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Original Article

Amelioration of Lipid Metabolic Profiles by Trigonelline a Bioactive Compound of *Trigonella Foenum-graecum* in Alcohol Induced Albino Rats

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ABSTRACT

Aim: Alcoholism leads to heart diseases, lung diseases and liver disorders. Alcoholism impairs heart homeostasis and causes cardio vascular diseases. The current investigation was carried out to know the hypolipidemic effect of Trigonelline (TG) a bioactive compound of *Trigonella foenum-graecum* (TGf) in alcohol intoxicated rats. **Materials and Methods:** The rats were randomized into five groups and treatment was given as per the experimental protocol. The lipid metabolic profiles like Malondialdehyde (MDA), Total Cholesterol (TC), Triglycerides (TGs), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Very-Low-Density Lipoprotein (VLDL), and liver markers Alkaline Phosphatase (ALP), Alanine Aminotransferase (AAT), Aspartate Aminotransferase (AST) were estimated in the serum of all experimental groups. **Results:** All the lipid metabolic profiles and AAT, AST, ALP elevated in alcohol treated group, except HDL. This may be due to the alterations in lipid metabolism, which leads to hypercholesterolaemia and alcohol induced liver damage. Whereas with trigonelline treatment in alcoholic rats, these lipid metabolic profiles and liver markers were normalized and came back to normal levels. Further histopathological observations also proved that trigonelline protected heart tissue from alcohol toxicity in rats. **Conclusion:** Our findings suggest that trigonelline modulated the lipid metabolic profiles in alcohol intoxicated rats, hence trigonelline might be effective for preventing or treating alcohol related disorders and lipid related diseases.

Keywords: Alcoholism, Lipid metabolic profiles, Trigonelline, Rats.

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INTRODUCTION

Alcohol is the most widely abused drug. As per World Health Organisation (WHO) statistics the total deaths of alcohol are 3 million every year and is also responsible for more than 200 diseases. Every 10 sec a human being dies because of alcohol intoxication. Alcohol consumption can damage each and every organ in the human and also some times it leads to organ failure. Alcohol is metabolized in the liver and kidney by enzymes like Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase (ALDH) and Cytochrome P450 2E1 (CYP2E1) monooxygenase. Alcohol metabolism produces toxic chemicals like acetaldehyde and Reactive Oxygen Species (ROS). Over production of ROS can cause oxidative stress that leads to liver damage, stroke, kidney tissue damage and neuronal damage.[1] Alcohol intoxication is associated with hyperlipidaemia, which leads to Cardio Vascular Disease (CVD). Alcohol alters the lipid metabolism that ultimately leads to organ failure. There was J- or U-shaped relationship between alcohol consumption and cardiovascular disease.[2]

World Health Organisation (WHO) reports that medicinal plants are treasure of nature, which are handed over from generation to generation, whether verbally or in writing and are used in the treatment of diseases, removal of physical, mental and social stress.[3] Indian Traditional system of medicine is one of the oldest medical systems in the world and it plays a main role in providing beneficial effects, applications of medicinal plants right from its inception. In India Ayurveda, Unani and Siddha are practiced since the dawn of civilization.[4] Medicinal plants like ginger, ocimum, trigonella, curcumin and many plants contain pharmaceutical compounds, antioxidant compounds and other nutraceuticals which helps in protection of tissue from toxicity.[5]

Trigonella foenum-graecum, is known as trigonella or fenugreek, and it is cultivated in many parts of the globe including India. It

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has many medicinal properties like antioxidant, hyperlipidemic, anti-diabetic, anti-microbial and anti-fungal.[6] It has been used to treat many diseases like diabetes, cancer and hepatitis. Trigonella seed contains many bioactive compounds like saponins (fenugrin B, and fenugreekine), alkaloids (trigonelline and choline), flavonoids (quercetin and vitexin), amino acids (isoleucine, histidine, leucine, and lysine) and cumarins (methyl coumarin and trigocumarin).[7]

The present study was carried out to know the hypolipidemic effect, hepatoprotective effect and cardioprotective of trigonelline in alcohol intoxicated rats. This is the first study to know the hypolipidemic activity of trigonelline in alcoholic subjects.

MATERIALS AND METHODS

Collection of Plant Material

Trigonella foenum-graecum was collected at Tirupati, Andhra Pradesh, India. *Trigonella foenum-graecum* seed was dried in the shade, powered and soaked in 90% methanol for three days at 37°C room temperature. All the extract was collected, filtered and this process was repeated for three times. Later these extracts were pooled together and concentrated to dry. The methanolic extract of *Trigonella foenum-graecum* was used for isolation of trigonelline by following the method of Ashihara.[8]

Chemicals

The chemicals used in this investigation are obtained from Merck, qualigens, fischer and sigma-aldrich (St. Louis, USA), etc.

Animal Model

Young wistar strain male albino rats (200±20 g, 3 Months age) were purchased from Indian Institute of Sciences, Bangalore and were housed in room at temperature of 25±2°C, humidity of 50–55%, with a 12 hr light/dark cycle. Water and food are available *ad libitum*. The experimental study was conducted following the internationally accepted laboratory principles for animal care. Sri Venkateswara University (No.01/2011–2012/(i)/a/CPCSEA/IAEC/SVU/MB SR/Dt20/06/2011), Tirupati.

Experiment Groups

Rats were divided into five groups and in each group six rats.

Group 1 (Normal control - Nc): rats received saline via orogastric tube for 30 days.

Group 2 (Trigonelline - TGt): rats received trigonelline (50 mg/kg) via orogastric tube for 30 days.

Group 3 (Alcohol control - At): rats given alcohol (5 g/kg body weight) daily for 30 days.

Group 4 (Alcohol treatment + Trigonelline treatment (At+TGt): rats received alcohol and trigonelline for 30 days.

Group 5 (Alcohol +Vitamin E treatment– At+VEt): rats received alcohol and Vitamin E for 30 days.

Treatment was given for 30 days. All the rats were sacrificed by decapitation and blood was collected by capillary tubes from retro-orbital plexus and used for biochemical estimations and heart tissue was used for histopathological observation.

Estimation of Lipid metabolic profiles in serum

Malondialdehyde (MDA), Triglycerides (TGs), Total Cholesterol (TC), Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL), Very-Low-Density Lipoprotein (VLDL) were estimated in the serum of all experimental groups by standard protocols.

Estimation of Serum markers

Serum ALP, AST and AAT activities were estimated in serum of all groups by standard protocol.

Preparation of heart tissue for pathological examination

Heart tissues of all the experimental animals were fixed in formalin, paraffin-embedded and stained with Hematoxylin-Eosin (HE) to assess the histopathological observations. Pathological observation and photography were taken by using an Olympus multi-function microscope (10X).

Statistical analysis

Results were analysed by using SPSS and expressed as mean ± standard deviation of mean. To check the difference between groups, A One-way Analysis of Variance (ANOVA) was performed and Dunnett multiple comparision test (for the difference between control and treated groups) was conducted. p-value < 0.05 was considered statistically significant.

RESULTS

Effect of Trigonelline on Lipid metabolic profiles in alcohol treated rats

Figure 1 depicts the Lipid metabolic profiles in all the groups. The levels of MDA, total cholesterol, triglycerides, LDL, VLDL were elevated in alcohol intoxicated rats. But HDL levels are depleted in alcoholic subjects. However, with trigonelline treatment for 30 days, all the lipid metabolic profiles were normalized in alcoholic rats. All the lipid profiles were come back to near to normalcy. Our study reported that trigonelline possess hypolipidemic activity in alcohol treated rats (Figure 1).

Effect of TG on Serum markers in alcohol treated rats

Table 1 depicts the serum markers in all groups. In alcohol treated rats AAT, AST and ALP activities were increased. However, with trigonelline supplementation in alcoholic rats the activities

of AAT, AST and ALP were decreased. Our study reported that trigonelline showed hepatoprotective effect in alcohol intoxication rats (Table 1).

Effect of trigonelline on Heart tissue in alcoholic rats

In normal control rats and trigonelline treated rats, normal architecture of the heart was observed. However, with alcohol intoxication, the heart tissue showed disrupted cardiac cells, severely damaged myofibrils and vacuolization. However, alcoholic rats which received trigonelline for 30 days reported the regeneration of cardiac cells, myocytes, regeneration of nucleus. Our studies proved that trigonelline protected heart tissue from alcohol intoxication in rats (Figure 2).

DISCUSSION

Medicinal plants contain many bioactive compounds that are used in the treatment of many diseases like diabetes, cancer, hepatitis and parkinsons. Herbal drugs are being used in many countries for the treatment of many diseases, due to low cost, easy availability, safety, as well as its effectiveness.[9] Trigonelline a bioactive compound of *Trigonella foenum-graecum* have anti-diabetic, antioxidant, anti-inflammatory, antibacterial and antiviral, antispasmodic and analgesic effects.[10]

Lipids metabolic profiles are the main component of cell membranes and they involved in signal transduction, energy storage and cell signaling. In our study, we analyzed lipid metabolic profiles in alcohol exposed rats. Excess alcohol intake

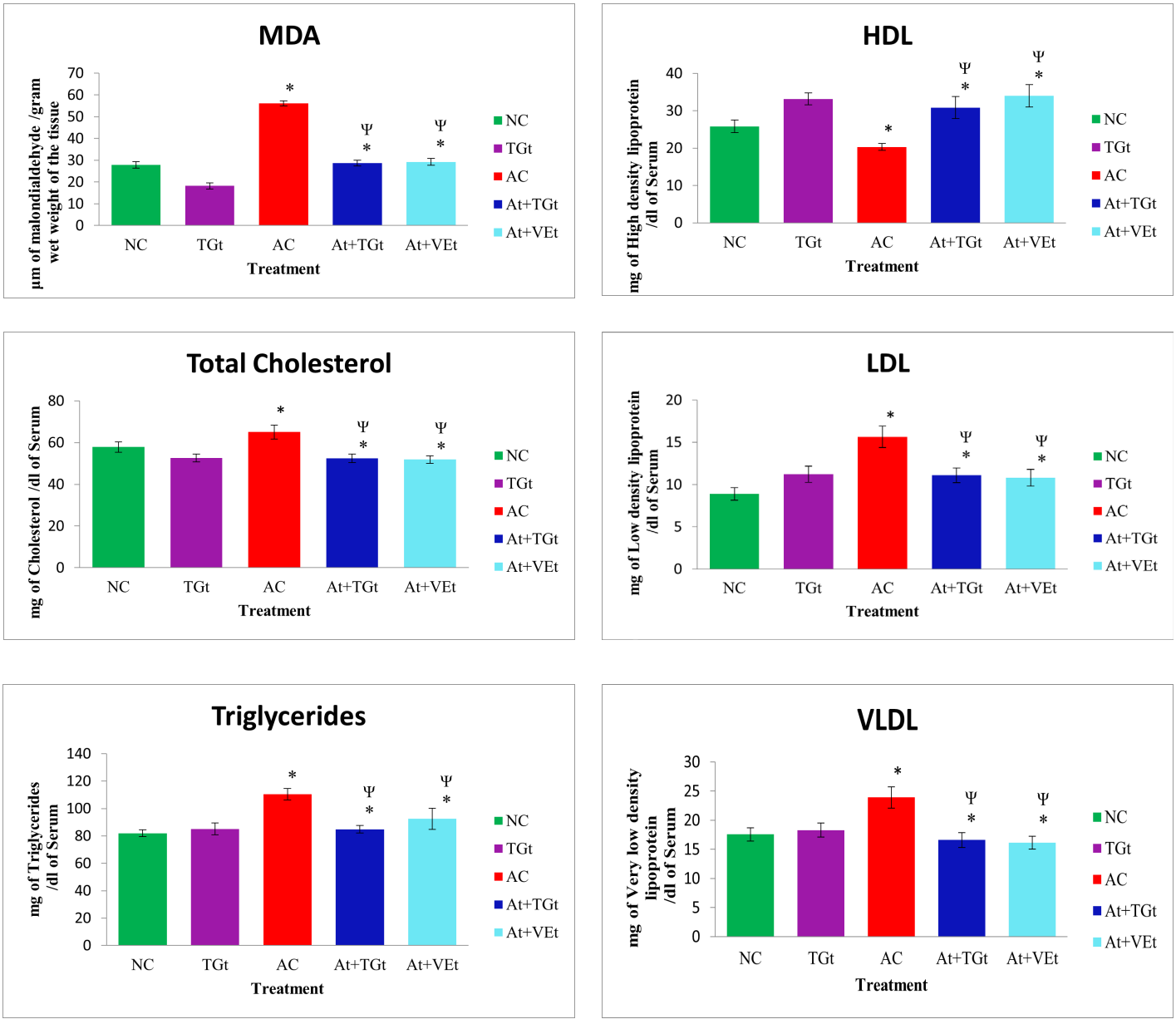

Figure 1: Effects of trigonelline on Lipid metabolic profiles in alcohol intoxicated rats. Data are expressed as means ± SD ($n = 6$). * The values are significant compared to the following: control (* $p < 0.001$), Alcohol ($^{\Psi}$ $p < 0.01$) (Dunnett's multiple comparison tests).

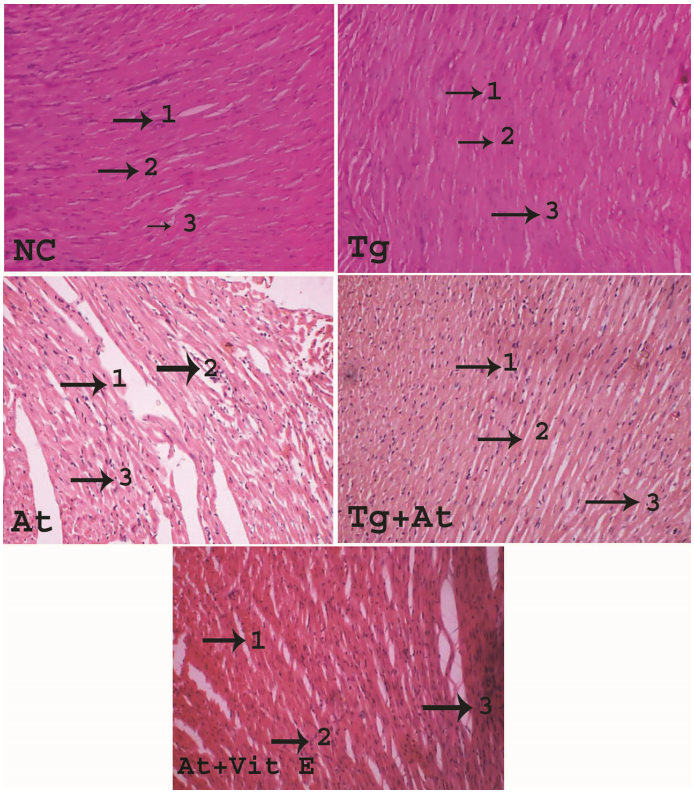

Figure 2: Effect of Trigonelline on Heart tissue in alcohol induced rats.

Photomicrograph of Heart tissue: 1) Normal control rat showed normal structure of heart tissue, 2) TG control group reported normal structure of heart tissue, 3. Alcohol-control rat showed disruption of cardiac cells, degeneration of myocytes and coagulation of blood, 4. Tg+At showed regeneration of cardiac cells, regeneration of myocytes. 5. At+Vit E: showed regeneration of cardiac cells, regeneration of myocytes and regeneration nucleus. (H&E, × 10).

Table 1: Effect of TG on Serum Markers enzymes in alcohol intoxicated rats.

Groups	AST	ALT	ALP
	(IU/L)	(IU/L)	(IU/L)
Group I (NC)	28.26	31.48	128.24
	(±4.26)	(±2.62)	(±8.46)
Group II (TGt)	32.68	32.52	128
	(±3.86)	(±2.82)	(±18.68)
Group III (At)	172.84*	104.26*	184.24*
	(±7.642)	(±10.54)	(±10.42)
Group IV (At)	40.62*	38.46*	138.64*
	(±3.42)	(±2.146)	(±8.64)
Group V	48.28*	43.46*	142.28*
(At+VitE t)	(±3.16)	(±1.42)	(±10.62)

All the values are means ± SD of six individual observations.* Significant at $p < 0.001$ with respect to normal control.

is known to cause liver cirrhosis, heart stroke, renal failure and brain haemorrhage.[11] Alcohol consumption is associated with hyper-triglyceridaemia in adult rats. Administration of alcohol for 30 days up regulated lipid peroxidation, TC and TGs levels in the serum of rats compared to control. The elevation of TC, MDA and TGs in rats observed in this study could be as a result of changes in lipid metabolism and alterations in cholesterol synthesis in alcohol intoxicated rats. The observed significant elevation of these profiles is indicative of alcohol-induced pathological changes and oxidative stress in the liver, brain, heart and kidney.[12] Our studies are in agreement with the report of Kim *et al.*[13] that rats fed with alcohol showed hyperlipidemia. However, with trigonelline supplementation in alcoholic rats MDA, TC and TGs levels were depleted in alcoholic rats. Recently Yeligar *et al.*,[14] and Tasnim *et al.*,[15] also reported high levels of triglycerides in alcohol intoxicated subjects (Figure 1).

In our study, we reported that LDL, VLDL levels were elevated and HDL levels were depleted in alcohol administered rats. Lipid metabolism is altered by chronic alcohol intoxication leading to the alterations in lipid metabolic profiles.[16] Alcohol consumption prompted the downregulation of genes involved in cholesterol synthesis, this may lead to alterations in lipid metabolic profiles. In our study, we reported a significant elevation in serum VLDL, LDL and depletion in HDL levels in rats, these values are risk for cardiovascular disease.[17] Shanmugam *et al.*,[18] also reported the same observation in rats which received alcohol. However, with trigonelline supplementation in alcohol treated rats, serum VLDL, LDL levels were decreased and HDL levels increased (Figure 1).

In this study, serum ALP, AST and AAT activities were estimated in all the experimental groups. In disease conditions liver tissue damage can be monitored by measuring serum enzymes like AAT, AST and ALP. These serum markers are important liver tissue damage subjects.[19] AAT, AST and ALP oozes from liver in to blood stream during disease condition, hence these activities were up regulated in alcohol intoxicated rats.[20] In our study, also we reported that AAT, AST and ALP activities were elevated in alcohol treated group. However, AAT, AST and ALP levels were significantly depleted with trigonelline supplementation in alcohol treated rats. Our study reported that trigonelline protects the liver tissue from alcohol induced toxicity in rats. This may be due to trigonelline antioxidant and pharmacological activities. Hence, AAT, AST, and ALP activities were decreased in alcoholic rats which received trigonelline (Table 1).

In our study, we reported that disrupted cardiac muscles, coagulation of blood, vacuolization, and severely damaged myofibrils in alcohol intoxicated rats. However, with trigonelline

supplementation in alcoholic rats, we reported the regeneration of myocytes, regeneration of nucleus and regeneration cardiac cells. This shows that trigonelline can protect the heart tissue from alcohol induced free radical toxicity in rats (Figure 2).

CONCLUSION

From our study, we reported that in alcoholic subjects all the lipid metabolic profiles were altered, however with trigonelline all these markers were normalized. This study, we may conclude that trigonelline possess hypolipidemic, cardio protective and hepato protective effect in alcohol intoxicated rats. The histopathological observations of this investigation also confirmed that trigonelline protected the heart tissue from alcohol induced intoxication in rats. Hence, trigonelline can be used to treat hyperlipidemic subjects.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TG: Trigonelline; **TGf:** *Trigonella foenum graecum*; **MDA:** Malondialdehyde; **TC:** Total cholesterol; **TGs:** Triglycerides; **VLDL:** Very low density lipoprotein; **HDL:** High density lipoprotein; **LDL:** Low density lipoprotein; **AST:** Aspartate aminotransferase; **ALP:** Alkaline phosphatase; **AAT:** Alanine aminotransferase; **ADH:** Alcohol dehydrogenase; **ALDH:** Aldehyde dehydrogenase; **CYP2E1:** Cytochrome P450 2E1 monooxygenase; **ROS:** Reactive oxygen species; **CVD:** Cardio vascular disease; **WHO:** World Health Organisation; **NC:** Normal control; **TGt:** Trigonelline treated; **At:** Alcohol treated; **At+TGt:** Alcohol treated+Trigonelline treated; **At+Vit E:** Alcohol treated+Vitamin E treated; **ANOVA:** One-way analysis of variance; **HE:** Hematoxylin-eosin.

SUMMARY

- In the present study, we have analyzed the anti-lipidemic effect of trigonelline in alcoholic intoxicated rats.
- In this study, we observed that in alcoholic rats, the lipid metabolic profiles were elevated, however with a trigonelline all the lipid metabolic profiles were normalized in alcoholic rats.
- Histopathological studies also proved that trigonelline have cardioprotective effect in alcohol treated rats.

AUTHORS CONTRIBUTIONS

Experimental design and Idea of the design: Dr. M. Guru Sekhar, Dr. K.R. Shanmugam and Dr. K. Siva Prasad, Ch Ramakrishana.

Biochemical estimations and interpretation of the data: Dr. M. Guru Sekhar, Dr. K.R. Shanmugam and Dr. K. Siva Prasad.

Drafting of the Manuscript and submission: Dr. M. Guru Sekhar, Dr. K.R. Shanmugam and Dr. K. Siva Prasad, Ch Ramakrishana.

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