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Original Research Article (Experimental)

Trigonelline, a Fenugreek Bioactive compound protects Heart tissue against alcohol intoxication: An *in-vivo* study focusing on antioxidant perspective

Meesala Guru Sekhar [a,b], Kondeti Ramudu Shanmugam [c,*], Inavolu Srinivasa Chakrapani [c]

[a] *Department of Zoology, Government Degree College, Cumbum, Prakasam District, A.P, India*
[b] *Sri Venkateswara University, Tirupati, A.P, PIN 517 501, India*
[c] *Department of Zoology, PRR & VS Government Degree College, Vidavalur, SPSR Nellore District, A.P, India*

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ABSTRACT

Background: *Trigonella foenum-graecum*, commonly known as fenugreek and it is used as a spice. It has antioxidant, anti-diabetic, antilipedemic and other pharmocological properties.
Objectives: The aim of the study was to detect the cardio protective activity of Trigonelline (TG) a bioactive compound of *Trigonella foenum-graecum* (TF) in alcohol intoxicated rats.
Material and methods: The young wistar strain albino rats are divided in to 5 groups and treatment was given as per the experimental protocol. Antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR), glutathione (GSH), malondialdehyde (MDA) levels are estimated in cardiac tissue of all experimental groups. Cardiac markers creatine kinase-MB (CK-MB), troponin-T (TT), troponin-I (TI), myoglobin (MG) and serum markers alanine transaminase (AAT), aspartate transaminase (AST) and alkaline phosphatase (ALP) are estimated. Free radical scavenging activities like 2,2-diphenylpicrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2) and hydroxyl radical are estimated in ethanolic extract of *Trigonella foenum-graecum*.
Results: SOD, CAT, GPx, GR, GSH activities are depleted and MDA, CK-MB, TT, TI, MG and AAT, AST, ALP activities are elevated in alcohol intoxicated rats. Trigonelline supplementation to alcoholic rats for 30 days elevated antioxidant enzymes, depleted MDA, cardiac markers and serum markers in alcohol intoxicated rats. Free radical scavenging assay also reported that *Trigonella foenum-graecum* possess free radical scavenging activity. Furthermore, our histopathological evidence also proved that TG protected the cardiac tissue from alcohol induced toxicity in all the experimental rats.
Conclusion: Our study concluded that TG may be useful to the alcoholic and myocardial infarction subjects.

1. Introduction

Alcohol is one of the most commonly abused drug. Alcohol intake damages liver, heart, kidney and brain. Cardiovascular disease, mainly myocardial infarction (MI) is the foremost leading cause of deaths around the world, accounting for 30% of all-cause deaths [1].Low dose of alcohol consumption is associated with protection against heart disease, reduced incidence of heart failure and atherosclerosis [2]. But high dose of alcohol is toxic to heart, liver and brain that leads to organ damage. Alcohol intoxication leads to changes in protein homeostasis in heart that are associated with impaired cardiac functions.

Cardiac biomarkers are important circulating proteins or molecules that are released into the blood from the heart. Creatine kinase-MB (CK-MB), troponin-T (TT), troponin-I (TI) and myoglobin (MG) are cardiac biomarkers. CK-MB is a cardiac marker for diagnosis of an acute myocardial infarction, myocardial ischemia or myocarditis. Troponin-T or Troponin-I are proteins present in the blood which are released during heart damage. These biomarkers are useful in the diagnosis of heart attacks. The more damage in the heart, the greater the amount of troponin-T and troponin-I observed in blood. Myoglobin (MG) is an iron and oxygen binding protein found in the heart and skeletal muscle tissue. Myoglobin is an early marker of acute myocardial infarction. Diagnosis of cardiac biomarkers help in determining person's cardiac health and also help to monitor and manage cardiac diseases.

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* Corresponding author.
E-mail address: krshanmugamphd@gmail.com (K. Ramudu Shanmugam).

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Medicinal plants are believed to have healing powers and people have been using them for many centuries in different parts of the globe. World Health Organization (WHO) recommends the use of medicinal plants for the treatment of various diseases. Recent investigations reported that there are many bioactive compounds in these plants, which shows the therapeutic applications. Medicinal plants like ginger, curcumin, trigonella and ocimum have cardioprotective properties. *Trigonella foenum-graecum* (TF), is a clover-like fragrant herb belonging to Fabaceae. Trigonella have many pharmacological properties like antioxidant, anti-diabetic, anti-cancer, anti-diuretic, anti-diarrhoeic, anti-allergic, anti-lipidemic, anti-bacterial and anti-viral activities etc. In Trigonella, there are many bioactive compounds like trigonelline, orientin, isoorientin and isovitexin [3,4].

Trigonelline (TG) is a major alkaloid isolated from *Trigonella foenum-graecum* (L). It has been confirmed that TG possess hypoglycaemic, cholesterol-lowering, anticancer and anti-oxidant effects [4].The aim of present investigation is to determine the antioxidant, cardio protective effect of trigonelline in alcohol intoxicated albino rats.

2. Material and methods

2.1. Plant material

Trigonella foenum-graecum was collected in Tirupati, A.P and identified by Dr. Madhav Chetty, Department of Botany, Sri Venkateswara University, Tirupati. TF seeds are air dried in the shade and the seeds are powdered. The powder was soaked in 70% ethanol for three days. Later ethanolic extract was filtered and the ethanol was removed from the extract by using rotary evaporator at 65 °C. The yield was 10% (w/w). The ethanolic extract of TF was used for free radical scavenging activities. Trigonelline was isolated and identified by using Aswar et al. [5], method.

2.2. Chemicals

The chemicals used in our study were brought from Merck, qualigens, sigma-aldrich (St. Louis, USA) and fischer scientifics etc.

2.3. Animals

Male albino rats (Wistar strain, 200 ± 50 gram, 3 Months age) were procured from Indian Institute of Science (IISc), Bangalore, India and the rats are kept at temperature of 25 (±2)°C, 12-h light/dark, relative humidity of 50–55%. Food and water were available *ad libitum*. All the experiments of our study were conducted by following guidelines and principles for laboratory animal care, (No.01/2011–2012/(i)/a/CPCSEA/IAEC/SVU/MB SR/Dt20/06/2011, Sri Venkateswara University, Tirupati).

2.4. Experiment groups

The animals were divided into five groups and each group containing six rats.

Group 1 (Normal control- Nc): Saline was given orally with gavage to six rats for a period of 30 days.
Group 2 (Trigonelline - TG treatment - TGt): Trigonelline (50 mg/kg) was given orally with gavage to six rats for a period of 30 days.
Group 3 (Alcohol control - AC): Alcohol (20%, 5 g/kg body weight) was given orally with gavage to six rats daily for 30 days.
Group 4 (Alcohol treatment + Trigonelline treatment At + TGt): This group of rats received both alcohol and trigonelline (50 mg/kg) for 30 days as described in group 2 and 3.
Group 5 (Alcohol + Vitamin E treatment– At + Vit E t): This group of rats received both alcohol and vitamin E orally with gavage for 30 days.

After completion of 30 days of treatment, all the rats were sacrificed and the blood was collected by capillary tubes from retro-orbital plexus and serum was separated, used for estimations of biochemical parameters and heart tissue was isolated from all the rats and used for the analysis of antioxidant enzymes and histopathological studies.

2.5. Preparation of heart tissue for pathological examination

Heart tissues were kept in paraffin-embedded block and tissues were cut in to sections and stained with hematoxylin-eosin (HE) to assess tissue damage. Pathological observation of all groups were examined under Olympus microscope.

2.6. Antioxidant enzymes estimation in heart tissue

SOD, GPx, GR, CAT, GSH, MDA respectively were estimated in heart tissue of all groups by the methods of Misra and Fridovich [6], Flohe and Gunzler [7],Carlberg, and Mannervik [8], Aebi [9], Akerboom and Sies [10], Ohkawa et al., [11]. (Fig. 1).

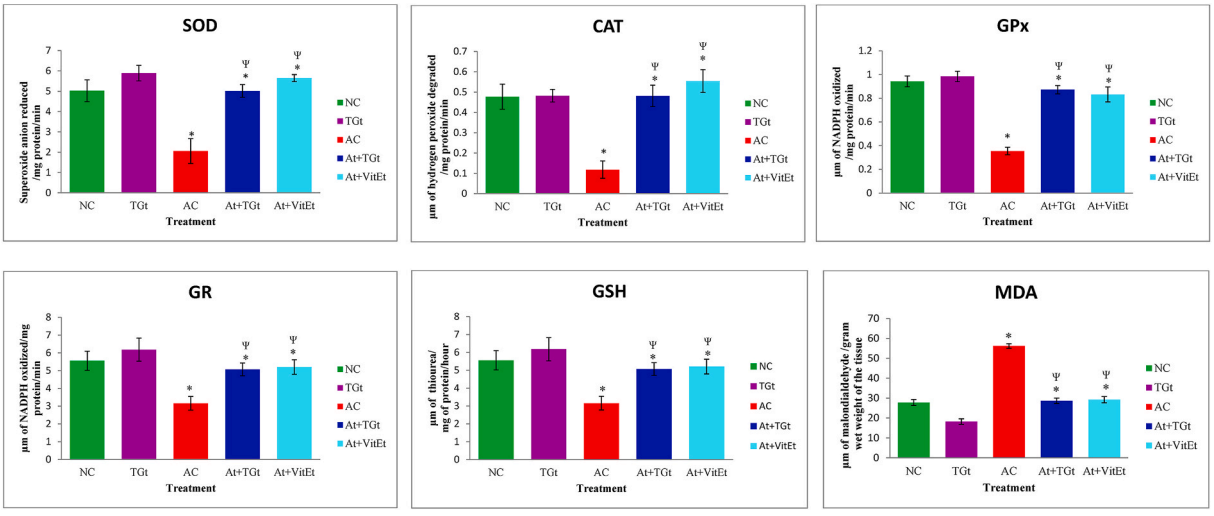

Fig. 1. Effect of TG on Heart Antioxidant enzymes in normal and alcohol intoxicated rats. Data are expressed as means ± SD (n = 6). * The values are significant compared to * Normal Control (NC), and (ψ) Alcoholic control (AC). (Dunnett's multiple comparison test).

2.7. Analysis of cardiac markers in serum

Creatine kinase-MB, troponin-T, troponin-I, myoglobin in serum of all experimental groups were analysed by standard kits using auto-analyzer. (Fig. 2).

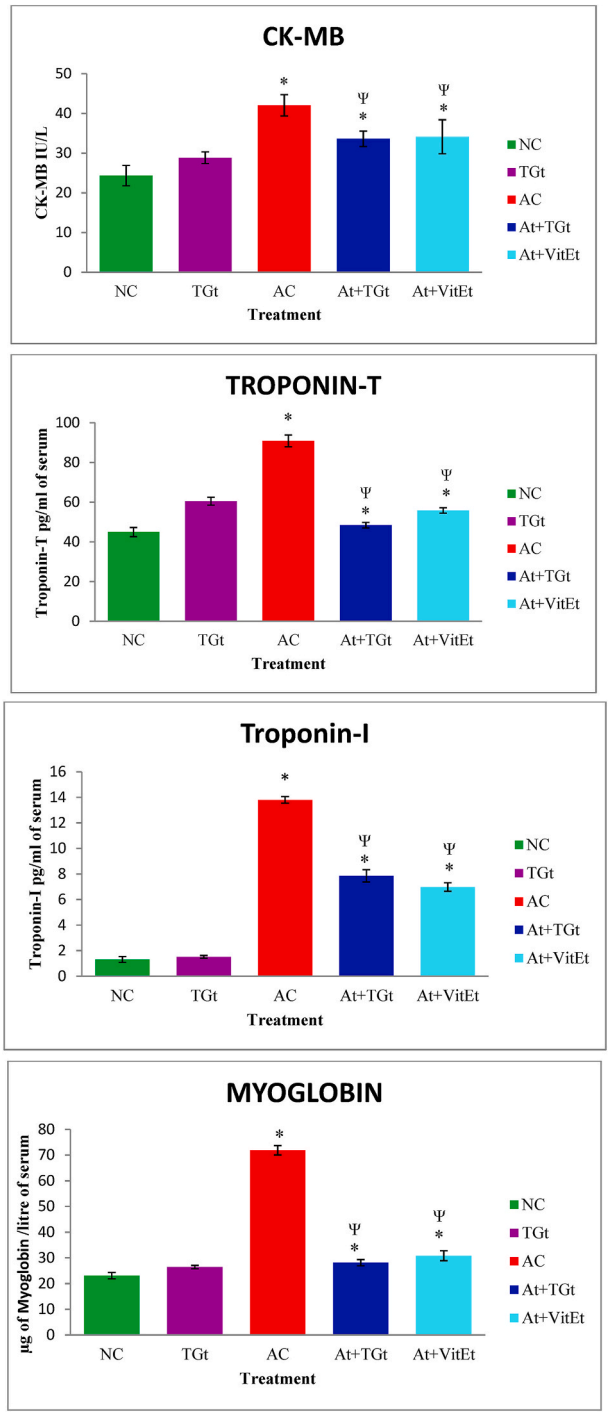

Fig. 2. Effect of TG on Heart Markers in alcohol intoxicated rats. Data are expressed as means ± SD (n = 6). * The values are significant compared to * Normal Control (NC), and (ψ) Alcoholic control (AC). (Dunnett's multiple comparison test).

2.8. Serum markers

ALP, AST and AAT in serum of all groups were estimated by standard kits using autoanalyzer. (Table 1).

2.9. Proteins analysis

Proteins was estimated in the heart by the method of [12].

2.10. Estimation of free radical scavenging activity

DPPH, H_2O_2 and hydroxyl radical were estimated in ethanolic extract of *Trigonella foenum-graecum* by the methods of Brand-Williams et al. [13], Ruch et al. [14], and Klein et al. [15].

2.11. Statistical analysis

The results of our study were calculated by a statistical package for social science (SPSS version 24) and data were expressed as mean ± standard deviation of mean (SDM). One-way analysis of variance (ANOVA) was carried out to know the difference between groups and dunnett multiple comparison test (for the difference between control and treated groups) was conducted. P-value <0.05 was considered statistically significant.

3. Results

3.1. Effect of TG on antioxidant enzymes and MDA in alcohol intoxicated rats

Fig. 1 depicts the results of antioxidant enzymes. SOD, CAT, GPx, GR, GSH activities, which are significantly depleted and MDA levels elevated in alcohol treated rats, when compared with normal control rats. However, with TG administration for 30 days, all these antioxidant enzymes increased and MDA levels decreased significantly in alcohol treated rats. (Figurer 1). ($P < 0.001$).

3.2. Effect of TG on cardiac markers in alcohol treated rats

Fig. 2 shows the cardiac markers of all groups. CK-MB, TT, TI and MG levels are significantly increased in alcohol treated rats, when compared with normal control rats. But with TG supplementation, all these cardiac markers are decreased in alcohol intoxicated rats. Our study shows the cardioprotective effect of TG in alcoholic rats. (Fig. 2). ($P < 0.001$).

3.3. Effect of TG on liver markers enzymes in alcohol treated rats

Table 1 represents the liver markers in all groups. In alcohol intoxicated rats AAT, AST and ALP activities are significantly up-regulated, when compared with normal control rats. However, TG supplementation for 30 days significantly down-regulated AAT, AST and

Table 1
Effect of TG on Liver Marker enzymes in alcohol intoxicated rats.

Groups	AAT (IU/L)	AST (IU/L)	ALP (IU/L)
Group I (NC)	40.24 (±4.261)	40.84 (±1.620)	146 (±9.826)
Group II (TGt)	41.768 (±4.28)	41.352 (±2.82)	132 (±10.68)
Group III (AC)	194.28* (±7.642)	124.42* (±11.398)	296* (±12.368)
Group IV (At + TGt)	82.46* (±3.42)	68.84* (±3.16)	186* (±10.26)
Group V (At + Vit E)	62.12* (±3.16)	48.84* (±2.94)	164* (±12.42)

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

ALP activities in alcohol group when compared to alcohol control group. Our investigation reports that TG possess hepatoprotective effect in alcohol treated rats. (Table 1).

3.4. Effect of TG on heart in alcoholic rats

Fig. 3 represents the histopathological observation of heart tissue of all groups. Normal control rats, and TG treated rats showed normal architecture of the heart. The heart tissue of control rats showed the normal cardiac cells, myocytes and normal myofibrils. Disrupted blood vessels, vacuolization, disrupted cardiac cells and severely damaged myofibrils are observed in alcohol intoxicated rats. However, with TG administration in alcoholic rats, regeneration of nucleus, regeneration of cardiac cells and myocytes are observed. From this histopathological studies, it is proved that trigonelline has cardio protective effect in alcohol intoxicated rats (Fig. 3).

3.5. Free radical scavenging effect of TF

Free radical scavenging activities are summarized in Fig. 4. In this study, DPPH, H_2O_2 and hydroxyl radicals are estimated in ethanolic extract of *Trigonella foenum-graecum* and they showed promising results. The antioxidant activities of the TF were compared with ascorbic acid. (Fig. 4).

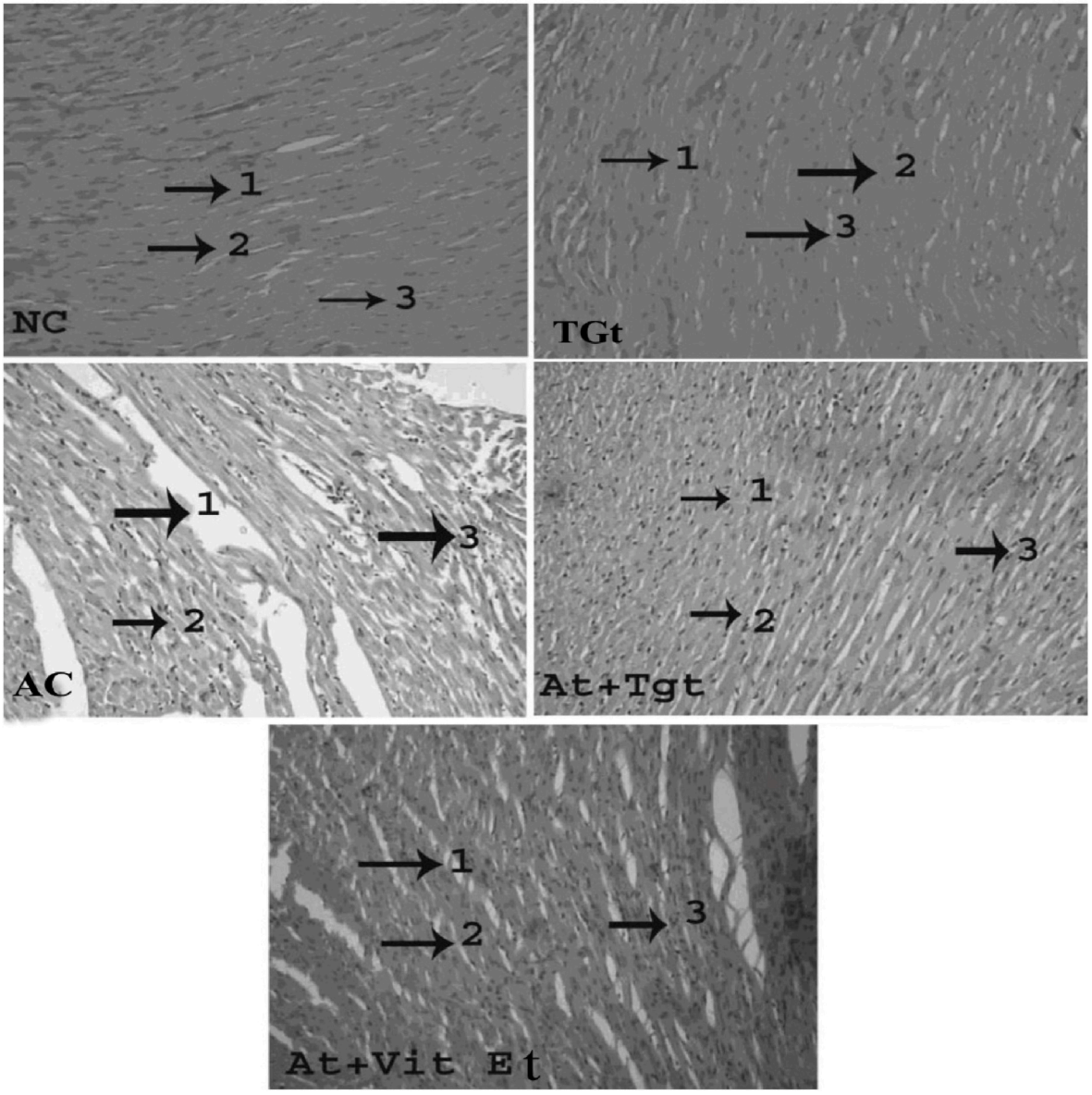

Fig. 3. Effect of TG on Heart tissue in alcohol intoxicated rats.
Photomicrograph of Heart tissue sections from: 1) Normal control rat showed normal structure of heart tissue, 1. Normal cardiac cells, 2. Normal myocytes, 3. Normal myocardial fibers, 2) TG t Control also showed Normal structure of heart tissue like 1. Normal cardiac cells, 2. Normal myocytes, 3. Normal myocardial fibers, 3. Alcohol-control (AC) rat showed 1. Disruption of cardiac cells, 2. Coagulation of blood, 3. Degeneration of myocardial fibers, 4. At + Tgt showed 1. Regeneration of cardiac cells, 2. Regeneration of myocytes, 3. Regeneration of myocardial fibers, 5. At + Vit Et: showed 1. Regeneration of cardiac cells, 2. Regeneration myocytes, 3. Regeneration of myocardial fibers. (H&E × 100).

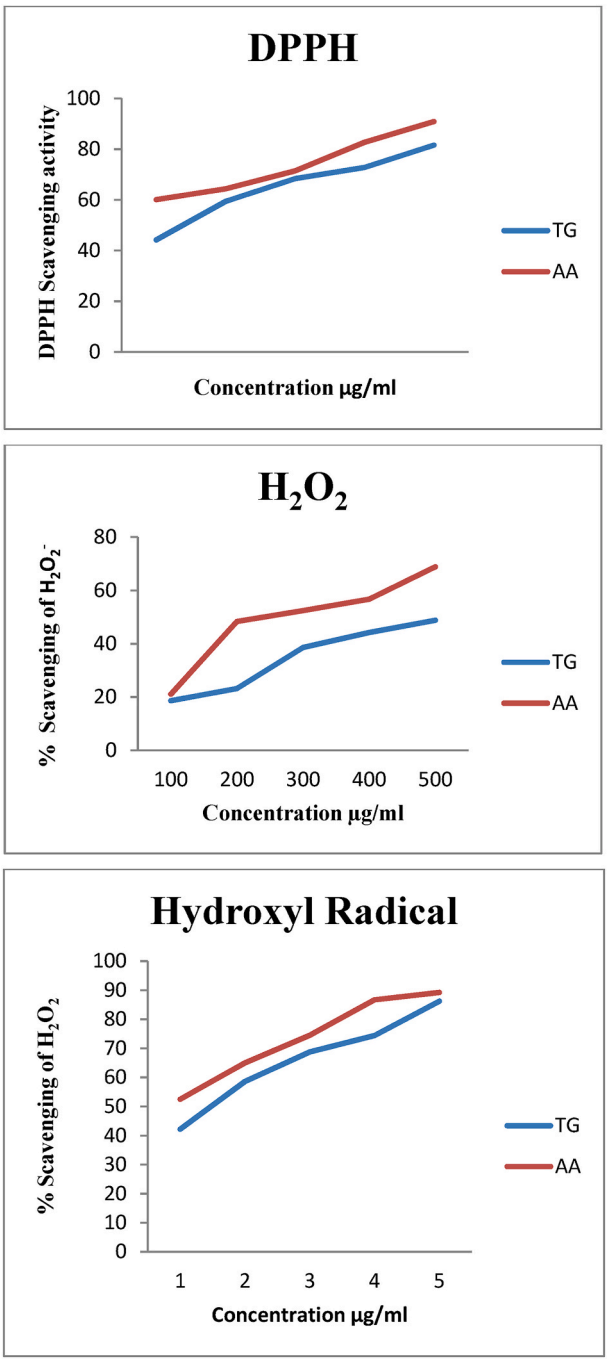

Fig. 4. DPPH, H_2O_2 and Hydroxyl radical effect of TG.

4. Discussion

Heart is being one of the important vulnerable organ and alcohol consumption can damage and cause MI and alters cardiac function. Hence, there is need for treatment for MI in alcoholic subjects. Continuous use of allopathic drugs for heart related disorders will have many side effects. It may damage heart and its function in alcoholic subjects. It also alters the functions of other organs like liver, kidney and brain. So there is need for alternative therapy instead of allopathy. Hence, herbal treatment is suggestable. Many medicinal plants like ginger, ocimum, curcumin and trigonella are used to treat heart related disorders. In our study *Trigonella foenum-graecum* bioactive compound trigonelline is used to study the cardio protective effect in alcohol toxicity rats.

Antioxidant defense mechanism is normal process that occurs in all animal cell including hepatic, cardiac cells and renal cells. Antioxidant enzymes act against free radicals during toxic conditions [16,17]. Free radicals like superoxide radicals, hydroxyl radicals, singlet oxygen, hydrogen peroxide are produced usually by the mitochondria. During alcohol toxicity oxidative stress produces free radicals, so antioxidants acts against to neutralize them. Cells use SOD, GPx, GR, CAT as antioxidants to defend themselves against the elevated free radicals in the body due to oxidative stress and protect the tissue from toxic conditions.

In our study, we reported that SOD and CAT activities are depleted in alcohol intoxicated rats. Superoxide dismutase scavenges the superoxide ions produced as cellular by-products of alcohol metabolism. Catalase acts as a preventive antioxidant and it plays key role in protection against the toxic effects of alcohol. Antioxidant enzymes SOD, GPx and CAT converts superoxide anions to hydrogen peroxide and hydrogen peroxide to water. Activation of antioxidant system is, therefore, referred as a promising approach to defend the heart against alcohol-induced oxidative stress [18,19]. However, with TG supplementation in alcohol treated rats, SOD and CAT activities are elevated. Trigonelline have antioxidant activity and it supress the production of superoxide anions and hydrogen peroxide, hence SOD & CAT activities are elevated with TG administration in alcohol intoxicated rats. (Fig. 1).

In our study, we observed a significant decrease in GPx, GR activities and GSH levels in alcohol ingested rats, that indicates the excess production of free radicals and these findings are similar with the reports of Shanmugam et al., 2010 [18]. Accumulating evidence also suggests that alcohol intoxication results in escalating free radical production, dropping of antioxidant enzymes and enhancing oxidative stress in many tissues, especially in the heart [20]. But with TG supplementation in alcoholic rats, these antioxidant enzymes activities are increased. Our results reported that the supplementation of TG can reduce free radicals and protect heart from alcohol intoxication. Tcheutchoua et al. [21], also reported that with Cymbopogon treatment in alcoholic rats, all these antioxidant enzymes came back to near to normal levels. (Fig. 1).

MDA is a secondary metabolite produced by lipid peroxidation and the amount of MDA is used as an indicator of lipid peroxidation [22]. In the current study, we found that heart MDA levels are upregulated in alcoholic rats. Alcohol administration induces the production of free radicals, which helps in the elevation of MDA levels. Alcohol induces tissue damage in liver, heart kidney and brain. In the present study, MDA levels are higher in alcohol intoxicated group due to the production free radicals. Mitha et al. [23] also reported that in alcohol fed rats, MDA levels are increased. However, with TG supplementation in alcohol intoxicated rats, MDA levels are reduced due to suppression of free radicals.

Cardiac markers are important for diagnosis of heart related disorders. In this study, we reported elevated activities of CK-MB, TT, TI and MG in alcohol intoxicated rats. Elevated levels of CK-MB, TT, TI and MG in alcoholic subjects are due to production of free radicals. Alcohol toxicity induces the production of free radicals and alters the function of heart. The free radical production during alcohol intoxication attacks the polyunsaturated fatty acids of the cardiac muscles and causes their rupture, resulting in the release of enzymes from cardiac tissue into the blood stream, hence these heart markers are up regulated in alcoholic subjects. However, with TG supplementation in alcohol administered rats, these cardiac marker levels are depleted.TG supplementation also reduced that production of free radicals in alcoholic subjects. Our results reported that TG possess cardio protective effect. (Fig. 2).

AAT, AST, and ALP are liver marker enzymes. These tissue markers help in diagnosis of liver related disorders. These serum markers are important biomarkers of hepatic tissue damage. Liver tissue damage results in the release of AAT, AST and ALP to the blood stream, hence serum marker levels are elevated in alcohol administered rats. Hence, in our study, we also observed that AST, AAT and ALP levels are upregulated in alcohol control group [24].Whereas with TG administration in alcohol intoxicated rats AST, AAT, and ALP levels are significantly down regulated. TG protected the hepatic tissue from alcohol induced

oxidative stress and toxicity in rats. This may be due to hepatoprotective effect of TG. Hence, in At + Tgt group AAT, AST, and ALP activities are decreased. (Table 1).

In this study, we observed coagulation of blood, degenerated cardiac muscles, vacuolization, and severely damaged myofibrils in alcohol toxicity rats. But in At + Tgt rats, we observed the regeneration of nucleus, regeneration cardiac cells and regeneration of myocytes. Our study reported that TG have cardio protective effect in alcohol intoxicated rats. (Fig. 3).

Free radical scavenging activities are very important to know medicinal plants possess these activities or not. It will help in preventing the deleterious effects of free radicals in many disease conditions like cancer, diabetes and hepatitis. DPPH, hydroxyl and H_2O_2 free radical scavenging activities are estimated in ethanolic extract of TF. Invitro studies of medicinal plants predicts antioxidant potential through free radical scavenging assays, which helps in the production of herbal drugs. Our results revealed that the ethanolic extract of *Trigonella foenum-graecum* showed promising free radical scavenging effect. The results are compared with standard ascorbic acid. The ethanolic extract of *Trigonella foenum-graecum* showed good DPPH, hydroxyl radical and H_2O_2 scavenging activity. Secondary metabolites, like alkaloids, phenolics, and flavonoids are present in TF, which posses' free radical scavenging effects, which in turn helps in protecting the tissue from oxidative damage from reactive oxygen species in alcohol intoxicated rats [25]. (Fig. 4).

4.1. Conclusion

From our study, we concluded that trigonelline have antioxidant, cardio protective and hepatoprotective effect in alcohol intoxicated rats. Histopathological studies also proved that TG protected heart tissue from alcohol toxicity in rats. Hence, trigonelline may be useful to treat myocardial infarction in alcoholic subjects.

Authors contributions

Experimental design and the Idea of the design: Dr. M. Guru Sekhar and Dr. K.R.Shanmugam.

Biochemical estimations and interpretation of the data: Dr. M. Guru Sekhar, Dr. K.R. Shanmugam, I.S. Chakrapani.

Drafting of the Manuscript and submission: Dr. M. Guru Sekhar and Dr. K.R. Shanmugam.

Source of funding

Nil.

Decleration of generative AI in scientific writing

None.

Declaration of competing interest

None.

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