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COMPARISON OF CATECHIN PROFILES (*CAMELLIA SINENSIS*) IN ALCOHOLIC SMOKERS' PLASMA AND URINE

Thippannagari Lokesh [*1], Chikati Rajasekhar [2], M. Abdul Kareem [3] and N. Ch. Varadacharyulu [4]

Department of Medical Sciences [1], NIEPID, Secunderabad - 500009, Telangana, India.
Department of Biochemistry [2], Yogi Vemana University, Kadapa - 516005, Andhra Pradesh, India.
Department of Biochemistry [3], IGNOU, Maidan Garhi - 110068, New Delhi, India.
Department of Biochemistry [4], Sri Krishnadevaraya University, Anantapur - 515003, Andhra Pradesh, India.

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Correspondence to Author:
Thippannagari Lokesh

Senior Biochemistry Technician, Department of Medical Sciences, NIEPID, Secunderabad - 500009, Telangana, India.

E-mail: lokeshthippannagari@gmail.com

ABSTRACT: Cigarette smoking and excessive chronic alcohol consumption are accepted risk factors for the causation of various diseases and disorders. Globally 2.5 billion alcoholics and 2.0 billion smokers exist (WHO, 2018). The comorbidity of alcohol and cigarette smoking is highly prevalent. This study is designed to investigate the influence of green tea an effective therapeutic agent to treat the damage caused by the co-use of cigarette smoking and alcohol in humans. Green tea, a water infusion prepared from the dried leaves of *Camellia sinensis*, is one of the most popular beverages in the world. The major tea catechins are Epigallocatechin-3-gallate (EGCG), Epicatechin (EC), Epicatechin gallate (ECG), Epigallocatechin (EGC). This study had a participation of 150 healthy adult male volunteers between 35 to 65 years of age and weighing between 50 to 75 Kg. In this study, EGCG catechin metabolites were detected in the plasma and urine of human volunteers after supplementation of green tea. The served green tea showed the concentrations of various catechins especially EGCG (81.24%), ECG (1.13%), EGC (1.57%), EGCG-3Me (0.36%) and EC (0.96%) present in the beverage. HPLC chromatograms of tea catechins and their metabolites in human found as higher in plasma where EGCG was recorded in smokers with green tea intake (45.4 nmol/L/100 µL) in comparison with alcoholics (18 nmol/L/100 µL), alcoholic smokers (21.2 nmol/L/100 µL) and control subjects (19.4 nmol/L/100 µL).Similarly, urinary excretion of catechins/degradatory products were more in alcoholics with green tea (128.9 nmol/L/100 µL) than other groups viz., smokers, alcoholic smokers and controls with green tea intake.

INTRODUCTION: Green tea is one of the most popular beverages consumed worldwide. However, people consume tea in different forms *viz.*, black tea, oolong tea, yellow tea, white tea, pu'erh tea and green tea in different parts of the world [1].

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Among this green tea has the most significant effects on human health like hypotensive, detoxification, lipid-lowering and anti-to the carcinogenic, inflammatory and obesity [2-10].

Green tea can effectively modulate the antioxidant capacity mainly in people subjected to oxidative stress and could improve the metabolism of glucose, lipid and uric acid [11]. Cigarette smoking and chronic alcohol consumption are risk factors for the causation of various diseases and disorders [12]. It is clear from the literature that green tea

constituents are excellent in combating with various pathologies associated with diseases, in particular, cardiopulmonary disorders and chronic diseases such as diabetes [10, 11]. Globally adult smoking prevalence in 2020 was 32.6% and 6.5% among men and women [13]. Green tea containing high quantities of polyphenolic components *viz*., EGCG, EC, ECG, EGC which have protective and therapeutic properties [14]. Green tea catechins comprise 80 to 90% of total flavonoids, with EGCG, being the most abundant catechin 48–55%, followed by the other catechins, EGC; circa 9–12%, ECG; circa 9–12%, and EC; circa 5–7% [15, 16]. These flavanoids are found in greater amounts in green tea [17]. The chemical structures of catechins presents in green tea are shown in **Fig. 1.**

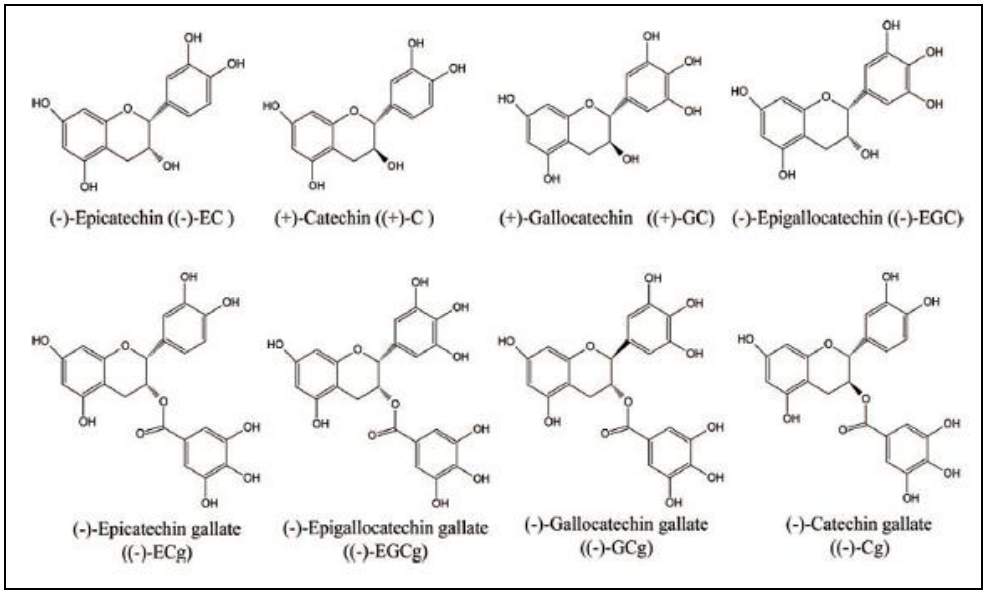


FIG. 1: CHEMICAL STRUCTURES OF CATECHINS PRESENT IN GREEN TEA. (Adopted from Kenichiro Otake *et al.*, 2015) [18]

This study chiefly focuses on the adverse health effect due to the alcohol and cigarette smoking and also to evaluate the role of green tea intake in human volunteers. The flow chart provided at **Fig. 2** will illustrate the harmful effects of alcohol and smoking.

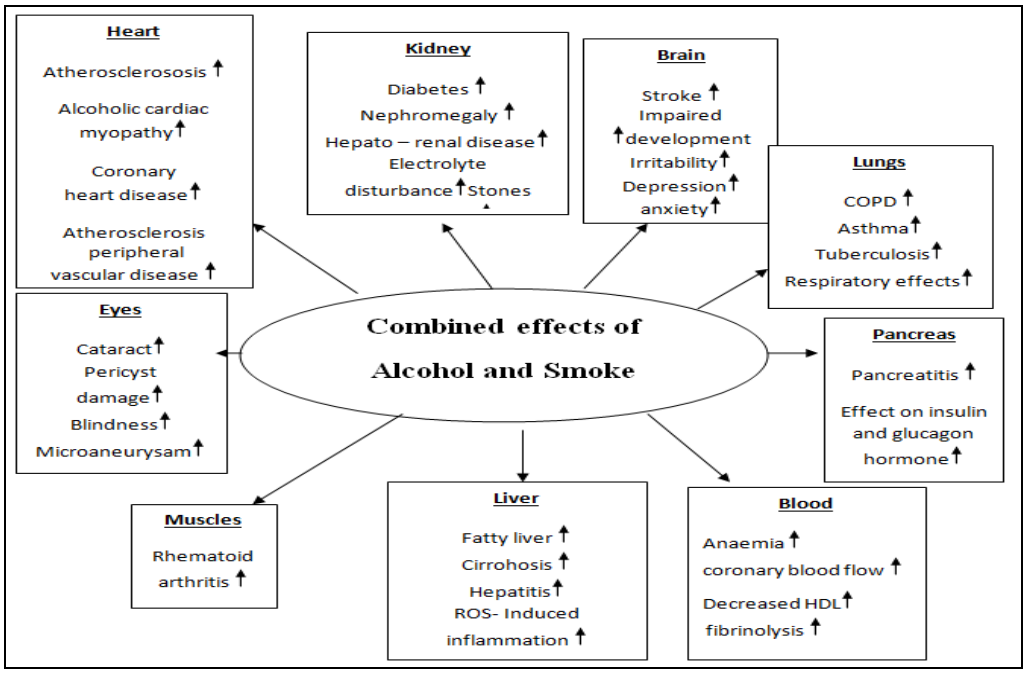


FIG. 2: SCHEMATIC REPRESENTATION OF EXACERBATION OF ALCOHOL INDUCED DAMAGE BY CIGARETTE SMOKE ON ALL BODY PARTS

MATERIALS & METHODS:

Experimental Subjects: One hundred fifty human male volunteers aged between 35 to 65, residing in Anantapuramu, Andhra Pradesh, India taking local diet, categorized into five groups **Table 1**. Each group consists of 30 human male volunteers were selected based on the certain baseline characteristics provided by them in a questionnaire **Table 2.** This study was approved by the Institutional Ethical Committee. All the volunteers were well explained about the experimentation and their written consent was obtained. Plasma and urine samples collected from human volunteers and were used immediately for biochemical analysis.

TABLE 1: GROUPS

Group I	Control (non-alcoholics and non-smokers without green tea)
Group II	Control (non-alcoholics and non-smokers with green tea)
Group III	Smokers (who consume more than 7-15 cigarettes/day and with green tea)
Group IV	Alcoholics (who consumed 40-120 g alcoholic beverage/day and with green tea)
Group V	Alcoholic Smokers (who smoke more than 12 -18 cigarettes/day with alcoholic beverage with green tea)

Ethical no.01, dated 27/06/2018 version 1.1

TABLE 2: BASE LINE CHARACTERISTICS OF THE SUBJECTS

Parameter	Group I Controls Without green tea	Group II Controls with green tea	Group III Smokers With green tea	Group IV Alcoholics With green tea	Group V Alcoholic smokers With green tea
No of Volunteers for each group	30	30	30	30	30
Age	35-65	35-65	35-65	35-65	35-65
Height (cm)	145-215	145-215	150-240	140-225	145-235
Weight (kg)	50-75	50-75	50-85	52-80	55-75
Socioeconomic status (per month in Rupees)	8,000-45,000	8,000-45,000	8,000-45,000	8,000-45,000	8,000-45,000
Green tea consumption (3 months)	No	Yes	Yes	Yes	Yes
Smoking history	NO	NO	For the past 7 years	No	For the past 7 years
Number of Cigarettes consumed per day	No	No	7-12	No	14-18
Alcohol historyfor the past 7 years and above	No	No	No	Yes	Yes
Alcohol consumed Or 40-50% alcohol containing alcoholic bevarages	No	No	No	3-4 *drinks per day	3-4 *drinks per day

(* One standard drink consists of ~15g of ethanol)

Preparation of Green Tea and Supplementation: Green tea (*Camellia sinensis)* leaves were obtained from Kanan Devan Hills Plantations Company (P) Limited, Munnar, Kerala, India. The leaves were air-dried at room temperature, powdered and stored in air-tight glass containers and protected from sun light until required for analysis. To 100 ml of mineral water, 1.5 gm of green tea leaf powder was added and boiled at 60°C temperature for 10 min. The liquid extract was separated by filtration and the filtrate was collected into a cup (100 ml) and the contents (tea) were mixed for 2 min and consumed by volunteers twice a day Once in the morning and again in the evening) for 3 months. After careful examination of earlier reports and consultations with physicians the dosage and duration in the present study were fixed. In earlier green tea supplementation studies, the dosage used and length of duration (or) period of supplementation were at the discretion of scientists' [19, 20, 21, 22]. In present study the volunteers received green tea twice a day for 3 months. Because this duration and dosage were found to be ideal and feasible for present experimentation.

Analysis for Specific Constituents and Therapeutic Efficacy of Green Tea: Green tea contains specific constituents of catechins were identified by HPTLC using the method of Amarowicz and Shahidi [23]. Crude catechins were extracted from 50 g of green tea leaves using 500 ml of hot water (80°C) under stirring over a 1 h period [24]. Separation and purification of catechins was done by the method of Tanizawa [25]. Chromatograms (TLC plates) were developed using various mobile phase systems in a

chromatographic chamber (12 × 10 × 8 cm; Sigma) until the solvent front advanced 8 cm. The spots were located on the plate under UV lamp.

Analysis of EGCG Concentration in Plasma and Urine: Venous blood samples were collected in EDTA-coated tubes from subjects. Urine samples were collected 3hrs after intake of green tea in the morning. The samples were processed immediately for further analysis. Plasma and urine samples were analyzed by adaptating of the method of Lee [26]. The dried sample was reconstituted with 200 mL of 10% (v/v) acetonitrile in 0.04 mol/L EDTA, centrifuged for 15 min at 16000 X 3 g and 50 mL were assayed by HPLC. Urine samples were processed as described above for plasma with the omission of the methylene chloride/water extraction step.

RESULTS & DISCUSSION:

Concentrations of Catechins in Served Green Tea: Green tea has drawn significant attention due to its increased health benefits such as antioxidant, anti-inflammatory, anticancer properties, protection against cardiovascular, kidney and other diseases [27, 28, 29, 30, 31].

Green tea constituents have the ability to ameliorate the toxicities induced by chemicals such as pesticides, carbon tetra chloride (CCl_4), as well as drugs and toxicants. These catechins exert antioxidant and therapeutic properties [32].

Green tea is rich in catechins (EGCG, ECG, EGC and EC) [2, 3, 33]. In this study the green tea has been analysed for individual catechin *viz.*, EGCG, ECG, EC, EGC using HPTLC.

Fig. 3 provides the information on separated catechins of the served green tea. Data in **Table 3** showed the concentrations of various catechins especially EGCG (81.24%), ECG (1.13%), EGC (1.57%), EGCG-3Me (0.36%) and EC (0.96%) respectively present in the green tea.

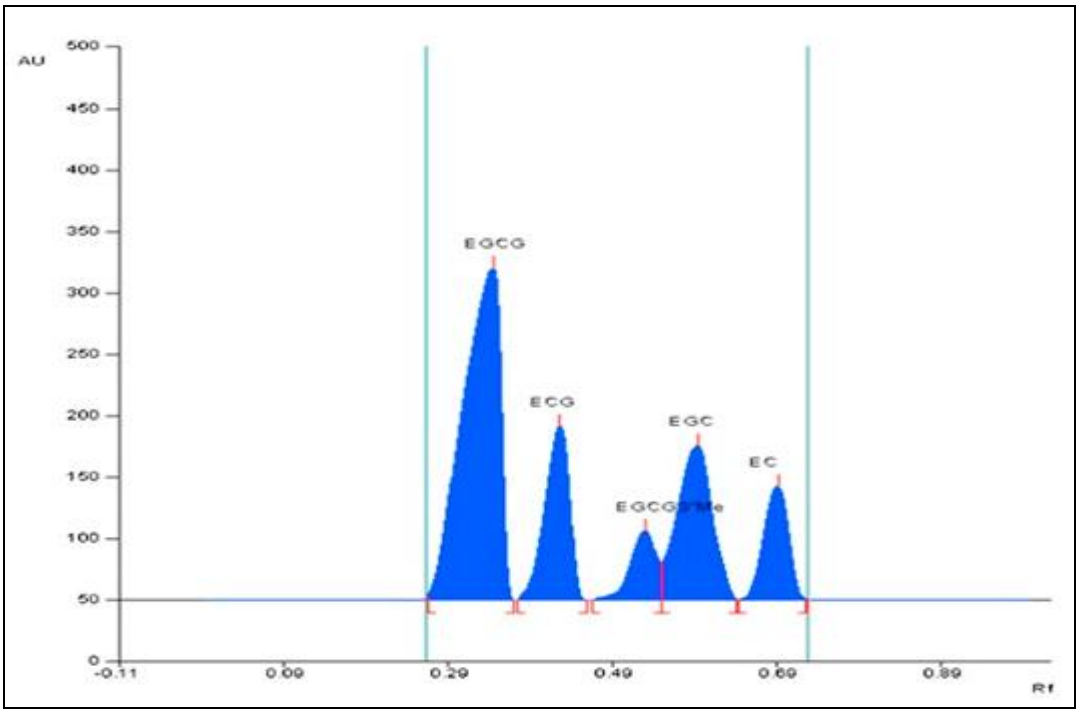


FIG. 3: SEPARATION OF CATECHINS FROM GREEN TEA AQUEOUS EXTRACT BY HPTLC

TABLE 3: RELATIVE MIGRATION OF GREEN TEA CATECHINS

Green tea Catechins	Relative migration (Rf)		Area	Concentration of catechin (%)
	Start	End		
Epigallocatechin-3-gallate (EGCG)	0.29	0.35	36820.6	81.24
Epicatechin gallate (ECG)	0.36	0.47	510.4	1.13
Epigallocatechin gallate (EGCG 3 Me)	0.49	0.52	163.4	0.36
Epigallocatechin (EGC)	0.52	0.64	710.6	1.57
Epicatechin (EC)	0.65	0.69	437.0	0.96

Chromatography Studies of Catechins on Experimental Subjects in Plasma (Human Male Volunteers): It is clear that there is an established relationship between cigarette smoking and rapid acute/chronic renal dysfunction and also between alcohol consumption and renal damage [33].

However there is paucity of information on renal damage in cigarette smokers using alcohol. Potential mechanisms of cigarette smoking induced renal damage are suggested by Orth and Hallan [34].

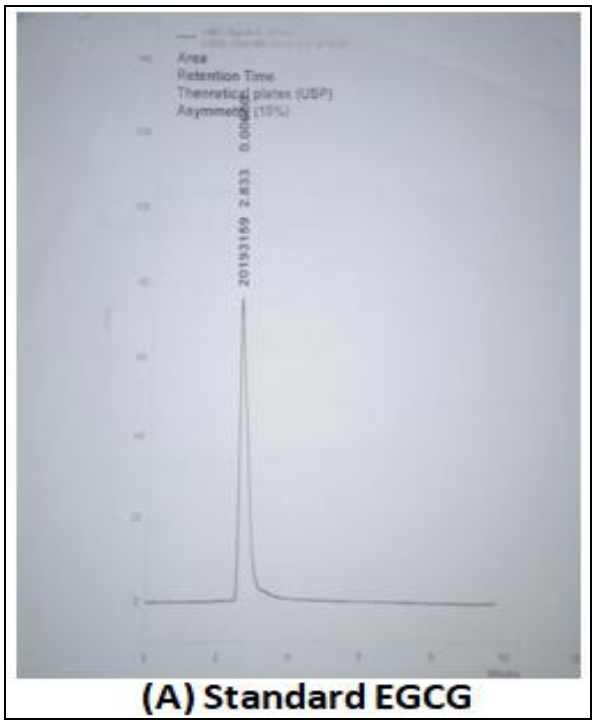
Hence, in present study HPLC quantification of plasma and urine EGCG levels in experimental groups compared with controls **Table 4** and **Table 5**. The chromatograms obtained from standard EGCG and of samples were shown in **Fig. 4-5**. In this study higher plasma concentration of EGCG was recorded in group III (45.4 nmol/L/100 µL) in comparison with Group IV (18 nmol/L/100 µL), Group V (21.2 nmol/L/100 µL) and Group II subjects (19.4 nmol/L/100 µL) **Table 4**.

These results showed that substances used in either cigarette smoke or alcohol use caused a significant increase in plasma EGCG concentration within stipulated time. Though Fung observed 10 fold increases in plasma after 1hr post ingestion [35].

Yang observed 2.7 fold increases in plasma EGCG in normal subjects in comparison with non green tea users [36]. In this study we observed 2-fold increase in plasma EGCG in controls. The difference is mainly due to the concentration of catechins administered and recorded timings of sampling were also different. Best to our observation we would be the first reporting plasma EGCG hike in smokers, alcoholics and alcoholic smokers when compared with controls.

TABLE 4: HPLC QUANTIFICATION OF PLASMA EGCG CONCENTRATIONS IN STUDIED HUMAN VOLUNTEERS

Groups	Retention time (min)	Concentration of EGCG nmol/L/100 µL	Peak height (Micro volts)
Green tea aqueous extract	2.79	15.08	1682657
Group I	3.30	10.21	385192
Group II	2.76	19.4	1880327
Group III	3.11	45.4	2079766
Group IV	2.79	18.0	1711528
Group V	2.75	21.2	2329494



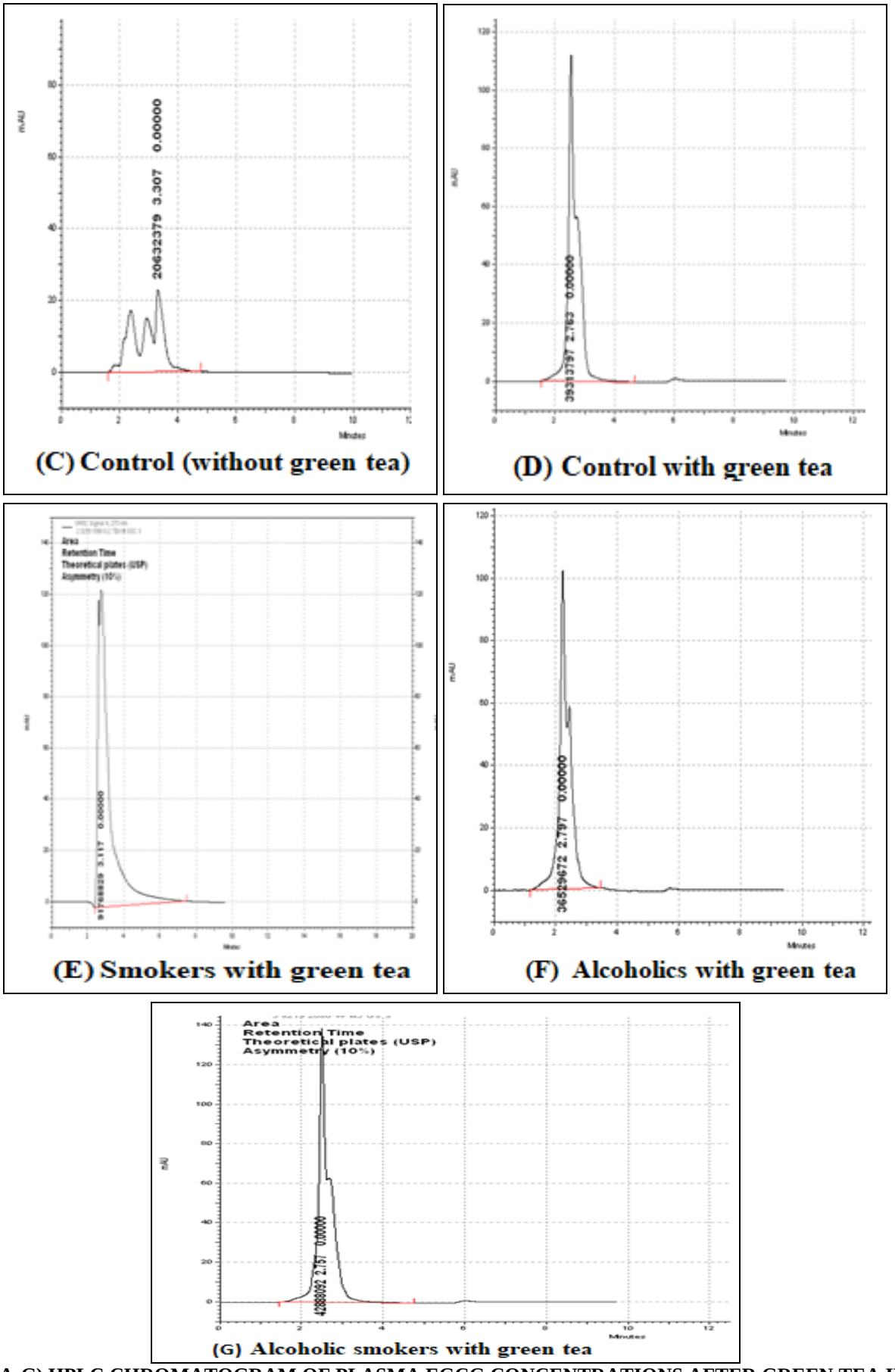


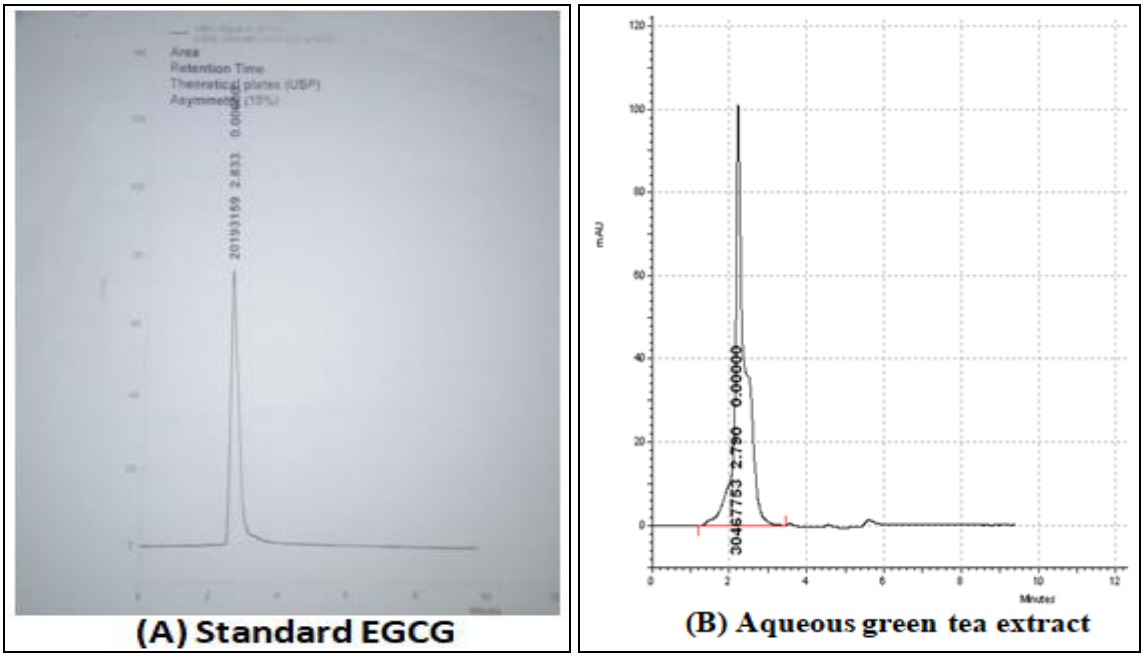
FIG. 4: (A-G) HPLC CHROMATOGRAM OF PLASMA EGCG CONCENTRATIONS AFTER GREEN TEA INTAKE IN DIFFERENT EXPERIMENTAL GROUPS

Chromatography Studies of Catechins on Experimental Subjects in Urine (Human Male Volunteers): Similarly urinary excretion of catechins was more in alcoholics with green tea (128.9 nmol/L/100 µL) than other groups viz., smokers, alcoholic smokers and controls with green tea intake **Table 5.** Plasma samples of smokers, alcoholics, alcoholic smokers consuming green tea were also analysed by HPLC which revealed high EGCG in plasma after 2 hours of green tea intake whereas the concentrations of degradatory products (ring fission products) were found in urine suggesting **Table 4 & 5.**

The difference is mainly due to the concentration of catechins administered and recorded timings of sampling were also different. The level of EGCG in urine was undetectable but the difference was not statistically significant [26]. The studies of Lee showed that except EGCG and ECG the remaining degraded catechins are the high concentrations in the urine samples when compared to prior consumption of green tea [37]. Yang *et al*. (1998) did not observe urinary catechin excretion in detectable amounts suggesting the maximal utilization of catechins. We found that urinary catechins were excreted significantly in particular in alcoholics, smokers and alcoholic smokers in this study. Green tea polyphenols can modulate both the structure and properties of biological membranes [36, 38]. EGCG also affects renal inflammatory signals [39]. Recent evidence revealed many direct actions of green tea catechins that are independent of antioxidative mechanism. The degradatory products of catechins in particular, ring fission products formed during biotransformation at second phase metabolism of catechins appear to be more potent than the parent catechins in bringing about various beneficial actions [40]. The concentrations of urinary degraded catechins (EGCG and ECG) had weak association with trigylcerides and glycated haemoglobin for both plasma and urine [41]. Though faecal excretion of catechins exists, the catechins reached the plasma are to be considered [42, 43, 44, 45].

TABLE 5: QUANTIFICATION OF URINE EXCRETORY EGCG CONCENTRATIONS IN STUDIED HUMAN VOLUNTEERS

Groups	Retention time (min)	Concentration of EGCG/ degraded products nmol/L/100 µL	Peak height (Micro volts)
Group I	3.30	67.2	2218330
Group II	2.75	12.70	1242481
Group III	3.11	55.3	2704964
Group IV	3.09	128.9	6790554
Group V	3.32	52.28	2524863



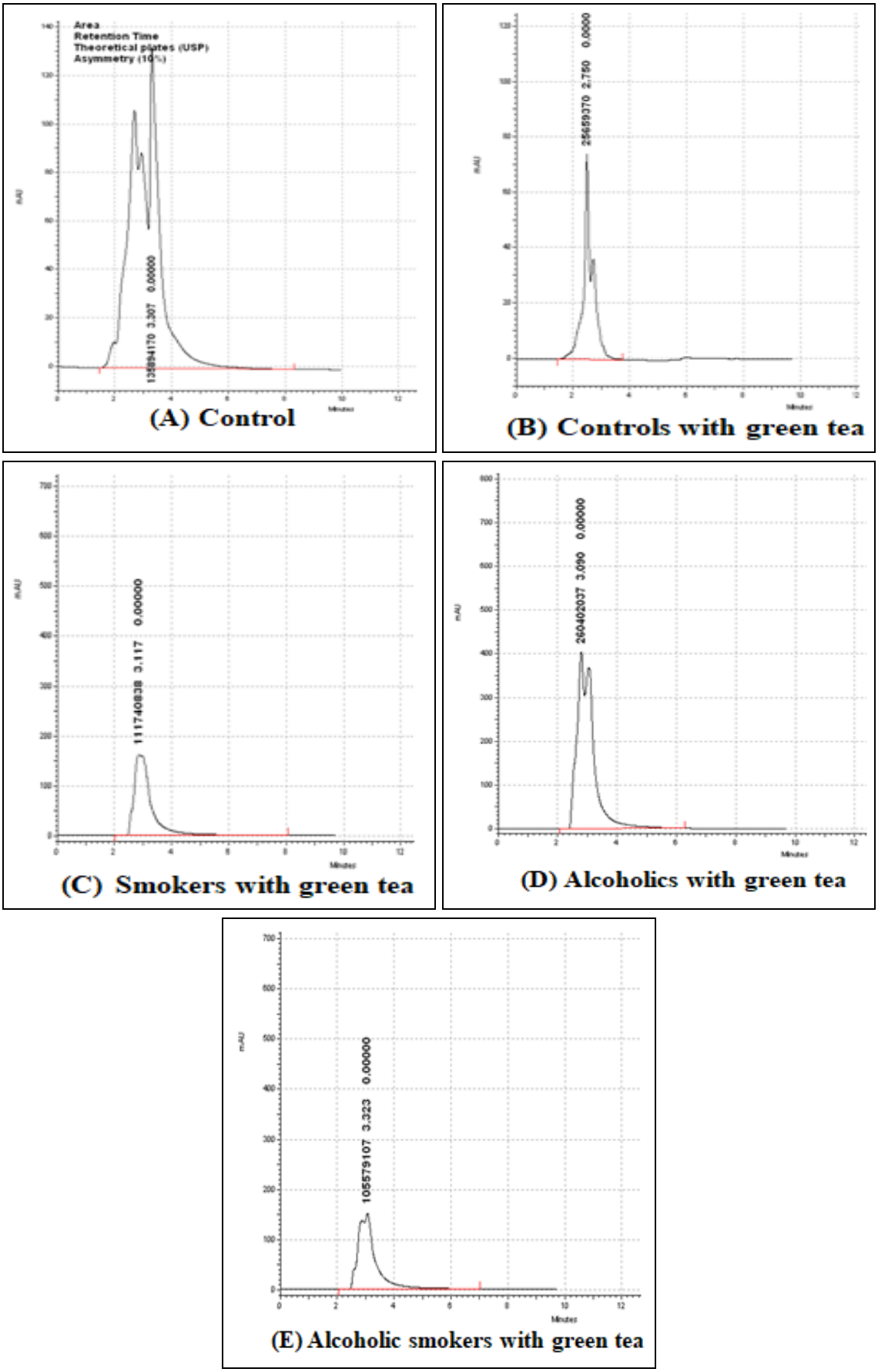


FIG. 5: (A-E) HPLC CHROMATOGRAM OF URINE EGCG CONCENTRATIONS AFTER GREEN TEA INTAKE IN DIFFERENT EXPERIMENTAL GROUPS

The major catechin is EGCG enters the circulation and antioxidant capacity increases after ingestion of green tea [4, 6, 47]. Though gastrointestinal absorption is limited, with rapid phase II metabolism (glucuronidation, sulphation and methylation) of catechins have also been identified as metabolites in liver and kidney of humans, rats and mice [9]. Valero lactones have also been identified as metabolites of catechins in humans in urine and plasma after oral ingestion of cups of green tea [37]. After single dose of green tea intake, at 2 hrs post ingestion the catechin EGCG levels are significantly increased (2- fold hike) compared with Group I. These catechin metabolites are by the metabolic reactions converted to hydrophilic compounds. In conclusion the served green tea shows high concentration of catechins especially EGCG. Maximal concentration of plasma EGCG was seen in smokers when compared to the remaining groups in the following order Group III > Group V > Group IV > Group II (smokers > alcoholic smokers > alcoholics > controls). Excretion of urinary metabolites, in particular concentrations of EGCG and metabolites were found to be more and rapid in alcohol consuming subjects than other groups and the order is Group IV > Group V> Group III> Group II (alcoholics > alcoholic smokers > smokers > controls) **Fig. 5A-E**. However, catechins could act *via* different mechanisms in alcoholics and smokers *viz*., carcinogen, redox switches and Mitogen activated protein (MAP) kinases.

CONCLUSSION: Nil

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CONFLICT OF INTEREST: The authors declare that they have no conflict of Interest.

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