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Potential Hepato-protective effect of Naringin and Propolis against Furan Toxicity: A Comparative study

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ABSTRACT

Smokers and negative smokers are exposed to a probable carcinogen named furan formed during tobacco burning. The purpose of this investigation was to assess the protective role of two natural antioxidants, naringin and propolis against furan-induced hepatocyte damage in rat livers. Rats were divided into 7 groups: control group; furan group (40 mg/kg b.w furan); furan + narnigin 50 (40 mg/kg b.w furan and 50 mg/kg narnigin); furan + narnigin 100 (40 mg/kg b.w furan and 100 mg/kg narnigin); furan + propolis 100 (40 mg/kg b.w furan and 100 mg/kg propolis); furan + propolis 200 (40 mg/kg b.w furan and 200 mg/kg propolis); furan + silymarin (40 mg/kg b.w furan and 37.8 mg/kg b.w silymarin); all treatments administrated orally for 28 days. Blood samples were obtained at the end of experiment for biochemical determination of hepatic enzymes, protein and lipid profiles. Furan administration leads to increasing in hepatic enzyme activities, reducing in albumin and total protein levels, and increasing in serum total cholesterol in addition to triacylglycerols compared to control group. In groups treated with narnigin (50 and 100 mg/kg) and propolis (100 and 200 mg/kg) the biochemical alterations induced by furan were significantly ameliorated compared to furan group. Interestingly, low dose of narnigin showed the closest results to silymarin treated group. Moreover, the high dose of propolis was more efficient than low dose compared to silymarin group. Thus, using narnigin and propolis as natural antioxidants markedly ameliorate adverse effects of furan.

1. INTRODUCTION

Furan is a heterocyclic, colorless, volatile compound. Furan (C_4H_4O) is an organic lipophilic compound. Food and Drug Administration (FDA, 2004) and the European Food Safety Authority (EFSA, 2004) have declared that this compound is found in food product that treated by heat. These products include canned or jarred baby food, squash, beans, sweet potatoes, canned meats, toasted bread, and roasted coffee beans, among other foods. Numerous products, such as pesticides, resin stabilizers, and pharmaceuticals, are made from furan. It may also be formed during accidental ignition, as well as in engine fumes and other situations like incompletely burned plastic or construction material (Hayes and Marnane, 2002).

Furan is present in almost every element of the environment, including the air, soil, water, and sediments. Up to 65 mg of furan can be discovered in cigarette smoke and cigarettes, noting that tobacco derivatives constitute the primary source of exposure to furan for the public people (Morehouse et al., 2008). Assert that heat treatment's actions on unsaturated fatty acids, carbohydrates, ascorbate, and carotenes result in the synthesis of furan (Van Lancker et al., 2009). However, chronic furan usage may harm children's health; this has not yet been fully researched (Scholl et al., 2013). Babies frequently ingest more furan than adults because they only consume breast milk or formula manufactured with high quantities of furan. Furan can also cross the placental barrier; thus, exposure can start while a woman is pregnant (Van Wijnen et al., 1990). The furanic compound is categorized as a Group 2B probable carcinogenic compound for human by the International Agency for Research on Cancer (IARC) (Zuckerman, 1995). The liver is the primary target organ for the toxicological effects of furan. Cytochrome P450 (CYP) 2E1 metabolizes furan into an extra toxic metabolite that induces an imbalance in the oxidant/antioxidant homeostasis with subsequent protein and DNA damage besides lipid peroxidation (Awad et al., 2018).

Citrus fruits, grapefruits, mandarins, etc., frequently contain naringin (4′, 5, 7-trihydroxyflavanone 7-rhamnoglucoside) (Kumar et al., 2010). Naringin exhibits a lot of biological effects, like anti-lipid peroxidation properties (Rajadurai and Prince, 2009), anti-inflammatory and anti-mutagenic activity (Kanno et al., 2006), antioxidant agent (Choe et al., 2001), inhibiting the growth of tumors, and causing suppression of the proliferation of tumor cells (Choe et al., 2001). Propolis (PRO), also known as bee glue, is a waxy, resinous building material used by bees. It has many clinical uses in traditional medicine and has a good reputation as a prophylactic strategy against hepatotoxicity (Majeed et al., 2016). The present study aimed to evaluate

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the efficacy of naringin and propolis as hepato-protective agents against the adverse effects of furan on liver tissues using silymarin as a standard hepato-protective pharmaceutical preparation.

2. MATERIAL AND METHODS

2.1. Experimental animals:
In this study, 49 male albino rats weighing between 160 and 220 g were used. They were obtained from Animal House, Faculty of Veterinary Medicine, Benha University. Rats were maintained in metal cages under suitable living circumstances like temperature at 21-24 °C, and at twelve hours light/dark cycle, with humidity does not exceed than 60%, furnished with a suitable meal, and given access to water for seven days before the study. The Ethical Committee of the Faculty of Veterinary Medicine, Benha University, authorized the study (BUFVTM 10-10-22)

2.2. Chemicals:
Tetrahydrofuran (CAS No. 109-99-9; 99.5% Extra pure; LOBA CHEMIE PVT.LTD.). Thermofisher Scientific provided the naringin (L09834 4', 5, 7-Trihydroxyflavanone, 97%) (CAS Number: 67604-48-2, Product Number: L09834), which was purchased as a white powder. Propolis was obtained from local herbal market. Silymarin was purchased from SEDICO, 6 October City, Egypt. The biochemical analytical kits were from Bio-diagnostics Co, Giza, Egypt.

2.3. Experimental design:
Rats were randomly divided into seven groups for the studies, and every single group contained 7 rats and received the prescribed therapy every day for 28 days. Control group: obtained distilled water orally; Furan group: administered 40 mg/kg b.w furan (Hamadeh et al., 2004); Furan + narnigin 50: obtained 40 mg/kg b.w furan and concurrently administered 50 mg/kg naringin (Adebiyi et al., 2015); Furan + narnigin 100: obtained 40 mg/kg b.w furan and concurrently administered 100 mg/kg naringin (Xulu and Owira, 2012); Furan + propolis 100: received 40 mg/kg b.w furan and co-treated with 100 mg/kg propolis (El Menyiy et al., 2018); Furan + propolis 200: received 40 mg/kg b.w furan and co-treated with 200 mg/kg propolis (Gogebakan et al., 2012); Furan + sylimarin: obtained 40 mg/kg b.w furan and concurrently administered 37.8 mg/kg b.w sylimarin (Paget and Barnes, 1964); all treatments administrated via gavage.

2.4. Blood sampling:
Under the influence of sodium pentobarbital anaesthesia (60 mg/kg), in order to pierce the tissue and reach the orbital sinus, blood samples were obtained from the medial canthus of the eye, where a capillary tube was placed at a 30° angle to the nose. The capillary tube was carefully withdrawn after blood flowed through it, and finger pressure applied softly could halt bleeding. The acquired samples were centrifugated for 15 minutes at 3000 rpm for serum separation. The serum was maintained at -20 °C for the biochemical evaluation of biochemical parameters.

2.5. Biochemical analysis:
Based on the manufacturer's instructions, enzyme activities as AST, ALT, and ALP were assessed via commercial kits. Serum activities of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were assayed following the way of (Huang et al., 2006). Alkaline phosphatase (ALP) activity was determined according to the procedures of (Schumann et al., 2011). The serum albumin and total protein were estimated following (Doumas et al., 1971; Koller and Kaplan, 1984). Total bilirubin was determined according to (Young et al., 1995).

2.6. Statistical analysis:
The one-way analysis of variance was utilized to analyze the data which shown as (Mean ± S.E.). Duncan's test was used as post hoc for multiple comparisons between groups using SPSS 25. The study's findings showed that mean values with various superscripts are significantly different at ($P<0.05$).

3. RESULTS

Oral administration of furan to rats significantly increased serum ALT, AST, and ALP enzyme activities, in addition to total bilirubin compared to control group (Figure 1 a-d) with a significant decrease in albumin and total protein levels (Figure 2 a-b). Moreover, a significant elevation in serum total cholesterol in addition to triacylglycerols levels was recorded (Figure 2 c-d).
Co-administration of Naringin 50 and 100 mg with furan significantly decreased ALT (40.9%-27.8%), AST (38.1%-29.5%), and ALP (22.5-17.3%) enzyme activities, in addition to total bilirubin (40.8%-37.2%) compared to furan group (Figure 1 a-d). Also, a significant increase in albumin and total protein was observed (Figure 2 a-b), besides a significant decrease in total cholesterol (27.1%-20.4%) and triacylglycerols (31.5%-29.5%) levels (Figure 2 c-d).
Concurrent treatment with Propolis 100 and 200 mg with furan exhibited a significant decrease in liver enzyme activities ALT by (13.7%-25.7%), AST (14.4%-24.0%), ALP (7.3%-13.63%) and serum total bilirubin (18.9%-29.1%) compared to furan group (Figure 1 a-d). A non-significant increase in albumin level was seen in propolis 100 while propolis 200 significantly increased serum albumin. Furthermore, a significant rising in the level of total protein was exhibited (Figure 2 a-b). Moreover, both doses of propolis showed a significant decrease in total cholesterol (10.5%-15.5%) and triacylglycerols (13.2%-23.5%) levels (Figure 2 c-d).

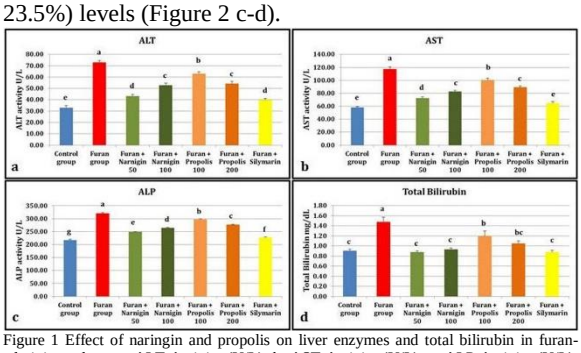

Figure 1 Effect of naringin and propolis on liver enzymes and total bilirubin in furan-administered rats.a: ALT Activity (U/L); b: AST Activity (U/L); c: ALP Activity (U/L); d: Total Bilirubin level (mg/dL).

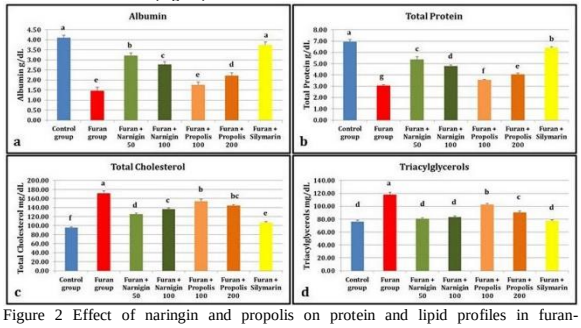

Figure 2 Effect of naringin and propolis on protein and lipid profiles in furan-administered rats.a: Serum Albumin level (g/dL); b: Serum Total protein level (g/dL); c: Serum Total Cholesterol level (mg/dL); d: Serum Triacylglycerols level (mg/dL).

4. DISCUSSION

The chemical molecule known as furan is created during the thermal heat of some foods by the three primary traditional heat processes: canning, regular cooking, and jarring. In the current study, rats orally administered furan displayed significant alterations in the activities of liver enzymes, protein, and lipid profiles. Liver as the main organ of detoxification metabolize furan via its detoxifying enzymes. Bis-electrophilic metabolite and cis-2-butene-1,4-dial are the products of furan detoxification by cytochrome P450. Furan metabolites alter oxidant/antioxidant balance, ending with lipid peroxidation to hepatic cells (Hickling et al., 2010). This can explain the elevation of serum hepatic enzymes in our experiment since lipid peroxidation adversely affects the membrane permeability of hepatocytes. Increased serum level of ALP is a sign of bile duct damage (Ramaiah, 2007), and this was supported and explained the increase in total bilirubin in the current study. Studies have reported a reduction in albumin production and total protein during liver damage because of the prevention of liver metabolic function. Furan may have disrupted albumin formation in the liver as a consequence of fluid retention inside the spaces of the intracellular (Yakubu et al., 2006). The detoxification function of the liver takes priority over the other functions of liver as albumin synthesis, as confirmed by our results. Additionally, liver is the master organ of lipid metabolism. Disturbance in lipid profile after furan administration was expected since liver is directed toward furan metabolism and detoxication. This was supported with the previous results of (Pekiner et al., 2002), who found that furan toxicity raised blood levels of triacylglycerols and cholesterol.

Increased antioxidant capacity is essential for hepato-protection and reversing the harmful effects of oxidative stress (Pushpavalli et al., 2008). As a type of antioxidant, naringin protects the rat's liver against damage caused by furan. The oxidative stress elicited by furan could be alleviated by the antioxidant capacity of naringin by scavenging furan reactive metabolites hence protecting cells from lipid peroxidation. This could explain the obvious decrease in hepatic enzyme activities since lipid peroxidation decreased and cellular permeability was preserved. This explanation is in line with with the previous study of (Pawar et al., 2001). Consequently, protein and lipid profiles returned near control levels as the other metabolic functions of liver performed normally. Our previous studies support this explanation considering an unbalanced oxidant/antioxidant state elicited by thioacetamide that was relieved by rosemary extract (Ragab et al., 2019; Said et al., 2019).

The results of our experiment came in agreement with previous studies in Nile Tilapia that recorded propolis's hepato-protective effect (de Oliveira et al., 2020). Moreover, our findings concur with (Jasprica et al., 2007; Wen et al., 2012), who noted normal cholesterol and Triacylglycerols levels following the propolis supplementation. Along the same line, the significant amelioration of the furan's adverse effects on liver functions by propolis in a dose dependent pattern could be explained on the basis of antioxidant efficacy of propolis against oxidative stress induced by furan.

5. CONCLUSION

We can conclude that elevation of hepatic enzyme activities and alterations in protein and lipid profiles after furan administration may result from oxidative stress induced by furan metabolites. This assumption was confirmed by ameliorating the above-mentioned parameters in the groups co-treated with naringin or propolis, which are known for their antioxidant properties which require further investigations on the effect of furan on antioxidant homeostasis in liver and the hepato-protective mechanisms of naringin and propolis via biochemical, molecular, immunohistochemical, and pathological studies.

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