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Effect of green tea and its polyphenols on mouse liver

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ABSTRACT

Increased consumption of green tea (GT) without enough scientific data has raised safety concerns. Epigallocatechin 3-gallate (EGCG) is the most prominent polyphenol of GT that has antioxidant activity. However, higher doses of EGCG have been shown to cause liver injury. This study was initiated to determine the effect of GT extracts in a mouse model. We also investigated the effects of EGCG in normal and health-compromised mice. Different doses of GT fractions and EGCG were administered for 5 days to mice. Also, a single dose of lipopolysaccharide (LPS) was combined with EGCG in order to investigate its effect in the presence of fever. Plasma ALT and ALP levels were determined along with liver histopathology. Combining a single high IG dose of EGCG with a single IP dose of LPS initiated liver injury. Furthermore, repeated administration of high IG doses of EGCG showed mild liver injury, but it was augmented under febrile conditions induced by LPS. This study confirms the safety of reasonable consumption of GT over a short term. However, it highlights a caution that high doses of EGCG can lead to mild liver injury, and this may be markedly enhanced under febrile conditions.

1. Introduction

Tea is one of the most popular beverages consumed worldwide. It is obtained from the plant *Camellia sinensis*, as green, black, or oolong tea [1]. Green tea (GT) is favored in Japan, China and the Middle East; therefore, initial research on its benefits was carried out in these countries [2,3]. Green and black teas are processed differently. Green tea is prepared from freshly harvested leaves that are steamed to prevent fermentation, yielding a dry, stable product. Fresh tea leaves are rich in the flavanol group of polyphenols known as catechins which may constitute up to 30% of the dry leaf weight. The most prominent catechins are: epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG). Other polyphenols include flavonols and their glycosides such as chlorogenic acid, coumarylquinic acid, and one unique to tea, theogallin (3-galloylquinic acid). Green tea leaves contain three main components which affect human health: xanthic bases (caffeine and theophylline), essential oils and especially, polyphenolic compounds. Caffeine is present at an average level of 3% along with very small amounts of the other common methylxanthines: theobromine and theophylline. The amino acid theanine (5-Nethylglutamine) is also unique to tea [4].

Green tea has been considered a healthy beverage since ancient times. Traditional Chinese medicine has recommended

Abbreviations: Fr., Fraction; GT, Green tea; Fig., Figure; IG, Intragastric; IP, Intraperitoneal; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; EGCG, Epigallocatechin-3-gallate; LPS, Lipopolysaccharide.

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this plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and, in general, to prolong life. Caffeine mainly affects the central nervous system, stimulating wakefulness and decreasing the sensation of fatigue [5]. Some of the effects caused by caffeine are influenced by theophylline as it induces psychoactive activity [6]. Theophylline also has a mild inotropic and vasodilator effect, and a much higher diuretic effect than caffeine [7]. However, on the broncho-pulmonary and respiratory tract, it causes non-specific relaxation of the bronchial smooth muscles and stimulates respiration [8]. Green tea has received considerable attention as a strong antioxidant due to its high level of polyphenols. Important biological properties have been linked to GT such as antimutagenic, antibacterial, anti-inflammatory, immunostimulant, neuroprotective and hypocholesterolemic effects as well as protection against dental caries, periodontal disease, and teeth loss [6] and the most important effect body weight control [9–12].

Overconsumption of tea (black or green) may lead to harmful effects that are attributed to three main factors: its caffeine content, the presence of aluminum and the effects of tea polyphenols on iron bioavailability [13]. Like most plants, GT contains different active constituents that compensate for the biological effects of the other constituents. For example, flavonoids are known to increase gastric motility and possess a laxative effect [14], while tannins have the ability to decrease the gastric motility and possess a constipating effect [15]. Similarly, if a separated fraction of GT is consumed rather than the whole GT, compensation for harmful effects may be lost and provoke a biological effect that does not appear with the common usage of the whole GT extract.

Marketing strategies of GT for weight loss and for augmenting physical performance has led to the availability of GT extracts in the form of capsules. Its consumption has increased significantly and thus becomes fashionable among weight watchers [16]. Moderate drinking of GT is not the cause of concern; it is the caplet/capsular form of the GT extract or the pure EGCG products that have raised queries about its safety. There is a lack of scientific data on the safe dose levels of these products. The use of GT supplements has led to sporadic adverse effects reported as case studies. Major adverse effects are associated with the consumption of high doses of tea preparations containing polyphenols. These case studies highlight hepatotoxicity related to the consumption of high doses of tea-based dietary supplements [17]. A case of acute liver failure linked to GT extract was reported from Canada where a GT supplement was taken as a weight reducing agent [18]. Several other reports in Europe which exhibited marked liver toxicity in the form of acute hepatitis were linked to the consumption of supplements containing GT extracts. One of these reports described a patient with recurrent episodes of acute hepatitis that were linked to the consumption of GT extract for a period of time before each episode, confirming the role of GT extract in hepatotoxicity [19]. The reported toxicity of green tea extract, although sporadic, led to the removal of "Exolise" (a supplement which contains GT extract) from the market by the French and Spanish authorities in 2003 [10]. In many of these reports, other predisposing factors had been suspected but were not thoroughly investigated.

Questions that arise from the literature cited above include: Is it safe to administer a single constituent of GT alone? If it is, what is the maximum dose that is efficacious and still not considered toxic? Why do GT supplements cause liver injury in some individuals while the majority of consumers do not suffer from this injury? What predisposing conditions or factors should be kept in mind when deciding the optimum dose for human consumption? With these queries in mind, we initiated screening studies of different extracts and fractions of GT as well as pure EGCG at different concentrations in mice to determine if any deleterious effects could be observed.

For the protection of consumers of GT or other supplemental products it is important to study the safe doses of these products under different health-compromising or predisposing conditions. Fever is a common symptom of many ailments and is generally treated with acetaminophen or other fever reducing drugs [20]. Thus it is appropriate to investigate the effects of GT supplement consumption under febrile conditions. Experimental studies have shown that the combination of non-toxic doses of pharmaceuticals and LPS (a known inflammagen and fever inducer) can cause liver injury [21]. We and others have previously shown that non-toxic doses of monocrotaline in combination with LPS can produce severe injury to the liver [22,23] and kidney [24]. In this study we have made an attempt to study the effect of EGCG under the influence of LPS.

2. Experimental

2.1. Plant material

Commercially available Chirag Green Tea was used for this study. A sample (no. 4915) has been deposited at the National Center for Natural Products Research, University of Mississippi. EGCG (95%) was purchased from Sigma-Aldrich USA.

2.2. Extraction and fraction preparation

Green tea (1.7 kg) was extracted with methanol (4.0 L × 3 × 24 h) at room temperature. The combined solutions were evaporated under reduced pressure to afford a dark-green residue (Fr. A, 490 g). Fraction A was partitioned between hexanes and methanol. The hexanes and methanol soluble parts of Fr. A were dried under reduced pressure to give the hexanes soluble fraction (Fr. A_1) and a methanolic fraction (Fr. A_2). Fraction A_2 was subjected to column chromatography over Sephadex LH-20 and eluted with methanol to obtain non-phenolic (Fr. A_2A) and phenolic (Fr. A_2B) parts. Green tea (200 g) was also extracted with distilled water (1 L) at 80°C for 1 h. Water solution after filtration was freeze-dried to yield a water soluble fraction (Fr. B).

2.3. Animal model

Male ND-4 mice were obtained from Harlan Lab (Indianapolis, IN) at 5 weeks of age and 23–28 g body weight, housed in micro isolator cages with corn cob bedding on 12 h light/dark cycle, at 72°F and 35–50% relative humidity. Mice were fed on Purina 5001 laboratory chow and water ad libitum. Before administering any treatment, mice were kept off feed for 12 h. After treatment, food was made available ad libitum (Tables 1 and 2).