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Developmental toxicity of orally administered pineapple leaf extract in rats

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ABSTRACT

The extract of pineapple leaves (EPL) has anti-diabetic and anti-dyslipidemic effects and can be developed into a promising natural medicine. This study was conducted to evaluate EPL's effects on developmental parameters in order to provide evidence of its safety before potential medical use. Five groups were included: a negative control that was given distilled water daily, a positive control that was dosed 7 mg/kg cyclophosphamide (CP) every two days, and three groups that were respectively dosed 2.0, 1.0, and 0.5 g/kg EPL daily. Female rats were dosed during the organogenesis period of gestation days (GD) 7–17 and terminated on GD 20. A series of parameters were examined. Data revealed that CP significantly reduced maternal body weight gains, caused maternal organ weight alterations, reduced female fertility, disturbed fetal growth and development, and caused marked teratogenic effects on fetal appearances, skeleton and internal organs. Distilled water and the three high doses of EPL did not cause any of the aforementioned effects. This study concluded that orally administered EPL is safe to rats during embryonic development.

1. Introduction

Pineapple (*Ananas comosus* (Linn.) Merr.) is the third most important tropical fruit, whose mean world production is over 13 million metric tons annually in recent years. It is extensively planted throughout the world, particularly by leading pineapple-producing nations such as Thailand, Philippines, Malaysia, India, Brazil, China and Africa (Williamson et al., 2008; Cuadra and Bjorklund, 2007; Pommer and Barbosa, 2009). Such a large amount of production and worldwide distribution demonstrates that progress made in comprehensive utilization of pineapple and its byproducts will potentially have significant value. In Thailand, pineapples are used in the treatment of dysuria (Sripanidkulchai et al., 2001). Recent research has shown that pineapple fruit, peel, and juice exhibit robust effects of antioxidant capacity, phenolic content, and polysaccharide content (de Oliveira et al., 2009; Ramadan-Hassanien, 2008; Alothman et al., 2009; Guo and Zhang, 2009; Mhatre et al., 2009). It was reported that pineapple juice inhibited cytochrome P450 2C9 activity (Hidaka et al., 2008) and that the major component extracted from pineapple, Bromelain, could reduce CD25 expression and inhibit COX-2 expression via anti-inflammation and anti-tumor activities (Secor et al., 2009; Bhui et al., 2009).

In China, pineapple yield is over 4 million tons annually; consequentially, over 12,000–18,000 tons of pineapple leaves per 1 km^2 are produced (Zheng et al., 2009). How to deal with this abundance of pineapple leaves and produce as much valuable use as possible has been a considerable problem for years. Up till now, pineapple leaves are used in the following ways: animal feeds, fiber and paper production, medicine and nutraceutical research (bromelain), food additives (furaneol), filter materials, etc. (Mohamed et al., 2009; Wenga et al., 2009; Wang et al., 2009; Threepopnatkul et al., 2009). It is reported that the developed nanocellulose is an exceptionally versatile material, having a wide range of biomedical and biotechnological applications, such as tissue engineering, drug delivery, wound dressings and medical implants (Cheriana et al., 2010).

In Chinese medicine, pineapple leaves are used as antidyspepsia or antidiarrhea agents (Song, 1999), which implies the potential medical significance of pineapple leaves. Previous research by our laboratory on the extract of pineapple leaves (EPL) revealed valuable pharmacological activities, including anti-diabetic, anti-dyslipidemic, and anti-oxidative activities in diabetic rats. EPL was even more effective than fenofibrate in activities of increasing HDL (high density lipoprotein) levels and anti-oxidation capacity, and was shown to be as effective as fenofibrate in activities of decreasing TG (triglyceride), TC (serum total cholesterol) and LDL-C (low density lipoprotein cholesterol) levels, which indicated the potential of developing EPL into an effective anti-diabetic medicine (Xie et al., 2005). Further work revealed that the hypoglycemic effect of EPL might be related to improvement of insulin

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sensitivity (Xie et al., 2006). EPL could improve insulin sensitivity in type 2 diabetic rats and experiments on HepG2 indicated that the effect might be associated with the enhancement of insulin action in hepatic cells. Another series of experiments showed that the hypolipidemic effect of EPL might be related to inhibition of the activity of HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase and activation of plasma LPL (lipoprotein lipase), which bears resemblance to the mechanism of action of statins but differs from that of fibrates (Xie et al., 2007). From chemical research conducted in our lab, the extract was mainly composed of phenolic constituents by LC/MS assay (Ma et al., 2007; Wang et al., 2006). Moreover, one of the main ingredients contained in EPL – *p*-coumaric acid (Meng et al., 2006), belonging to phenolic acid which is existed in many fruit and wine (Luthria et al., 2006; Brettonnet et al., 2010; Salameh et al., 2008), believed to be an antioxidant that can prevent oxidation of LDL and, thus, reduce serum LDL-C levels (Zang et al., 2000; Biswick et al., 2010), which might elucidate the anti-oxidative activities of EPL.

Considering the compelling anti-diabetic and anti-dyslipidemic effects with their mechanisms partially revealed and the profuse sources of pineapple leaves, EPL can be developed into a promising natural medicine. Since the leaves of the pineapple plant have potential use in biomedicine based upon our previous studies and the history of medicinal use in China, evaluation of its safety is important. Therefore this study presents an evaluation of the developmental toxicity of pineapple leaf extract as a first step in the evaluation of reproductive and developmental safety, which is defined by the thalidomide tragedy in the 1950s and early 1960s (Collins, 2006).

2. Materials and methods

2.1. Animals

Wistar rats (C.L. strain, 13–15 weeks old, females weighed 220–250 g, males weighed 230–260 g) were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (Beijing, China). Animals were kept in an environmentally controlled breeding room (temperature: 23–25 °C, relative humidity: 40–70%, 12-h dark/light cycle). They were fed standard laboratory chow diet with water ad libitum.

The rats were acclimated to the laboratory for a week before the experiment. Those in good health were selected for use. Female rats were time mated to males (2:1), and the day on which a vaginal plug and sperm in a vaginal smear were observed was regarded as day 0 of gestation (GD 0). Mated females were randomly distributed to one of 5 study groups (16 rats/group) based on body weight and each rat was assigned a unique identification number by coat staining.

2.2. Materials

EPL was obtained in our lab. The preparation and phytochemical screenings of EPL were conducted by Dr. Wei Wang. Dried powder of pineapple leaves was refluxed with 80% ethanol. After a series of processes, the powder form of ethanolic extract of pineapple leaves was obtained (Xie et al., 2007). EPL contained total phenols (60%, w/w in terms of the extract). There were several phenols known as *p*-coumaric acid (1.5%), 1-*O*-*p*-coumaroylglycerol (0.3%), caffeic acid (1.0%), 1-*O*-caffeoylglycerol (0.2%) and ananasate (1,3-*O*-dicaffeoylglycerides) (0.066%) by HPLC analysis (Shen et al., 2007; Ma et al., 2007), with the chemical structures displayed in Fig. 1. The homogeneity of the EPL was verified by HPLC using 1,3-*O*-dicaffeoylglycerides as quality control (Shen et al., 2007). The EPL was dissolved in H_2O as an 800 mg/ml aqueous suspension. During use, it was dissolved in distilled water and diluted to certain concentrations in order to be administered to animals.

Cyclophosphamide (CP), used as a teratogenic agent(Mirkes et al., 1984; Gomes-Carneiro et al., 2003; Latorre et al., 2007), was purchased from Hengrui Medical Corp. Ltd. in the form of injection. CP was diluted with normal saline to a certain concentration before injected into animals. All others reagents were of analytical grade and obtained from standard commercial suppliers.

2.3. Experimental procedure

2.3.1. Dosage and administration of doses

The doses were administered from GD 7 to GD 17. Five study groups were included: three dose levels of EPL, a concurrent negative control, and a concurrent positive control (Gonzalez et al., 2007). The dosages of EPL groups were 2, 1 and 0.5 g/kg per day, respectively, which were correspondingly 5, 2.5 and 1.25 times of the pharmacological effect dosage of 0.4 g/kg per day (Xie et al., 2005).

For intragastric (IG) administration, 1.0 ml of EPL was given for every 100 g of rat body weight. The concentration of EPL was 0.4 g/ml. The rats were observed for 2 weeks. We altered the procedure for oral delivery to give multiple administrations to increase the overall dosage of EPL. We administered EPL once every 2 h (for accumulated dosage of 12.0 g/kg in three times), observed and recorded the number of dead rats in each group. We also weighted and recorded the body weight of the rats alive. None of rats died during the toxic experiment and there were not body weight reduced apparently compared with the negative control (distilled water). So the maximum tolerated dose (MTD) was 12.0 g/kg (data not published).

The negative control received distilled water by oral administration. The positive control received CP at a dosage of 7 mg/kg every two days by intramuscular injection to produce teratogenic effects on fetuses. The dose of CP was set according to related literature (Mirkes et al., 1984) and adjusted according to the toxic effects observed in our preliminary test.

The method of administration was consistent with the method in which each material was used clinically. The dosage volume for each animal was based on the most recent individual weight. EPL and distilled water, at a volume of 1 ml/100 g body weight, were administered by gavage. CP was injected at a volume of 0.5 ml/100 g body weight.

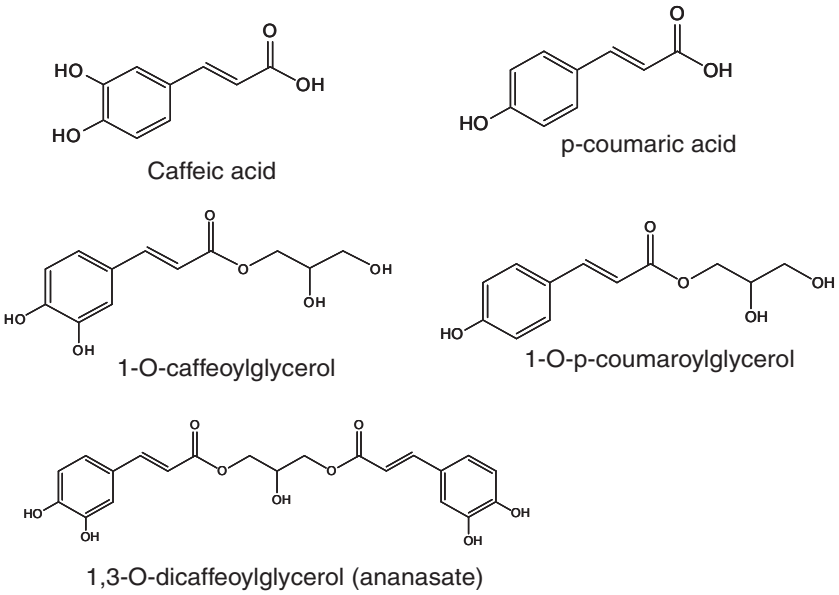

Fig. 1. Chemical structure of five compounds from the extract of pineapple leaves.