U/L) **
20.23 ± 0.82 C	26.43 ± 0.72 B	37.67 ± 0.53 A	16.66 ± 0.54 D	ALP(U/L) **
5.67 ± 0.25 C	7.56 ± 0.40 B	10.46 ± 0.72 A	3.31 ± 0.22 D	Malonaldehyde (nm / ml) **

NS: Non significant. * significant difference at 0.05. ** high significant difference at 0.01.

Statistical analysis

The statistical results of the data were analyzed according to Complete Randomized Design (C.R.D.) (AL- Rawi *et al.*, 2000). The mean differences between the averages of the studied traits were determined at the probability level of (0.01) using the Duncan test (Duncan, 1995). Statistical data were analyzed using the (SAS, 2010).

Results

Body weight

No fatalities were observed in the groups of rats that were given GNT either alone or in summation with BRB, with reduced appetite, decreased activity and progressive physical fatigue were observed in the rats from the GNT group. I/P injection of GNT produced significant ($P \leq 0.01$) decrease in the body weight compared to control. The animals were weighed before and after the experiments, whereas, treatment with BRB (50 mg/kg) produced significant ($P \leq 0.01$) improved on the body weight compared to GNT control rats, while BRB (100 mg/kg) produced more effects Table 1.

Effect of BRB on GNT induced alterations in hepatic function parameters

Effect of GNT induced alterations in liver function in rats. A significant ($p \leq 0.01$) increased in serum AST, ALT, ALP levels compared to the control group and significant ($p \leq 0.01$) increase in MDA levels compared to control was observed after (7) days of treatment with GNT Whereas, treatment with BRB (50 mg/kg) prevented GNT induced significant ($p \leq 0.01$) increased in serum AST, ALT, ALP levels and produced significant ($p \leq 0.01$) reduction on the MDA compared to GNT control rats. However, BRB (100 mg/kg) it has more effect than, BRB (50 mg/kg) in reduction serum AST, ALT, ALP and MDA level compared to GNT control rats Table 2.

Effect of BRB on GNT induced histopathological alteration in hepatic tissue

Light microscopic of liver examination using H&E (400X) stain in control rats shown the normal structure in (Fig. 1:a). Histopathological effects of GNT on liver of treated rats are presented in rats treated with GNT for (7) days shown extensive vaculation like the fatty changes of the hepatocytes in (Fig. 1:b). Liver rats treatment with GNT and BRB (50 mg/kg) shown congestion of the central vein and the sinusoids and presence of inflammatory cells in the liver parenchyma in (Fig. 1:c). Liver rats treatment with GNT and BRB (100 mg/kg) show dilation of sinusoids and filled with the RBCs with increase in the numbers of Kupffer cells in (Fig. 1:d).

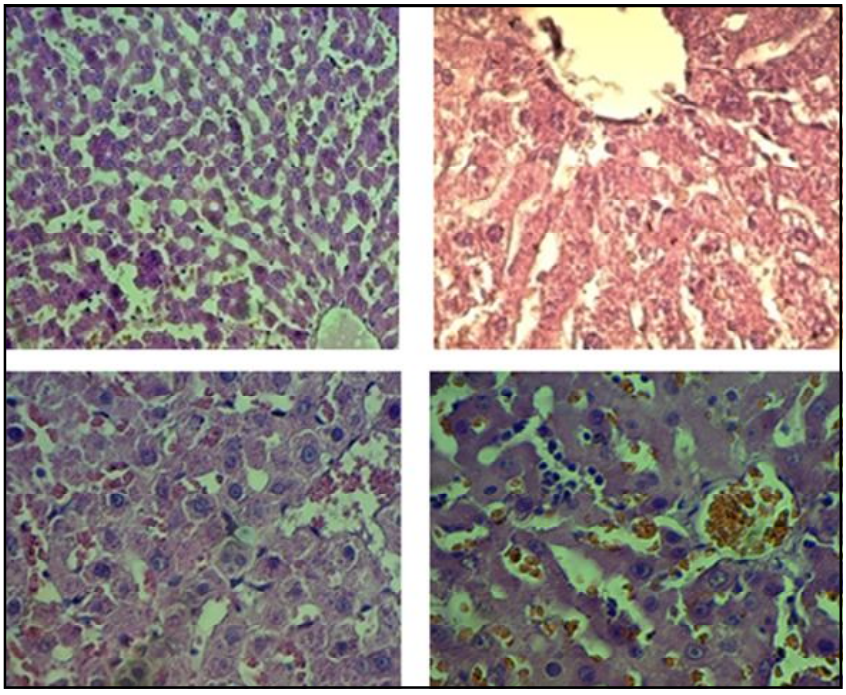

Fig. 1: Histopathological alteration in hepatic tissue.

Discussion

This study is the first to show that BRB could mitigate GNT induced liver toxicity. In this study, BRB has an ameliorative effect against hepatotoxicity induced by GNT as explained by reduction in the body weight , when compared to control . Our results are in acceptance with previous findings of (Samah *et al.*, 2018) . According to (Erdem *et al.*, 2000), increased catabolism and anorexia are responsible for decreased food intake and causes body weight loss Further, following loss of the tubular cells, involved in renal water reabsorption leads to dehydration and decreases body weight (El-Zawahry - Abu El Kheir *et al.*, 2007). In the present study, the administration of GNT for (7) days, produced a significant elevation of serum AST, ALT, ALP and MAD levels. These results are in acceptance with those obtained by other investigators (Rashid-Khan *et al.*, 2017; Al-Elewi *et al.*, 2014; Ahsan *et al.*, 2017). Elevated levels of these enzymes in the serum are presumptive markers of drug-induced alternations in the hepatocytes (Shabana *et al.*, 2012). Estimation of the activity of ALT, AST and ALP are good marker of assessment liver function. These enzymes

are normally located in the cytosol of hepatocytes, when liver cells are damaged, these enzymes are released in the plasma and increased their activity in plasma is a useful marker of the extent and type hepatocellular damage (Atta *et al.,* 2010). We found that GNT treatment caused increased MDA levels, these results are in acceptance with those obtained by other investigators (Zakiah, 2017; Ahsan *et al.,* 2017). This increase of MAD due to increased accumulation of superoxide free radicals inducing more lipid peroxidation (Al-Hashem *et al.,* 2009; Ademiluyi *et al.,* 2013). These alterations might differ depending on exposure time and the dose given. Histopathological changes , extensive vaculation like the fatty changes of the hepatocytes induced by GNT which add with clearly elevated levels of liver biochemical markers AST, ALT, ALP and MDA activities. Similar findings were noticed by (Ahsan *et al.,* 2017; *et al.,* Muhammad *et al.,* 2011). Rats treated with GNT mediates the generation of Reactive Oxygen Species (ROS) that play an important role in progression of hepatic and renal injuries, as well as an array of biomolecules such as membrane lipids, protein and nucleic acids, especially in mitochondria and lysosomes of renal tissues. Peroxidation of polyunsaturated fatty acids on bio membranes is due to an increase in ROS propagation. The GNT caused lipid peroxidation, cellular function impairment and led to necrosis (Reiter *et al.,* 2003). The index of endogenous lipid peroxidation are TBARS, an indirect evidence of increased free radical production (Reiter *et al.,* 1995). The results of the present study suggest that, the accumulation of free radicals, and that increased oxidative stress is a basis for cellular damage. Through the aforementioned findings, we conclude that there is hepatic damage due to the use of GNT. Treatment with BRB for (7) days reduced the GNT injured liver produces a significant increased in body weight and significant decrease in serum ALT, AST and ALP, levels and significant decreased in MDA levels. Histopathological changes induced by GNT were modulation the liver extensive vaculation like the fatty changes of the hepatocytes through the formation perivascular cuffing with dilation of sinusoids and filled with the RBCs. Our results demonstrate the ameliorative effect BRB (100 mg/kg) on GNT for (7) days induced liver toxicity in the rats. This can be explained on the anti-inflammatory activity (Zhou - Mineshita *et al.,* 2000). BRB may inhibit the release of arachidonic acid from cell membrane phospholipids and exerts an effect on arachidonic acid metabolites (Huang *et al.,* 2000) . BRB could protect experimental liver fibrosis through enhancing anti-oxidant system, inhibiting lipid peroxidation and hepatic stellate cell proliferation (Sun *et al.,* 2009; Zhang *et al.,* 2008) and also the preventive effect against paracetamol and carbon tetrachloride (ccl4), which causes hepatotoxicity in mice (Gilani- Janbaz *et al.,* 1995) Analgesic, antinociceptive and antipyretic activity (Esra *et al.,* 2002). Anti microbial (Iwasa *et al.,* 1998). Also effective antioxidant and free radical scavenger that prevents ROS formation and exerts protective effects on cardiac, hepatic and renal functions (Lee *et al.,* 2010). As for BRB (50 mg/kg) , it had less effect than(100 mg/kg). Based on the advance findings, it can be conclude that, GNT had adverse effects on the liver. BRB (100 mg/kg) administration showed a marked hepatoprotective activity. The protective effects of BRB (100 mg/kg) may be due to the its anti-inflammatory effects or antioxidant effects or analgesic effects or antimicrobial effects, individually or synergistically.

Conclusion

This study explained that BRB declines GNT induced hepatotoxicity. The effect of BRB against GNT induced hepatotoxicity could be mediated through its anti-inflammatory, antimicrobial, antioxidant and anti-apoptosis action. However, BRB treatment was able to alleviate liver damage associated with GNT treatment and this is assign to its antioxidant activity and its ability to prevent inflammation.

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