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Diclofenac induced gastrointestinal and renal toxicity is alleviated by thymoquinone treatment

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ABSTRACT

The aim of this study was to investigate whether thymoquinone (TQ) could alleviate diclofenac (DCLF)-induced gastrointestinal and renal toxicity in rats. Diclofenac was administered via intramuscular injection twice daily for 5 days and TQ was given by gavage for the same period. Hematological and biochemical profiles were measured with autoanalyzers while reactive oxygen/nitrogen species (ROS/RNS) generation and total antioxidant capacity (TAC) were assayed by standard kits. Tissue injuries were evaluated by microscopy and histopathological scoring. Diclofenac treatment caused kidney and liver function test abnormalities, reduced hematocrit and hemoglobin levels but increased WBC and platelet counts. Histopathological findings showed renal tubular damage, gastrointestinal lesions and increased fibrosis in DCLF treated rats. Thymoquinone administration, along with DCLF treatment, attenuated hematological test abnormalities and DCLF induced renal functional impairment as evident by significantly restored serum creatinine and blood urea nitrogen levels. Similarly, TQ treatment significantly alleviated liver function test abnormalities and decreased tissue injury in the stomach and duodenum. Diclofenac treatment caused increased ROS/RNS formation and decreased TAC in the kidney, stomach and duodenal tissue. Thymoquinone administration increased gastrointestinal and renal TAC in DCLF treated rats. These results indicate that TQ could ameliorate gastrointestinal and renal toxicity induced by high dose DCLF treatment.

1. Introduction

Diclofenac is a lipophilic, nonsteroidal anti-inflammatory drug (NSAID) frequently used to treat pain in musculoskeletal injuries, rheumatoid and osteoarthritis (McCarberg and Argoff, 2010; van Walsem et al., 2015; Tieppo Francio et al., 2017). Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase (COX) pathway and lead to suppression of prostaglandin synthesis from arachidonic acid (Shin et al., 2017). Normal therapeutic doses of DCLF is safe, effective, and widely used (Franceschi et al., 2016) however, higher doses for longer interval leads to nephrotoxicity (Ungprasert et al., 2015), drug-induced enteropathy (Shin et al., 2017), liver and bone marrow toxicity (Bessone, 2010; Ibáñez et al., 2005) which results in acute kidney injury, gastrointestinal bleeding, ulceration, fulminant hepatic failure, hepatitis or aplastic anemia.

The mechanism of DCLF induced toxicity is reported to involve mitochondrial dysfunction and increased oxidative stress (Galati et al., 2002). Diclofenac is reported to covalently bind to macromolecules in situations where intracellular levels of NADH, NADPH, GSH, and other reducing agents are low (Shen et al., 1999). Covalently bound protein adducts of DCLF have also been detected in the small intestine of rats (Ware et al., 1998). Diclofenac is eliminated following conjugation by sulfate and glucuronic acid (Sarda et al., 2012). Excretion and accumulation of DCLF conjugates have been correlated to renal function and end-stage renal disease (Davies and Anderson, 1997).

Previous studies have demonstrated that DCLF is associated with progressive increase in epithelial permeability, marked increase in enteric gram-negative bacteria numbers and intestinal ulceration (Reuter et al., 1997). High incidence of acute erosions has been reported to occur early in DCLF treatment and administration of DCLF has been reported to cause immediate ultrastructural gastric surface epithelial damage, obvious endoscopic gastroduodenal subepithelial hemorrhages and erosions within several hours of ingestion (Hawkins and Hanks, 2000). To our knowledge there are no literature about the protective

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Abbreviations

ALB	albumin
ALT	alanine aminotransferase
AST	aspartate aminotransferase, serum urea nitrogen, blood urea nitrogen
COX	cyclooxygenase
CRE	creatinine
DCFH	dichlorodihydrofluorescin
DCF, 2′, 7′	dichlorodihydrofluorescein
DCLF	diclofenac
DCLF-AG	diclofenac acyl glucuronide
GI	gastrointestinal
HCT	hematocrit
HGB	hemoglobin
H_2O_2	hydrogen peroxide
NO	nitric oxide
NSAID	nonsteroidal anti-inflammatory drug
ONOO	peroxynitrite anion
RBC	red blood cell
RNS	reactive nitrogen species
ROO	peroxyl radical
ROS	reactive oxygen species
TAC	total antioxidant capacity
TBIL	total bilirubin
TQ	thymoquinone
UAE	uric acid equivalents
WBC	white blood cell

role of TQ on gastroduodenal toxicity induced by DCLF, but TQ has been reported to counteract gastrointestinal toxicity accompanying cisplatin (Shahid et al., 2017) and acetylsalicylic acid (Zeren et al., 2016) chemotherapy in rats. Thus, TQ may have a potential for clinical application to counteract the accompanying gastrointestinal toxicity in DCLF chemotherapy.

The frequency and severity of DCLF toxicity can be underestimated (Zeino et al., 2010). The most common approach used clinically to minimize NSAID induced gastropathic injury has been the co-administration of a proton pump inhibitor (Wallace, 2012). However recent animal studies suggest that these gastroprotective drugs synergistically exacerbate NSAID-induced small intestinal injury and bleeding (Satoh et al., 2012). Thus, it is necessary to find novel therapeutic agents to prevent the NSAID-induced gastroenteropathy as well as the proton pump inhibitor induced exacerbation of NSAID enteropathy. Glucocorticoids are one of the choices for the management NSAID-induced acute renal failure (González et al., 2008). However, considering that glucocorticoid treatment induces severe side effects, it is also imperative to find novel therapeutic agents to alleviate NSAID-induced acute kidney injury.

Thymoquinone, a bioactive compound derived from black seed (Nigella sativa) oil, has been reported to have anti-oxidant and anti-inflammatory effects against renal injury (Ragheb et al., 2009). Immunomodulatory effects of TQ have also been demonstrated (Salem, 2005). Previous studies have shown that TQ suppresses inflammatory reactions and oxidative stress and is protective on streptozotocin-induced diabetic nephropathy (Kanter, 2009). Likewise, TQ has novel gastroprotective mechanisms via inhibiting proton pump, acid secretion and neutrophil infiltration, while enhancing mucin secretion, and nitric oxide production (Magdy et al., 2012). Oral administration of TQ has been reported to mitigate gastrointestinal toxicity of cisplatin on brush border membrane enzymes, energy metabolism and antioxidant system in rat intestine (Shahid et al., 2017).

Given that the mechanism of DCLF induced toxicity is reported to involve increased oxidative stress (Galati et al., 2002) and inflammation (Morise and Grisham, 1998), and that TQ has been proven previously to have a considerable antioxidant and antiinflammatory effects (Ragheb et al., 2009), the present study was designed to evaluate the possible protective role of TQ in DCLF toxicity. The therapeutic effects of TQ on DCLF -induced gastrointestinal and renal toxicity was investigated in rats. We determined hematological and biochemical profiles, reactive oxygen/nitrogen species generation and total antioxidant capacity in DCLF treated rats and examined the possible protective effects of TQ administration. Gastrointestinal and renal injury were also evaluated by light microscopy and histopathological scoring in order to observe the therapeutic potential of TQ in alleviating gastrointestinal and renal toxicity induced by DCLF treatment.

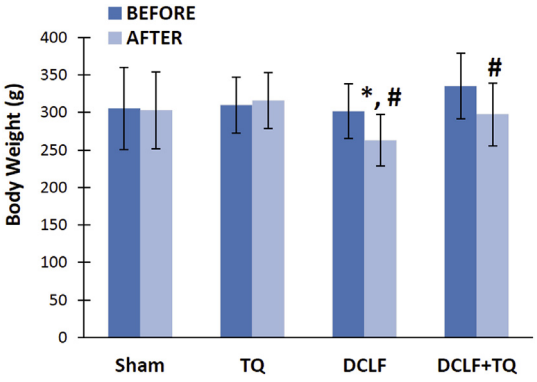

Fig. 1. Changes in body weight during the treatment period. Data are mean ± SD (n = 10). TQ, thymoquinone treated; DCLF, diclofenac treated. Body weights were measured before (day 0) and after (day 5) treatment. *, $p < 0,01$, vs. sham and TQ groups. Statistical analysis was done by One Way Analysis of Variance with Multiple Comparisons by Dunnett's Method. #, $p < 0,001$ vs. before treatment. Statistical analysis was done by Paired *t*-test.

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Akdeniz University Medical School. Male Wistar rats weighing 350–450 g were housed in stainless steel cages and given food and water ad libitum. Animals were maintained at 12 h light-dark cycles and a constant temperature of 23 ± 1 °C at all times. Rat were randomly divided into sham (n = 10), DCLF treated (n = 10), TQ treated (n = 10) and DCLF + TQ treated (n = 10). Diclofenac sodium (RODINAC, VEM Pharmaceuticals, İstanbul, Turkey) was administered (9 mg/kg) via intramuscular injection twice daily for 5 days as previously described (Singh et al., 2016). This dose and duration of DCLF administration has been shown to induce gastroenteropathy in rats (Singh et al., 2016). Thymoquinone (Sigma-Aldrich, St. Louis, MO, USA) was given by gavage (15 mg/kg per day) for the same period as previously described (Aycan et al., 2014). Water solubility of TQ is reported to be > 0,5 mg/mL which is enough to exert pharmacologic effects (Salmani et al., 2014). Sham group animals received an equal volume of i. p. saline and distilled water via gavage. Body weight was determined before and after the treatment period of 5 days. Rats were anesthetized intraperitoneally with a mixture of ketamine (100 mg/kg, Richter Pharma AG, Wels, Austria) and xylazine hydrochloride (10 mg/kg, Bioveta, Czech Republic). An incision on the abdominal skin was performed to expose the abdominal cavity. Blood was collected from the abdominal aorta and animals were sacrificed. Dissected stomach,