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Mitigating potential of *Ginkgo biloba* extract and melatonin against hepatic and nephrotoxicity induced by Bisphenol A in male rats

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ABSTRACT

Bisphenol A is one of the anthropogenic chemicals produced worldwide, currently released into the environment and causes endocrine-disruption. The largest environmental compartments of BPA are abiotic associated with water and suspended solids that becomes an integrated part of the food chain. The present study aimed to examine the possible protective role of *Ginkgo biloba* extract (GBE), melatonin and their combination against BPA-induced liver and kidney toxicity of male rats. Fifty rats were divided into five equal groups: control, BPA, BPA plus GBE, BPA plus melatonin and BPA plus GBE plus melatonin. The elevated activities of plasma ALT and AST in addition to increased levels of urea and creatinine concomitant with the decreased total plasma protein could reflect the injurious effect of BPA. Liver and kidney levels of TBARS were significantly increased, while GSH, SOD and GPX were decreased in BPA-treated rats. Also, CAT and GST activities were significantly disrupted in the liver and kidney of rats treated with BPA. Moreover, BPA significantly increased the proinflammatory cytokine TNF-α in the liver and kidney tissues. The histopathological analysis confirmed these results. All the previous alterations in the liver and kidney could be ameliorated when BPA-treated rats were co-administrated either with GBE, melatonin or their combination. These natural substances could exhibit protective effects against BPA-induced hepato- and nephrotoxicity owing to their antioxidative and anti-inflammatory potentials.

1. Introduction

Bisphenol A (2,2-bis (4-hydroxyphenyl) propane, (BPA) is an environmental chemical contaminant that is widely used in the manufacture of polycarbonate plastics and epoxy resins [1]. Also, BPA is used in the production of thermal stabilizers, plasticides, paints and dental materials [2]. Human extensive exposure to BPA through the food chain is widespread because BPA is released by food and beverage containers [3]. High doses of BPA altered liver weights and decreased the viability of rat hepatocytes [4]. BPA is a nephrotoxic agent due to the accumulation of its toxic metabolites and inability of the kidney to eliminate those metabolites [5]. Also, BPA induce the formation of reactive oxygen species (ROS) which cause tissue injury in the liver, kidney and other organs [6]. Furthermore, the low doses of BPA generate ROS by means of decreasing the activities of the antioxidant enzymes and increasing lipid peroxidation thereby causing oxidative stress [7]. Nowadays, natural supplements are used in the treatment of many diseases either by improving the efficacy of the drug or by minimizing the toxic side effects [8].

Ginkgo biloba leaf extract (GBE) is one of the most widely used herbal supplements in the traditional Chinese medicine for centuries [9]. Several investigators reported that *G. biloba* polysaccharides (GBP) have different biological actions, such as antioxidation, anti-inflammation, immunomodulation and anti-tumor [10]. It could protect the liver from an induced injury by means of reducing lipid peroxidation and GSH depletion concomitant with enhancing gene expression of the antioxidant enzymes [11] and inhibiting TNF-α expression [12]. *In vivo* results showed that GBL-treatment is a potent protector against uranium-induced toxicity [13].

Melatonin (5-methoxy-N-acetyltryptamine) is natural hormone secreted mainly from the pineal gland [3]. Also, other organs such as eyes, brain, gut, skin and immune cells can synthesize melatonin [14]. Melatonin is also synthesized in the gastrointestinal tract [15]. Melatonin participates in the regulation of many physiological processes. Melatonin can reduce oxidative stress via stimulating the activities of the anti-oxidative enzymes; superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) [16]. Addi-

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tionally, melatonin can protect DNA, protein and lipids [17]. Moreover, melatonin reduces the level of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), inteleukin-6 (IL-6) and interleukin 1beta (IL-1β) [18]. Consequently, the present study was carried out to evaluate the protective effect of *G. biloba* extract and/or melatonin against Bisphenol A-induced hepatotoxicity and nephrotoxicity in adult male rats. This is accomplished by the assessment of liver and kidney function tests, apoptotic marker (TNF-α), lipid peroxidation end product (TBARS), enzymatic and non-enzymatic antioxidants in addition to histological examination of the liver and kidney tissues.

2. Materials and methods

2.1. Experimental animals

Adult male rats weighing (180–200 g) were used in the current study. They were obtained from the animal house, Faculty of Medicine, Alexandria University, Egypt. Rats were housed in stainless steel cages and maintained at 25–28 °C with 12-h light-dark cycle. Animals were allowed to food and water *ad libitum*.

2.2. Experimental design

Rats were randomly distributed into five groups as follows: Control group; rats were used as negative control. Bisphenol A (BPA)-treated group (positive control); rats were orally treated with BPA at a dose of 40 mg/kg body weight [19] and [20]. Bisphenol A + *G. biloba* extract (BPA + GBE)-treated group; rats of this group were treated with BPA (40 mg/kg b. w.) and GBE used as 100 mg/kg b. w. [21]. Bisphenol A + melatonin (BPA + Mel)-treated group; rats were treated with BPA (40 mg/kg b. w.) and Mel of a dose 10 mg/kg b. w. [22] Bisphenol A + *G. biloba* extract + melatonin (BPA + GBE + Mel)-treated group; rats were treated with a combination of BPA, GBE and Mel. All the selected doses were orally and daily administered for 70 days.

2.3. Blood collection

At the end of the experimental period, rats were fasted for 12 h then they were scarified by decapitation under diethyl ether anesthesia. Blood samples were collected by heart puncture in tubes containing EDTA (anti-coagulant) and placed immediately on ice. Plasma was obtained by centrifugation of samples at 860 × *g* for 20 min and stored at −80 °C until used for analyses.

2.4. Tissue preparation

Livers and kidneys were excised and washed using saline solution (0.9%). Parts from the livers and kidneys of each group were fixed in 10% formalin for histological examination. Other parts were minced and homogenized in ice-cold sodium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl (10% w/v) using a polytron (Tekmar model TR-10, West Germany) homogenizer. The homogenate was centrifuged at 10,000 × *g* for 20 min at 4 °C using high speed cooling centrifuge (Universal 32 R, Germany). The resultant supernatants were used for the different investigations.

2.5. Assessment of the biochemical parameters

Total plasma protein was determined using biuret reaction [23]. Urea and creatinine plasma levels were also measured [24] and [25], respectively. Plasma and hepatic Aspartate aminotransferase (AST; EC 2.6.1.1) activity was assayed [26]. While, plasma and hepatic alanine aminotransferase (ALT; EC 2.6.1.2) activity was assayed using the methods of International Federation of Clinical Chemistry [27]. Glutathione peroxidase (GPX; EC. 1.1.1.9) activity was assayed in hepatic tissues [28]. Also, reduced glutathione level was determined in both livers and kidneys [29]. Hepatic and renal Glutathione-S-transferase (GST, EC 2.5.1.18) activities were assayed using p-nitrobenzyl chloride in 95% ethanol as a substrate [30]. Moreover, Superoxide dismutase (SOD; EC 1.15.1.1) activity was estimated in Hepatic and renal tissues [31]. Also, Catalase activity (CAT; EC1.11.1.6) was assayed in livers and kidneys [32]. The level of the lipid peroxidation end products, Malondialdehyde, which react with thiobarbituric acid-reactive substances (TBARS), was also evaluated in both livers and kidneys [33]. The pro-inflammatory cytokine TNF-α (K0331196) level was quantified by the enzyme-linked immunosorbent assay (ELISA) using ELISA kit specific for rat cytokines (Biosource International, Nivelles, Belgium).

2.6. Histological section preparation

Liver and kidney sections were fixed in formalin (10%), treated with alcohol and xylol then embedded in paraffin and sectioned at thickness of 4–6 μm. These sections were stained with Haematoxylin and Eosin stain (H&E, X 400) for the examination of histopathological changes [34].

2.7. Statistical analysis

Results were reported as means ± SE and data were statistically analyzed [35]. The statistical significant difference in values of the experimental animals was calculated by (F) test at 5% significance level. All parameters were calculated using the general linear model produced by Statistical Analysis Systems Institute [36]. Beside, Duncan's New Multiple Range Test was used in assessment of the significant differences between groups. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Assessment of biochemical parameters

The current study indicated that plasma activities of ALT and AST were significantly ($p < 0.05$) increased while hepatic ALT and AST specific activities were significantly ($p < 0.05$) decreased in BPA-treated group compared to the control. On the other hand, plasma ALT and AST activities were significantly ($P < 0.05$) decreased whereas hepatic ALT and AST specific activities were significantly ($p < 0.05$) increased in groups treated with BPA + GBE, BPA + Mel and their combination compared to BPA-treated ones (Table 1).

It was observed that hepatic GST and CAT activities were significantly ($p < 0.05$) increased in BPA-treated rats when compared to control, while significantly ($p < 0.05$) decreased after BPA co-treatment with GBE, Mel and their combination in comparison to BPA-treated ones (Table 1). Liver activities of GPx and SOD were significantly ($p < 0.05$) decreased in BPA-treated group as compared to control. On the contrary, treatment with GBE, Mel and their combination together with BPA resulted in significant ($p < 0.05$) increases in the activities of these enzymes (Table 1).

In addition, our results indicated that renal activities of GST, SOD and CAT showed significant ($p < 0.05$) decreases with BPA treatment compared to control. While, these enzyme activities were significantly ($p < 0.05$) increased when BPA co-administered with GBE, Mel and their combination (Table 1).

Furthermore, the results revealed that BPA induction caused significant ($p < 0.05$) increases in the plasma urea and creatinine