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RESEARCH ARTICLE

Impact of High EGCG Consumption on Mouse Liver

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ABSTRACT

Tea is one of the most popular beverages especially among Middle East countries. Epigallocatechin 3-gallate (EGCG), the principal component of green tea is known to have many beneficial effects in therapeutic doses. However, higher doses of EGCG may have a damaging impact on liver. This study was initiated to explore the effects of high doses of EGCG alone and under predisposing factors. One of these predisposing factors is fever that can be induced by lipopolysaccharide (LPS). Mice (ND4) were given single or multiple intragastric (IG) doses of EGCG 1500 mg/kg and(LPS) was injected intraperitoneal (IP) as a single dose of 6 mg/kg. Histopathological examination of liver was done and survival rates of animals were observed and statistically analyzed. A single high IG dose of EGCG alone did not show liver toxicity, while combination of a single dose of EGCG with a single dose of LPS, initiated liver toxicity as observed on histopathology. Repeated administration of high IG doses of EGCG without LPS showed mortality among treated mice; but mortality was increased under the influence of LPS. It can be concluded from this study that administration of high doses of EGCG alone can initiate liver toxicity. However, under febrile condition (induced by LPS), this toxicity can lead to increased mortality.

INTRODUCTION:

Epigallocatechine3-Gallate (EGCG) is the most active and best researched of all green tea (GT) ingredients [1]. Animal and human studies have shown its beneficial effect in their potential to reduce the risk of cardiovascular disease and cancer [2]. In addition, a number of reports have been published showing that regular consumption of GT, may influence energy metabolism, body weight and body fat content [3]. On the contrary, many case reports indicate hepatotoxicity related to the consumption of polyphenols containing supplements especially in high doses [4]. The use of GT based dietary supplements taken as weight reducing agents, has been implicated in liver failure [5]. Another case report with recurrent episodes of acute hepatitis has been linked with consumption of GT extract [6]. Similar cases of GT related hepatotoxicity have been reported from Europe. It is important to note that most of these reports indicate the use of GT supplements under some predisposing condition or in combination of other pharmaceutical drugs or supplements [6]. Oral administration of green tea commercial product containing 90% EGCG in dogs for 13 weeks resulted in liver toxicity and death. Similarly intraperitoneal administration of catechins in rats caused liver damage [7]. We have previously published hepatotoxicity model in mice with Lipopolysaccharide (LPS) and monocrotaline (MCT), a pyrrolizidine alkaloids of Crotalaria plant [8]. Similarly LPS with pharmaceutical drugs has shown to cause liver toxicity [9]. We initiated this study to explore the possible effects of high doses of EGCG administration with or without LPS in a murine model.

METHODOLOGY:

TREATMENT PROTOCOL AND STUDY DESIGN:

ANIMAL MODEL:

Male ND-4 mice were obtained from Harlan Lab (Indianapolis, IN) at 5 weeks of age and 25 - 30 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 fasted for 12 h. Food was made available ad libitum after treatment.Animals were allotted in 7 groups as shown in (Table 1).

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Group No.	Treatments	Doses	Routes of administration
1	15% DMSO (vehicle)	once daily (5 days)	IG
2	LPS	6mg/kg single dose	IP
3	EGCG	1500mg/kg single dose	IG
4	LPS EGCG	6mg/kg single dose 1500 mg/kg single dose	IP IG
5	EGCG	1500 mg/kg once daily(5 days)	IG
6	LPS EGCG	6mg/kg single dose 1500 mg/kg once daily (5 days)	IP IG
7	EGCG LPS	1500 mg/kg once daily (5 days) 6mg/kg single dose (4th day)	IG IP

Table No. 1: Animal groups, treatments, doses and routes of administration.

NOTE: All animal study protocols were approved by the IACUC, University of Mississippi.

HISTOPATHOLOGIC EVALUATION:

Liver samples were outsourced to Oxford pathology lab, for histopathological examination and imaging.

STATISTICAL ANALYSIS:

Data were analyzed with Kaplan-Meier test of survival, using Graph Pad Prism software (La Jolla, CA). A P-value of less than 0.05 was considered to show a significant difference between vehicle and other groups.

RESULTS:

1. ANIMAL SURVIVAL:

Administration of EGCG in daily doses of 1500 mg/kg IG for 4 days (group 5) showed 25% mortality of animals on day 3 and 100% mortality on day 4 post treatment. Administration of LPS in a single dose of (6 mg/kg IP) prior to EGCG in daily doses of (1500 mg/kg IG) for 3 days (group 6) showed 66.7% mortality of animals on day 2 and 100% mortality of animals on day 3 of treatment. Administration of EGCG in daily doses of (1500 mg/kg IG) for 4 days in combination with a single dose of LPS (6 mg/kgIP) on day 4 (group 7) caused 100% mortality of animals within 4-6 hours post LPS administration.

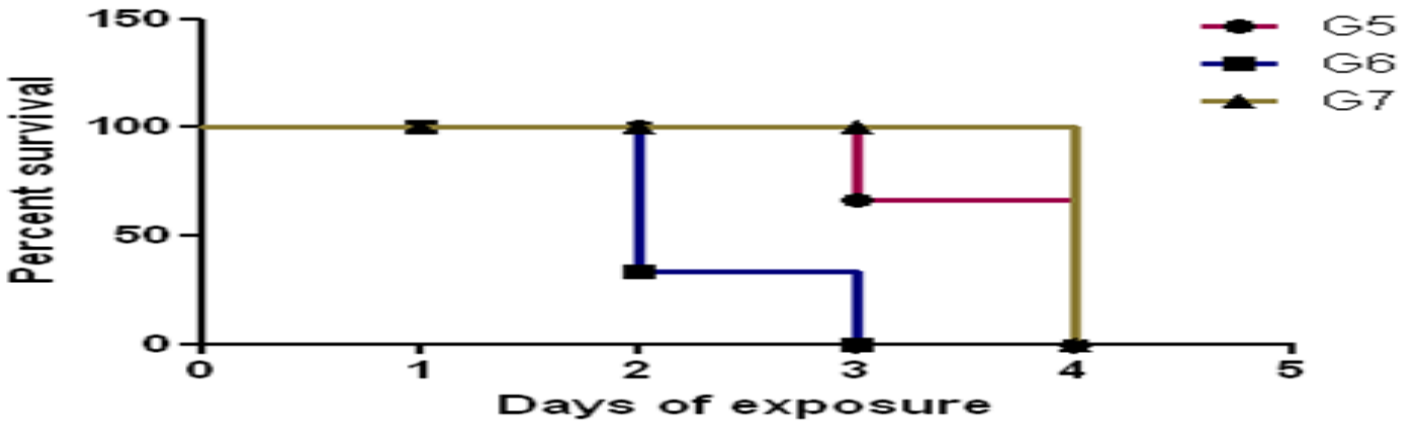

Figure No. 1: Survival of animals after treatment with high doses of EGCG with/without LPS

G5: Daily dose of EGCG (1500 mg/kg IG) for 5 days.
G6: Daily dose of EGCG (1500 mg/kg IG) for 5 days after a single dose of LPS (6 mg/kg IP).
G7: Daily dose of EGCG (1500 mg/kg IG) for 5 days + a single dose of LPS (6 mg/kg IP) on the 4th day.

- Data analysis was done usingKaplan-Meier test of survival.
- $P < 0.05$

1. Histopathology examination:

Histopathology of liver sections after treatment with different doses of EGCG intragastric and/or LPS intraperitoneal (IP) is shown in figure 2 :(G1) Vehicle treated group showed normal liver structure. (G2) LPS treated group showed minimal congestion of sinusoids. (G3) Animals that received 1500 mg/kg EGCG single dose IG showed degenerative hepatocytes and sinusoidal congestion. (G4) Animals that received 1500mg/kg EGCG single dose IG combined with one dose of LPS (6 mg/kg IP) showed sinusoidal congestion, increased vacuoles, increased number of degenerative hepatocytes and prominence of Kupffercells in hepatic lobules (Fig.2)

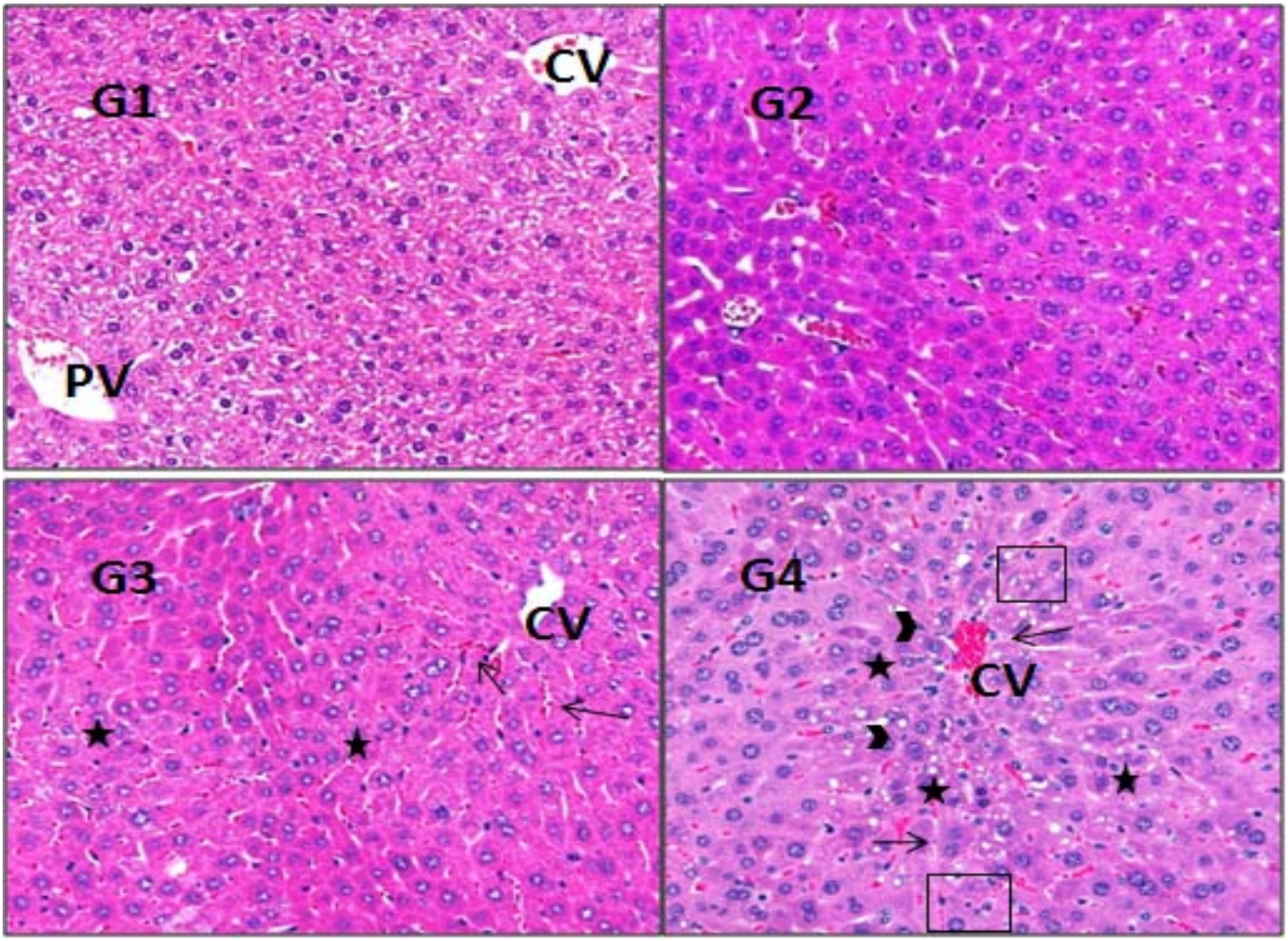

Figure No. 2: Histopathological evaluation of liver sections of mice after various IG treatments

Representative micrographs of liver sections from mice after various treatments with EGCGintragastric and/ or LPS intraperitoneal show different degrees of liver injury such as: congestion (arrows), vacuoles (arrow heads), degenerative hepatocytes (stars) and prominence of Kupffer cells and other inflammatory cells (rectangles).

G1: Vehicle 15% DMSO (IG).
G2: LPS one single dose (6 mg/kg IP).
G3: One single dose of EGCG (1500 mg/kg IG).
G4: A single dose of LPS (6 mg/kg IP) + a single dose of EGCG (1500 mg/kg IG).

DISCUSSION:

Green tea has been considered a medicine and a health fulbeverage since ancient times. The traditional Chinese medicine has recommended this plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and, in general, to prolong life [10]. Nowadays, green tea is considered one of the most promising dietary agents for the prevention and treatment of many diseases and consequently, it is being studied extensively worldwide [11]. Numerous studies in a variety of experimental animal models have demonstrated

that aqueous extract of GT and its catechins (EGCG, EGC, ECG and EC) possess skin protecting [12], antioxidant [13], antimutagenic [14], antidiabetic [15], anti-inflammatory [16], antibacterial, antifungal [17], antiviral [18], and above all, cancer-preventive [19] properties. Consumption of high doses of EGCG is found to have harmful effects on human health. Many reports show the exposure of subjects to marked liver toxicity in the form of acute hepatitis attributable to the consumption of GT containing supplements [20-22]. The toxic effect of pharmaceutical drugs or even natural products may be different if the health of the subject is compromised or affected by predisposing factor [23]. Endotoxins such as LPS of gram negative bacteria are among these predisposing factors that can alter hepatic detoxification mechanisms and can lead to liver injury [24]. LPS is known to decrease the toxic threshold of many chemicals and natural products [8,9]. In our study, single administration of EGCG at a high dose of 1500 mg/kg IG did not show significant liver pathology except for a low degree of sinusoidal congestion. No mortalities occurred in animals of the group treated with EGCG alone at this dose. However co-administration of EGCG IG at the same dose with a single IP dose of 6 mg/kg of LPS showed more lesions on histopathology such as increased vacuoles, increased number of degenerative hepatocytes and prominence of Kupffer cells in hepatic lobules. These observations did not appear in animals that were treated with either EGCG or LPS separately, neither was any deaths observed in this group of animals. These findings substantiate the impact of LPS as a predisposing factor in liver toxicity. This impact of LPS on EGCG induced liver injury is similar to many previous studies where LPS was combined with natural compounds such as Monocrotaline, the pyrrolizidine alkaloids of Crotalaria plant [8] and Usnic acid (UA), the natural botanical product which is a component of some weight reducing supplements [25]. In our study, IG administration of EGCG at 1500 mg/kg daily caused 100% mortality on day 4. This result is similar to those reported earlier where a single dose of 1500 mg/kg EGCG orally led to 85% mortality of mice over a period of 5 days [26]. Pre-administration of LPS in a single dose of LPS (6 mg/kg IP) followed by daily doses of EGCG caused 100% mortality on day 3. These results in addition to those of earlier studies underscore the impact of LPS on liver physiology. In conclusion, high doses of EGCG can initiate liver injury that can become severe under the influence of LPS or similar predisposing factors.

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