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Original article

Acetaminophen-induced hepatotoxicity: Preventive effect of *trans* anethole

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ABSTRACT

The hepatotoxicity induced by APAP is caused by the excessive production of *N*-acetyl-para-benzoquinone imine (NAPQI), which, when reacting with hepatic proteins proved to cause irreversible lesions. Associated with this process, an intense inflammatory process is also evidenced, characterized by the increased cell influx and production/release of inflammatory mediators. *Trans* anethole, an aromatic compounds has been showed anti-inflammatory efficacy by inhibit the cellular recruitment and synthesis/releases of many proinflammatory mediators such as prostaglandin (PGE_2), cytokines (TNF, IL-1) and nitrico oxide (NO). The aim of this study is to investigate the effect of *trans* anethole on some inflammatory parameters that are involved in hepatotoxicity induced by high doses of acetaminophen. Our results demonstrate that treatment with AN at doses 125 and 250 mg/kg once a day for seven days prevented the changes caused by the APAP overdose, showing less intensity in the histological changes (necrosis, size of hepatocyte area and inflammatory infiltration), and corroborating the findings of serum activities of transaminases and phosphatases and the activity of the enzyme myeloperoxidase. In addition, the treatment prevented the up-regulation of proinflammatory mediators such as NO, TNF, IL-1α, MIP-1α and MCP-1 and induced the up-regulation of anti-inflammatory cytokines (IL-4 and IL-10). Thus, our results demonstrate a possible protective effect of *trans* anethole on the hepatotoxicity induced by APAP.

1. Introduction

Among the drugs with potentially hepatotoxic effects, acetaminophen (APAP) stands out. APAP is an analgesic and antipyretic drug commonly prescribed in clinical practice, and can also be found in numerous prescription-free preparations [1]. APAP, at therapeutic doses, is metabolized in the liver by glucuronidation and sulfonation reactions and oxidation by cytochrome. However, in supra-therapeutic doses the oxidation pathway produces a highly reactive and toxic metabolite, *N*-acetyl-para-benzoquinone imine (NAPQI) can react directly with hepatic proteins, causing damage to the hepatocytes [2,3]. Associated with this process of liver injury, there is an severe inflammatory process characterized by intense cell influx, increased production and release of inflammatory mediators (cytokines, reactive oxygen and nitrogen species), which, in turn, increase liver damage [4–6]. This process is

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Table 1
Serum parameters: AST, ALT, ALP and γGT.

Activity Of Serum Transaminases And Phosphatases				
Treatment groups	AST (U/L)	ALT (U/L)	ALP (U/L)	γGT (u/l)
N	75.69 ± 4.6	56.87 ± 3.7	290.9 ± 6.8	22.05 ± 0.9
$AN_{62.5}$	57.17 ± 4.3	46.11 ± 3.5	309.5 ± 19.6	24.90 ± 1.5
AN_{125}	60.12 ± 2.5	40.95 ± 1.6	268.0 ± 11.2	22.29 ± 1.4
AN_{250}	55.30 ± 2.1	34.76 ± 1.8	250.2 ± 21.2	19.92 ± 2.3
CMC + APAP	4974 ± 1174*	7211 ± 1055*	473 ± 50.2*	34.72 ± 2.5*
$AN_{62.5}$ + APAP	1581 ± 440.3**	2728 ± 794.3**	315.5 ± 18.4**	23.35 ± 2.2**
AN_{125} + APAP	845.8 ± 249.4**	1070 ± 326.3**	320.1 ± 25.9**	20.86 ± 2.4**
AN_{250} + APAP	861.9 ± 229.4**	1209 ± 335.2**	337.1 ± 19.4**	21.63 ± 1.5**

Serum parameters of normal (N), pretreated with *trans* anethole at doses 62.5; 125 or 250 mg/kg without received APAP ($AN_{62.5}$, AN_{125} and AN_{250}), pretreated with CMC vehicle or pretreated with AN at doses 62.5; 125 or 250 mg/kg that received APAP (CMC + APAP, $AN_{62.5}$ + APAP, AN_{125} + APAP and AN_{250} + APAP, respectively). The serum parameters were determined after day seven of treatment. Results represent mean ± SEM. (ANOVA followed by Tukey's test).

* $P < 0.05$ compared to N group.

** $P < 0.05$ compared to CMC + APAP group.

closely related to increased activity of transaminases and phosphatases hepatic, being indicative of hepatotoxicity [7–9].

The sequence of events which leads to APAP-induced hepatotoxicity is well known [2–6] and for this reason APAP has been used in experimental approaches aiming the discovery of new hepatoprotective agents [10,11]. Within this context, we chose to study the *trans* anethole is a substance that has numerous biological activities.

Trans anethole is a phenylpropanoid, the major constituent of essential oils of plants such anise and star-anise. Many works showed anti-metastatic, anti-oxidative and anti-microbial activities [12–14]. In researches of our group showed anti-inflammatory, antinociceptive and immunomodulatory efficacy of *trans* anethole [15–19]. The activity anti-inflamatory of *trans* anethole is due it's ability in inhibit the prostaglandin synthesis and the levels of pro-inflammatory cytokines as tumor necrosis factor (TNF-α) [17,19]. Additionally, the *trans* anethole inhibits the rolling and adhesion leukocyte in postcapillar venules reducing the cellular recruitment [18]. In a recent study, Cho et al. showed that anethole exert a protective effect on hepatic ischemia/reperfusion injury by suppressed kinases protein and nuclear translocation factor [20]. Thus, this research aimed to investigate the effect of *trans* anethole on some inflammatory parameters that are involved in hepatotoxicity induced by high doses of acetaminophen.

2. Materials and methods

2.1. Animals

Male Swiss mice, weighing 25 ± 2 g. The animals were housed at 22 ± 2° C under a 12/12 h light/dark cycle. Prior to the experiments, the animals fasted overnight, with water provided *ad libitum*. The experimental protocols were approved by the Ethical Committee on Animal Use (CEUA 8912160715).

2.2. Animals treatment and APAP-induced hepatotoxicity

Animals were divided into eight groups (n = 5–7): (I) normal untreated animals (N); (II–VII) animals pretreated with *trans* anethole dissolved in vehicle carboxymetilcellulose (CMC) at doses 62.5, 125 or 250 mg/kg (II – $AN_{62.5}$, III – AN_{125}, IV – AN_{250}, V – $AN_{62.5}$ + APAP, VI – AN_{125} + APAP and VII – AN_{250} + APAP); (VIII) animals pretreated with CMC (CMC + APAP). The treatments *per os* with CMC or AN was carried once daily for seven days. At day 7, groups V–VIII received 250 mg/kg of p.o. APAP, after fasting for 8 h. After 12 h of APAP administration, serum samples and liver tissue were collected and biochemistry and histological analyzes.

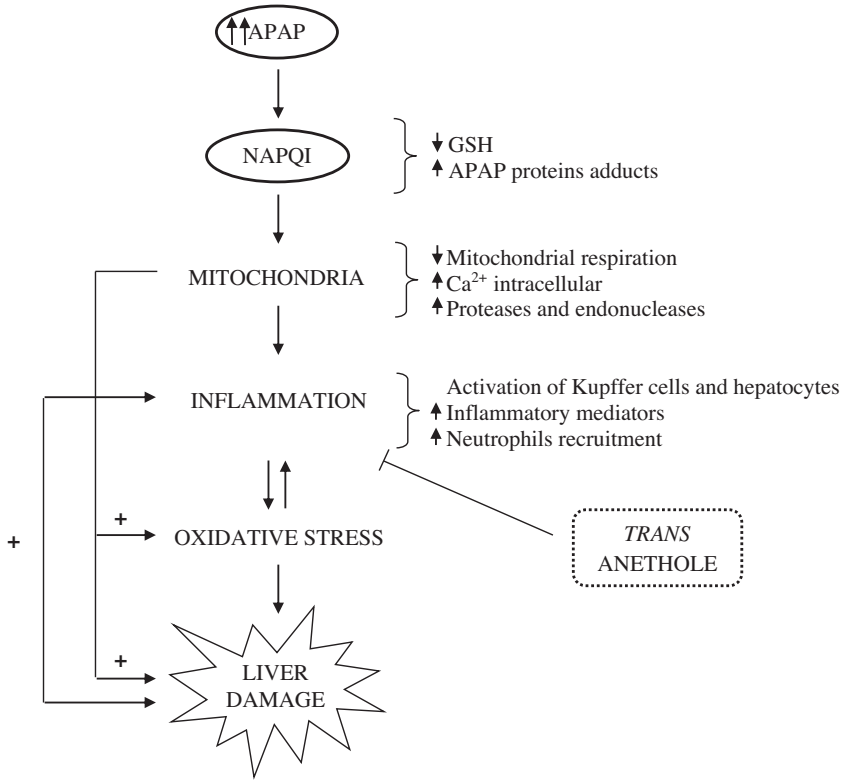

Fig. 1. Possible *trans* anethole effect on the hepatotoxicity induced by overdose of APAP.