



Transient Hyperbilirubinaemia and Sustained Elevation of Alkaline Phosphatase after the Ingestion of *Lemongrass Tea*

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ABSTRACT

This study assessed the effect of lemongrass tea (LGT) on liver function indices in humans. One hundred and five participants ingested the LGT prepared from 2, 4, and 8g of LGL powder once daily for 30 days. Serum ALP level increased throughout the study period; although only the increase at day 30 reached statistical significance, while serum AST and ALT were not significantly different from baseline values. At days 10 and 30, serum total bilirubin significantly increased and decreased respectively. Ingestion of LGT may be associated with transient hyperbilirubinemia, and sustained elevation of ALP.

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Introduction

Herbal and homeopathic medicines are common in third-world regions and are gaining more popularity in industrialized nations [1]. Data exist suggesting a rise in the consumption of herbal medicines, including medicinal and aromatic plants. In fact, it is posited that >70% of the world population depends on herbal medicine for their healthcare needs [2], leading to the increased annual growth rate of herbal industries from 5% to 15% [3,4]. Currently >200 000 natural products of plant origin are known and are used, and many more are being identified from higher plants [5,6,7].

However, <10% in the world market is truly standardized to known active/toxic components [8]. In addition, the lack of a systematic approach to assess their efficacy, safety, and potential toxicity limits their use. The existing growing interest in alternative medical practices underlines the need for more thorough investigation into the safety and efficacy of herbal therapies. Plant food-based nutritional studies are required to assess the effect of plants on human health and well-being to exploit the full therapeutic and nutritional potential of these natural products.

Recent advances in phytopharmacology have provided a greater insight into the therapeutic and toxic potential of some herbs and natural plant products. Several hundred plants and herbs initially considered to be less detrimental to health are now showing evidence of interference with some bodily functions, including liver function. A typical example is the Chinese herbal product Jin Bu Huan, which has been used for >1000 years for its sedative, analgesic, and antispasmodic properties without any evidence of hepatotoxicity, but was thereafter discovered to possess acute and chronic hepatotoxic potential with resultant chronic hepatitis and liver fibrosis [9,10].

Existing literature indicates that drug-/herb-induced hepatotoxicity is one of the significant causes of liver failure, accounting for approximately 50% of cases [11], and remains a major reason for drug withdrawal from pharmaceutical development and clinical use [12]. Also, herbal hepatotoxicity and associated complications are common and suggest the need for a continuous toxicity screening of some commonly consumed herbs/vegetables such as lemongrass (*Cymbopogon*

citratus), which has been extensively consumed in many countries for >2000 years [13].

Lemongrass (LG) is a perennial robust herb [13] of the Poaceae family, with long slender leaves of about 90 cm in length and 1.5 cm in breadth. The leaves give off a slight aroma of ginger when crushed and contain various bioactive constituents, including phytochemicals such as saponins, tannins, and flavonoids; minerals such as calcium, copper, manganese, and selenium; electrolytes such as potassium (K⁺) and sodium (Na⁺); and vitamins such as A, C, E, and folate [14]. Other constituents include macronutrients such as carbohydrates, protein, and fat, as well as variable concentrations (75%-85%) of essential oil (EO) and constituents.

In several animal models of experimentally induced hepatotoxicity, prophylactic administration of LGL extracts has time and dose dependently mitigated liver damage as evidenced by improvement in biochemical markers of liver function (AST), (ALT), (ALP) and histopathological features [15,16,17].

Common features of these studies include animal-based designs. The relevance of these animal studies to humans is questionable. In addition, reports on serum bilirubin and other markers of bilirubin metabolism in the body are lacking. Empirical data on the effect of LG ingestion on markers of hepatic function and bilirubin metabolism would be critical for a better understanding of the effect of LG on the human hepatobiliary system.

The aim of this study was to accurately evaluate the effect of infusion prepared from LGL powder on hepatobiliary system markers.

Materials and Methods

Study Design

A total of 105 volunteers aged 18 to 35 years who were selected by a simple random technique participated in this study. Informed written consent was obtained from all participants. All participants underwent a thorough pre-survey medical screening performed by a Medical Doctor to ensure medical fitness and to exclude those who did not meet the inclusion criteria. The exclusion criteria are as follows: inappropriate age, a history of kidney or liver disease, failure to satisfy the pre-research clinical and biochemical

assessment, pregnancy or lactation, allergy to any lemongrass constituents, and use of drugs known to affect or to be metabolized primarily in the liver. Screening included determination of medical history, lifestyle assessment (such as smoking, drinking, physical activity, diet, and drug history), BP and heart rate (HR), weight, blood glucose level, full blood and platelet count, and urine and blood indices of renal and hepatic function. The participants were advised to avoid excessive physical activity and ingestion of drugs or alcohol, and to remain on their regular diet throughout the study period. The study protocol was approved by the Institutional Research Ethics Committee, and the study was conducted at the University of Uyo, Nigeria, according to the rules set forth in the Declaration of Helsinki governing the conduct of human research.

Preparation of plant materials the Infusion of lemongrass leaves

Fresh LG leaves were obtained from an agricultural farm in Uyo, Akwa Ibom State, Nigeria few days prior to utilization, in the month of May 2012. The leaves were identified and authenticated by a taxonomist in the department of Botany at the University of Uyo. Voucher specimen NO. UUH3276/UYO was deposited at the herbarium in the department of Botany of the University. The leaves were rinsed, sun-dried, and pulverized into powder using electric blender to give a gram weight of 200g. This was soaked in a container with 2 liters of hot water and allowed to stand for about 8 hours. Thereafter the solution was filtered using No. 2 Whatman filter paper. The filtrate was evaporated by heating in water bath at 40°C to obtain the solid extract. The solid extract was weighed with an electric weighing balance (ACS-ZE14, Surgifriend Medicals Ltd, England) to obtain a yield of 70 grams (35% w/w), which was stored in clean bottles at room temperature until required for use. Similar procedures were repeated using 2, 4 or 8g of LG powder and yields of 410mg, 810mg and 1570mg extract were obtained respectively.

Determination of bioactive natural constituents from lemongrass leaves extract

The phytochemical analysis of the extract was carried out using standard procedures to determine the levels of saponins, phenolics, alkaloids, tannins, flavonoids, glycosides, steroids, deoxy sugars, and anthraquinones [18,19].

The nutrient constituents of the lemongrass leaf extracts was evaluated using standard procedures as described previously [14].

Dose Determination and Administration of Infusion

The participants were subdivided into 3 groups (n=35/group). Groups 1, 2 and 3 received infusions prepared from 2, 4 or 8g of LGL powder in 150ml of hot water respectively, given once daily for 30 days. This infusion was prepared in this pattern to correspond to the pattern usually employed by the population to prepare lemongrass tea [20]. The dose range was adapted from previous human studies [20,21], in which participants exhibited no obvious clinical or biochemical evidence of toxicity. To further confirm the safety of this dose range, the investigators of the present study conducted a pilot survey on 10 human volunteers using 2, 4, 8 and 10g of LGL powder. Participants who had received at least a dose of the infusion prepared from 2, 4, or 8g of LGL powder in equal volume of hot water (150ml) daily for 1 week showed no evidence of adverse/toxic effect, as judged by the results of the tolerability evaluation.

The evaluation consisted of determining clinical and laboratory test abnormalities (liver function test-aminotransferase activities) and renal function test serum

creatinine and clearance rate (Cr), serum urea levels and hematological indices. Physical examinations were also performed on each participant to check for presence of jaundice or pallor (evidence of hepatotoxicity or hemolysis) as well as abnormal skin reaction. Adverse events reported by the participants or observed by the investigators during the clinical evaluation were recorded. Also, participants were closely monitored during the period of the study. They were given a daily symptomatology and fluid intake assessment questionnaire which contained 25 questions and was designed by the author. The presence of symptoms such as; lightness of the body, blurring of vision, insomnia, headache, dizziness, sweating, frequent micturition, belching, dyspepsia, diarrhea, constipation, vomiting and conscious awareness of fast heart beating (palpitation) were to be reported.

Two grams of LG leaves powder was chosen as the starting dose to correspond to the quantity usually employed by the Brazilian population to prepare their lemon grass tea [20]. Studies by others have shown that in Brazil, LGT, prepared by pouring 150ml of boiling water over 2-3g fresh or dried leaves of LG, is one of the most popular remedies in their folklore for treating "nervous disturbances such as insomnia, irritability and anxiety" [20,22]. In order to evaluate dose effect, the 2g was doubled and quadrupled, to give 2, 4 or 8g of LGL powder used in the present study.

Biochemical Analysis

Venous blood samples were collected at days 0, 10 and 30 and used for biochemical analysis. The parameters measured included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin (total and conjugated) using automatic analyzer (Reflotron (R) plus system, Roche, Germany).

Statistical Analysis

Data (Mean \pm SEM) were analysed using one-way analysis of variance (ANOVA) followed by pair-wise comparison using the least significant difference (LSD) test. Differences were considered statistically significant at $P < 0.05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS 20.0).

Results

The baseline demographic and clinical characteristics of the study participants showed that most of them (52%) were males with ages between 18 and 35 years and with Ibibio ethnicity. The mean weight, BMI, SBP, DBP, MAP, and HR were 60.7 ± 1.93 kg, 23.5 ± 0.75 kg/m², 120.5 ± 1.89 mmHg, 74.6 ± 1.6 mmHg, 85.7 ± 1.13 mmHg and 77.7 ± 1.99 beats/min, respectively, whereas the mean PP, mean respiratory rate (RR), and eGFR were 45.9 ± 1.04 , 18.6 ± 1.52 /min and 99.9 ± 1.52 ml/min, respectively. The phytochemical constituents of LGL extracts revealed a relatively high concentration of saponins; moderate concentrations of tannins, flavonoids, and phenols; and relatively low concentrations of alkaloids, deoxysugars, and anthraquinones. Serum total bilirubin significantly increased in subjects treated with LGT prepared from 2g LGL powder at day 10 ($p < 0.05$). Other markers of liver function were not significantly different from baseline values. At day 30, a significant increase in serum ALP was obtained ($p < 0.05$). Serum albumin (ALB) total bilirubin and direct bilirubin significantly decreased from baseline values. Serum total protein, ALT, AST were not different from baseline values (Table 1).

Table 2 shows that at day 10, participants treated with LGT prepared from 4 g of LGL powder had a significant increase in serum total bilirubin ALP and protein, compared with baseline values. Serum direct bilirubin significantly

decreased ($p < 0.05$), whereas serum levels of AST, ALT and ALB were not significantly different from baseline values. At day 30, serum total bilirubin, direct bilirubin and ALB significantly decreased ($p > 0.05$), whereas ALP significantly increased ($p < 0.05$) compared with baseline values. Serum AST, ALT and total protein were not significantly different from baseline values.

Table 3 shows that at day 10, participants treated with LGT prepared from 8g of LGL powder had a significant increase in serum total bilirubin, whereas direct bilirubin, AST, ALT, ALP, total protein and albumin were not significantly different from baseline values. At day 30, serum total bilirubin, direct bilirubin, AST and albumin significantly decreased ($p < 0.05$). The significant increase in ALP observed at lower doses was sustained, whereas serum ALT and total protein were not different from baseline values.

Discussion

The present study findings showed that the consumption of LGT was associated with a significant dose-dependent increase and decrease in serum total bilirubin levels at days 10 and 30, respectively, while a sustained increase in serum ALP was observed in both study phases. However, only the increase at day 30 reached statistical significance. Serum aminotransferases (AST and ALT) were not significantly different from baseline values.

These findings are similar to those seen in acute cholestatic liver disorders [23]. Although previous studies in models evaluating hepatotoxic potentials of LG leaf/stem extracts found slight elevation of direct bilirubin and amylase in humans [20] and an increase in total bilirubin in animals [24], the absence of increased ALP in these studies failed to support cholestasis. In another animal study by Tarkang et al, [13] treatment of animals for 28 and 90 days with ethanolic and aqueous extracts from LGL showed biochemical and histopathological changes suggestive of mild hepatotoxicity at higher doses for both extracts (aqueous and ethanolic), and in particular on prolonged administration of the aqueous extract. The inconsistent results across studies may be due to the confounding effects of individual susceptibility factors leading to differences in the pharmacokinetic and pharmacodynamic properties of LG secondary metabolites. Also, differences in the inherent properties of the plant, such as the chemical constituents, which can vary by geographic region, genetic differences, the part of plant used for extraction, the method of extraction, experimental conditions, the age/stage of maturity, the season of harvest, and even the health status of the plant [25,26,27,28,29,30], can impact results.

The biochemical findings of this study (including the mild-to-moderate increases in ALP at days 10 and 30, the significant fluctuations in serum total bilirubin at days 10 and 30, and the non-significant changes in serum aminotransferase throughout the study period, as well as the absence of clinical evidence of hepatic disorder, suggest a less severe and self-limiting/reversible type of acute cholestatic liver disorder (bland cholestasis) [31]. This differs from a severe form of portal chronic inflammation characterised by hepatocellular cholestasis, and markedly raised levels of these enzymes [31].

Although the mechanisms by which LGL extracts induce biochemical indices suggestive of mild cholestasis and, hence, hyperbilirubinaemia have not been previously elucidated, the results of phytochemical analysis of the extract in this and previous studies [32,33,34] indicate the presence of several bioactive natural constituents, including saponins, tannins, flavonoids, phenolics, anthraquinones, alkaloids, proteins,

electrolytes, citral, and monoterpenoid compounds. Accumulating data suggest the role of bioactivation of some of these constituents with a resultant formation of reactive metabolites/intermediates, which subsequently interfere with the enzyme systems involved in bile synthesis, transport, metabolism, and excretion. Such enzymes include glutathione (GSH) synthesizing enzymes, $\text{Na}^+\text{-K}^+$ adenosine triphosphatase (ATPase), angiotensin-converting enzyme (ACE) system, and cytochrome P_{450} enzyme system. Regarding the effect of these constituents on $\text{Na}^+\text{-K}^+$ ATPase, it is now known that some of these constituents (saponins, tannins, flavonoids, phenolic compounds, and K^+) can induce diuresis and natriuresis through the inhibition of $\text{Na}^+\text{-K}^+$ ATPase activity by directly binding the enzyme, impairing its activity or causing changes in membrane fluidity [30,35,36]. Such interference with sinusoidal membrane fluidity and $\text{Na}^+\text{-K}^+$ ATPase activity is known to cause bland cholestasis, leading to decreased hepatocellular uptake of bile acids from the sinusoidal blood [37].

Additionally, inhibition of ACE could partly contribute to the observed biochemical markers of cholestasis because some of the aforementioned constituents (especially the polyphenolic compounds) and other antioxidant constituents present in LGL extracts, are known ACE inhibitors (ACE-I) and possess the potentiality to inhibit ACE in vitro [38,39,40]. Such inhibition has been shown by many investigators to be associated with cholestasis [37,41,42,43]. The hypothesized pathophysiological mechanisms of such inhibition include idiopathic hypersensitivity and inhibition of bradykinin activation, leading to arachidonic acid release and conversion into prostaglandins (e.g. 16, 16-dimethyl prostaglandin E_2) that reduces bile flow and, hence, cholestasis. Such stasis could lead to hepatocellular and biliary toxicity due to increased concentration of leukotrienes. ACE inhibitors can also induce liver disorder through their inhibitory actions on ACE-2, which is known to act as an endogenous negative regulator/brake to the detrimental effects of renin-angiotensin system in causing tissue remodelling and scarring.

Generation of angiotensin II (Ang II) is the speculated pathogenic action. ACE-2 converts this toxic Ang II to Ang_{1-7} , which is less harmful. Downregulation of ACE-2 by LG secondary metabolites and ACE-inhibitory peptides in LG extract may decrease the endogenous ACE-2 levels and, hence, activity leading to liver pathology. Conversely, upregulation of ACE-2 has been shown to limit hepatic fibrosis [44]. Moreover, previous studies [45,46,47] have collectively shown that the EO from LG has the high potentiality to inhibit cytochrome P_{450} enzyme isoform due to its high aldehyde content.

Accordingly, Chen et al [47] demonstrated the inhibitory effect of LG EO on $\text{CYP}2\text{E}_1$, whereas others have shown the inhibitory effect of β -myrcene in LG EO on liver $\text{CYP}_{450}\text{-2B1}$ isoform. These two enzymes are involved in the excretion of a variety of metabolites from phase II detoxification pathways, including bilirubin diglucuronide and glutathione conjugates [48]. Their inhibition has been positively correlated with a reduction in messenger ribonucleic acid (mRNA) expression of GSH-synthesizing enzymes and hepatic mRNA transport protein (MrP^2), leading to reduced protection against oxidative stress and reduced bile acid-independent bile flow [48]. Downregulation of MrP^2 could lead to accumulation of a cholestatic reaction [49]. The association of inhibition of CYP_{450} isoforms with cholestasis has been well established in the literature. In a study by Tanaka et al [50], inhibition of CYP_{450} caused cholestasis and a large increase in serum bilirubin (47-fold), total bile acid (8-fold), and ALP (2.5-fold).

George et al [51] showed low activity of CYP_{2E1} in patients with cholestatic liver disease.

The significant decrease in serum bilirubin at day 30 may in part be attributable to the effect of cholehepatic shunting, which is known to constitute an alternative escape route for bile salt following cholestasis and entails the flow of bile salt from the bile duct lumen via cholangiocytes and the peritubular plexus. Under normal physiological conditions, cholehepatic shunting probably constitutes a minor pathway for bile salt escape, but may constitute a major escape route for bile salt under cholestatic conditions when the bile duct epithelium proliferates. In addition, there is increased bile salt uptake into cholangiocytes and gallbladder epithelial cells in cholestasis. Such an increase is known to initiate cell signalling in the regulation of secretory and proliferatory events within the biliary tree, and can account for the decrease in serum bilirubin in the sub-chronic phase of the study. Similarly, ACE inhibitors associated with cholestatic liver disorder are usually self-limiting and may resolve once the offending substance is removed.

Furthermore, Chen et al [47] have shown that the inhibitory effect of LG on cytochrome CYP₄₅₀2E1 is reversible. Collectively, these effects can partly account for the significant decrease in serum bilirubin in the sub-chronic phase of this study.

Table 1. Acute and sub-chronic effects of lemongrass tea prepared from 2g of lemongrass leaf powder on serum markers of hepatic function.

Parameters	Baseline	10 Days	30 Days
Total Bil (µmol/L)	9.59 ± 0.68	12.57 ± 0.95 ^a	5.89 ± 0.40 ^{ab}
Direct Bil (µmol/L)	5.23 ± 0.39	4.48 ± 0.33	3.84 ± 0.36 ^a
AST (µ/L)	12.94 ± 0.58	11.17 ± 0.87	10.83 ± 0.93
ALT (µ/L)	7.40 ± 0.42	8.06 ± 0.33	8.71 ± 0.61
ALP (µ/L)	23.34 ± 0.63	24.34 ± 0.59	31.51 ± 1.95 ^{ab}
Total protein g/dl	67.09 ± 0.53	68.69 ± 0.66	67.63 ± 0.72
Albumin g/dl	42.66 ± 0.49	42.86 ± 0.43	40.17 ± 0.14 ^{ab}

a = significantly different from baseline.

b = significantly different from 10 days post treatment.

Table 2. Acute and sub-chronic effects of lemongrass tea prepared from 4g of lemongrass leaf powder on serum makers of hepatic function.

Parameters	Baseline	10 Days	30 Days
Total Bil (µmol/L)	12.55 ± 0.93	15.83 ± 1.32 ^a	5.33 ± 0.72 ^{ab}
Direct Bil (µmol/L)	7.46 ± 0.78	5.68 ± 0.50 ^a	2.95 ± 0.33 ^{ab}
AST (µ/L)	12.00 ± 0.42	13.20 ± 1.49	11.00 ± 0.89
ALT (µ/L)	7.87 ± 0.47	8.74 ± 0.59	7.49 ± 0.57
ALP (µ/L)	22.54 ± 0.58	24.71 ± 0.61 ^a	30.80 ± 2.13 ^{ab}
Total protein g/dl	67.11 ± 0.62	69.23 ± 0.75 ^a	68.29 ± 0.60
Albumin g/dl	43.46 ± 0.57	42.89 ± 0.58	40.80 ± 0.34 ^{ab}

a = significantly different from baseline

b = significantly different from 10 days post treatment

Table 3. Acute and sub-chronic effects of lemongrass tea prepared from 8g of lemongrass leaf powder on serum markers of hepatic function.

Parameters	Baseline	10 Days	30 Days
Total Bil (µmol/L)	10.17 ± 0.17	19.32 ± 2.17 ^a	4.34 ± 0.24 ^{ab}
Direct Bil (µmol/L)	5.57 ± 0.49	6.33 ± 0.67	2.34 ± 0.17 ^{ab}
AST (µ/L)	12.54 ± 0.60	10.63 ± 1.09	7.80 ± 0.95 ^{ab}
ALT (µ/L)	7.57 ± 0.59	8.31 ± 0.42	7.49 ± 0.54
ALP (µ/L)	23.23 ± 0.42	23.83 ± 0.76	28.77 ± 1.45 ^{ab}
Total protein g/dl	67.77 ± 0.88	68.71 ± 0.68	67.57 ± 0.49
Albumin g/dl	42.31 ± 0.56	42.71 ± 0.46	40.14 ± 0.44 ^{ab}

a = significantly different from baseline

b = significantly different from 10 days post treatment

The non-significant changes in serum liver enzyme levels observed in the present study at days 10 and 30 provide additional supportive evidence to the previously postulated hepatoprotective effect of LGL extracts. However, ingestion

of LGT may be associated with reversible hyperbilirubinemia due to its varied bioconstituents and their activities.

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Conflict of Interest: None

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