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Moderate alcohol consumption in chronic form enhances the synthesis of cholesterol and C-21 steroid hormones, while treatment with *Tinospora cordifolia* modulate these events in men

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ABSTRACT

Chronic and heavy alcohol consumption disrupts lipid metabolism and hormonal balance including testosterone levels. However, studies doubt the relationship between moderate alcohol intake and sex hormone levels. Therefore, the aim of the present investigation was to establish the direct impact of chronic and moderate alcohol intake on cholesterol homeostasis and steroid hormone synthesis. Asymptomatic chronic and moderate alcoholics (n = 12) without chronic liver disease and healthy volunteers (n = 14) were selected for the study. Furthermore, effects of standardized water extract of *Tinospora cordifolia* (Willd) Mier. (Menispermaceae) (TCJ), a well reported anti-alcoholic herbal drug, on urinary steroids was studied. This study included four groups, i.e. a) healthy; b) healthy + TCJ; c) alcoholic; d) alcoholic + TCJ. The blood and urine samples from each group were collected on day 0 and 14 of the post-treatment with TCJ and analyzed. Alcoholic blood samples showed the significantly higher values of traditional biomarkers γ-GT and MCV along with cholesterol, LDL, TGL and urinary methylglucuronide compared to healthy. Qualitative analysis of steroids showed that moderate alcohol intake in a chronic manner increased the cholesterol synthesis and directed its flow toward C-21 steroids; shown by increased levels of corticosterone (2.456 fold) and cortisol (3.7 fold). Moreover, alcohol intake also increased the synthesis of estradiol and clearance rate of other steroids through the formation of glucuronides. Therefore, it decreased the synthesis and increased the clearance rate of testosterone (T) and androstenedione (A). Quantitative analysis confirmed decreased T/A ratio from 2.31 to 1.59 in plasma and 2.47 to 1.51 in urine samples of alcoholics. TCJ intervention normalized the levels of steroids and significantly improved the T:A ratio to 2.0 and 2.12 in plasma and urine. The study revealed that TCJ modulated lipid metabolism by inhibiting cholesterol and glucuronides synthesis.

1. Introduction

Chronic and heavy alcohol intake led to impotence and infertility. These effects of alcohol were due to inhibition of testosterone synthesis in testis [11]. Decreased levels of testosterone have been observed in short as well as long term heavy drinkers [41]. Chronic (moderate and heavy) alcohol intake has been reported to be associated with a degree of elevation in plasma estrogen levels in male alcoholic by extending the conversion of androgenic hormone into estrogen [21]. However, reports available on the effects of low or moderate dose alcohol intake contradict these findings [32,35]. This shows that alcohol consumption may have different effects on both genders and according to age [26]. Moderate alcoholism has been reported to prevent atherogenesis through change in lipid metabolism [1], inflammation [19] and hemostasis [8]. An inverse relationship between aortic atherosclerosis and levels of testosterone has been reported in a 55 year old man [16]. Subsequently, aortic stiffness have been related inversely to testosterone [12]. Therefore, to understand the effects of alcohol on steroid homeostasis, it is important to study the effects of moderate alcohol intake on the steroid levels, in a controlled environment.

Presently few drugs are available in the market with various side effects to prevent alcoholism or disorders of alcoholism [22]. Herbal medicines may be the alternate option of these drugs or to develop new therapies. In Ayurveda, *Tinospora cordifolia* (Willd) Mier. (Menispermaceae) has been reported to possess rejuvenating

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properties with no *in vivo* toxic effect [39,14]. Several bioactive compounds, including berberine, cordifoliosides, tinosporoids etc have been reported from the plant [37]. Berberine and other metabolites have been reported as anti-hyperlipidemic and anti-diabetic agents [17,31]. Hence, it is the drug of choice for hepatic aliments [5,33], the most affected part in alcoholics.

The objectives of this study were to analyze the effect of moderate alcohol consumption on urinary steroids in men and co-relation between urinary with plasma testosterone levels. Furthermore, efficacy and the mechanism of *T. cordifolia* water extract (TCJ) in prevention of alcoholic effects on the steroid metabolism in moderate alcohol consumers male was also studied.

2. Material and methods

2.1. Chemicals

Standards of steroidal compounds, their conjugates and solvents i.e. water, acetonitrile, formic acid, were purchased from Sigma (St. Louis, MO). Internal and external calibrates and standards were purchased from Agilent Technologies. All other reagents used in the study were LC-MS grade.

2.2. Drug preparation

Spot identified fresh stems of *T. cordifolia* plants were collected in September 2011 from garden of National Research Institute of Basic Ayurvedic Sciences, CCRAS (Department of AYUSH), Nehru Garden, Kothrud, Pune. The voucher specimens (No. 207) were kept at the medicinal plant museum of the Institute. The stems were washed 3 times with reverse osmosis (RO) water and homogenized with RO water in 1:5 ratio and kept overnight at room temperature under septic conditions. Stem juice (TCJ) was prepared under the guidance of an Ayurvedic physician. The LC–MS profiles of the at least three extracts prepared at different time were analyzed for the standardization purpose and no significant difference was observed in the chemical constituents of stems [25,25].

2.3. Study subjects

Twelve alcoholic and fourteen non-alcoholic male volunteers of age 40.5 ± 3.8 (Mean + SD) years, having a BMI 30.9 ± 3.5 (Mean ± SD) were selected for this study from the local areas of Pune, Maharastra, India. All were apparently healthy and hadn't recorded any disease or any kind of regular medication. All of them reported a typical alcohol consumption of 60 ml of 80 proof liquor (40% alcohol) (24–30 g) per day from last 4–5 years, and were classified as moderate chronic drinkers. An informed written consent was taken from all the individuals who entered into the study. Exclusion criteria included body mass index >30 kg/m^2, blood pressure >160/90 mm Hg, total cholesterol >7.5 mmol/L, present or prior history of cardiovascular disease, diabetes mellitus, respiratory, gastrointestinal, hepatic, renal, endocrine, or reproductive disorders; or use of lipid-lowering agents, antihypertensive agents. All the volunteers underwent the questionnaire of alcohol use disorders identification test (AUDIT) for assessment of alcohol abuse. The Human Ethics Committee of the DYP Ayurveda College, Pune, India has approved the study protocols wide letter no. NRIBAS/2011/HEC/2023 dated 18-11-2011.

2.4. Study design

All volunteers were asked to maintain their usual vegetarian diet throughout the study and not to take any medicine without consulting. Fig. 1 shows the workflow of treatment and analysis period. After one week pre-watch analysis period, volunteers further divided into four groups (Healthy; Healthy + TCJ; Alcoholic; Alcoholic + TCJ). Volunteers were given 100 ml TCJ (3.0 g solid extract) early in the morning with an empty stomach for 14 days in the presence of a physician. Most of the standard drugs for treatment of alcohol induced disorders take 2 weeks to synthesize sufficient unbound enzyme to metabolize alcohol adequately. TCJ has also been reported to lower the cholesterol levels after two week treatment [30]. Therefore, the 14 day trial period was chosen to analyze the effect of TCJ on moderate alcohol intake. The alcoholic subjects continued 60 ml of 80 proof liquor (40% alcohol) consumption per day (0.5 g/kg/body weight corresponding to two to three standard drinks) during the TCJ treatment period of 14 days. All volunteers admitted to the day care center were given standard vegetarian diet. Fasting blood samples for analysis were collected at day 0 (after 7 day observation) and 14 after administration of TCJ. In the same way, first pass urine samples were collected on day 0 and 14 after 12 h of alcohol intake and treated with sodium azide to a final concentration of 2.5 mM. The entire urine sample was centrifuged filtered through 0.2 m filters and stored at −80 °C until analysis.

2.5. Hematology and blood biochemistry

Blood samples were collected into cold tubes containing EDTA and reduced glutathione and centrifuged immediately at 1000*g* for 10 min at 4 °C. The plasma samples were added with butylated hydroxytoluene (40 μg/ml plasma) to protect them from oxidation and stored at −80 °C until analysis. γ-GT, serum glutamic oxaloacetic transaminase (*SGOT*), serum glutamic-pyruvic transaminase (SGPT) and the red blood cell volume (mean corpuscular volume: MCV) were measured to confirm alcohol intake and the effect of treatment. Hematology and blood biochemistry was performed at the start (seven days after alcohol consumption), end of the

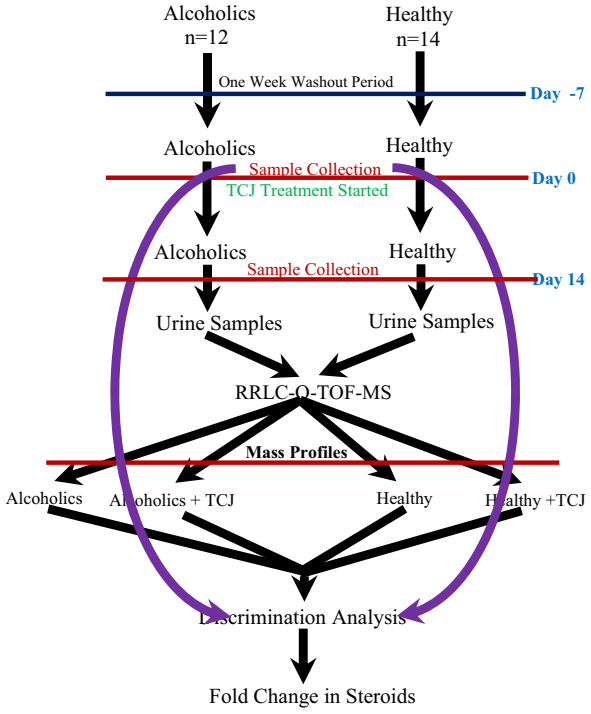

Fig. 1. Work flow diagram showing pre-watch, TCJ treatment, sample collection time timelines, mass profiles, discrimination analysis and quantization in the samples against control or healthy group.