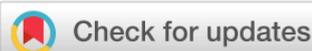


Original Research

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## Effects of *Euphorbia thymifolia* and *Euphorbia hirta* leaf extracts on membrane-bound, mitochondrial enzymes and lipid profile of carbon tetrachloride-induced hepatotoxicity in rats

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**ABSTRACT:** The present investigation was aimed to identify the potentiality of *Euphorbia thymifolia* Linn. and *Euphorbia hirta* Linn. leaf extract on the toxin-induced (carbon tetrachloride - CCl<sub>4</sub>) Albino Wistar rats. The animals were grouped into 7 categories including control (basal diet, G1), CCl<sub>4</sub>-induced (1.5 mL/kg, b.w., i.p.) (G2), G1 administered with 300 mg/kg b.w., extract of *E. thymifolia* (G3) and *E. hirta* (G4), G2 administered with 300 mg/kg b.w., extract of *E. thymifolia* (G5), *E. hirta* (G6), and standard drug (silymarin 25 mg/kg b.w.; G7) for 21-days trial period with each group contains 6 rats. The samples were collected and the following parameters including mitochondrial enzymes, different ATPase and lipid profiles were analyzed. The membrane-bound enzymes, the mitochondrial enzymes levels and the lipid profiles were reduced in the toxin-induced rats but the levels of enzymes were restored, significantly increased and lipid profiles are returned to the normal in the treatment of both extracts.

## 1. INTRODUCTION

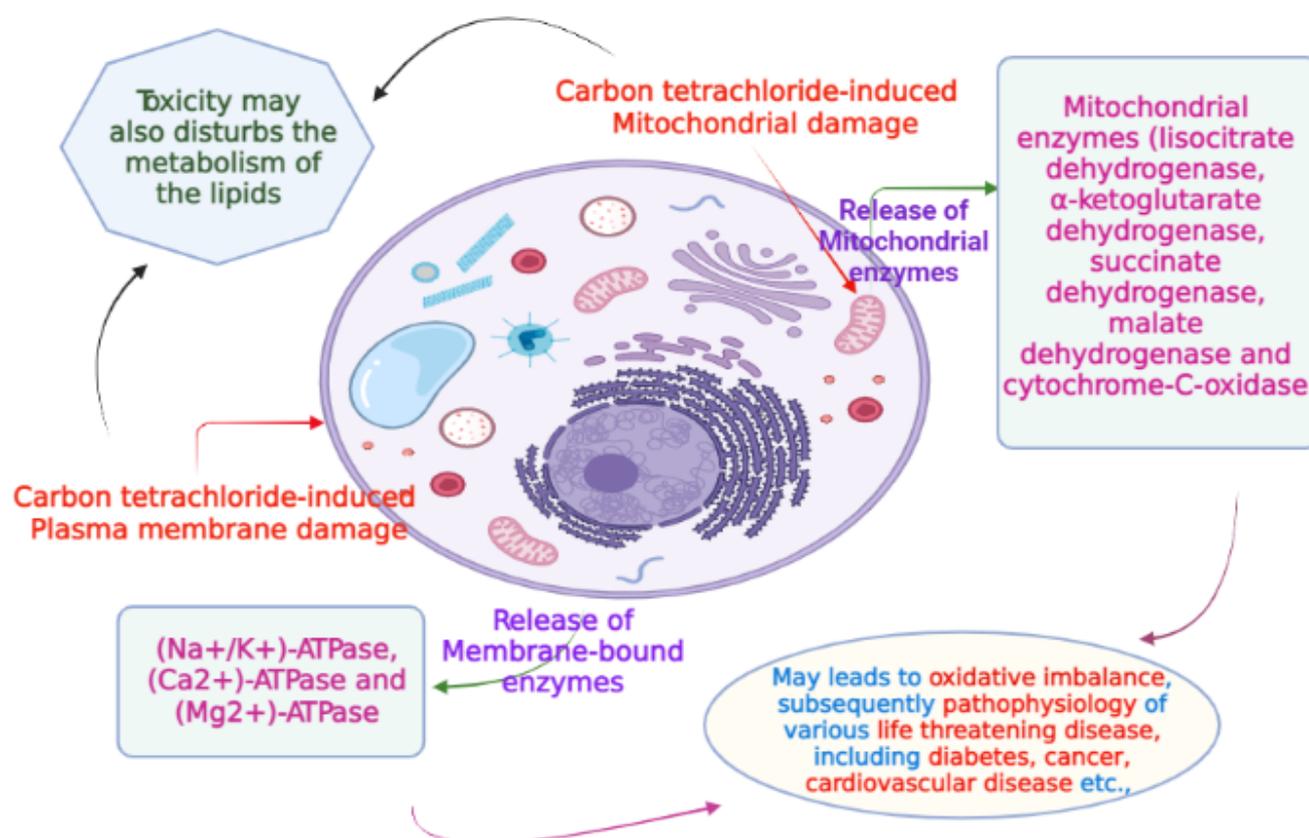
The liver is one of the most important organs and mainly involves the metabolism of biomolecules, protein synthesis, production of various biochemical compounds, detoxification and regulating homeostasis in the body (Rose, 2001; Udomsinprasert & Jittikoon, 2019). In addition, aids in the bile secretion and the storage of minerals as well as vitamins (Ahsan et al., 2009; Tavakoli et al., 2019). The liver has been exposed to substances from exogenous origin like environmental toxins, drugs and alcohol, which

may lead to complications in the liver, generally presenting as a distinct pattern of diseases such as cirrhosis, hepatitis, haemochromatosis, cholestatic, coronavirus (Dinesh et al., 2014; Li & Fan, 2020). In the case of disturbed liver functions due to toxins, may alter the chemical composition of the liver and its subcellular organelles (Figure 1). The modification in the structure of the liver and its function may result in jaundice, increased bleeding, portal hypertension and causes multiple metabolic changes which may affect the functions of the other organs (Ibrahim et al., 2008).

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**Figure 1.** Carbon tetrachloride-induced membrane damage.

Synthetic antioxidants like butylated hydroxyl anisole and butylated hydroxyl toluene are having various toxic effects in animals, including human beings (Madhavi & Salunkhe, 1995). The  $\text{CCl}_4$ -toxicity, which increases the cytochrome  $\text{P}_{450}$  system, induces free radical formation, affects the liver microsomes and consequently causes lipid peroxidation in the liver (Hamed et al., 2019). Due to the adverse effect of allopathic drugs, research is interested to find safer drugs from medicinal plants. Medicinal herbs and their extracts are widely used for the identification and development of new therapeutic agents for treating liver complications including hepatitis, cirrhosis, and loss of appetite (Lee et al., 2018; Recknagel, 1983). *Euphorbia thymifolia* Linn. and *Euphorbia hirta* Linn. plants belong to the family of Euphorbia are widely used in their general medicinal activities.

The *E. thymifolia* is found in tropical regions. The traditional use of this *E. thymifolia* is mainly due to its actions involving laxative, aromatic, sedative, blood purification, antiviral, antihelminthic, anti-inflammatory, anti-spasmodic, antifungal, anti-bacterial, anti-microbial, diuretic properties (Mani et al., 2013, 2016). The *E. hirta* has been commonly used as a medicinal plant for treating diseases including gastrointestinal disorders, inflammations of the skin and mucous membranes and respiratory system disorders by the use of whole plant and weed extract. The plant is generally found in India, China,

Malaysia, the Philippines, Australia and Africa (Kausar et al., 2016; Mani et al., 2016). Hence, the present study focuses on the protective effect of the membrane-bound, mitochondrial enzyme activity and lipid profile levels of the ethanolic leaf extract of *E. thymifolia* Linn. and *E. hirta* Linn. on hepatotoxic rats induced by carbon tetrachloride ( $\text{CCl}_4$ ).

## 2. MATERIALS AND METHODS

### 2.1. Plant materials collection and extracts preparation

The *E. thymifolia* and *E. hirta* were collected from Kanchipuram, India and the species were identified by a botanist from St. Joseph's College, Tiruchirappalli, India and the same were deposited at the Rapinet Herbarium with voucher numbers GDMM 001 and GDMM 002. The shade dried *E. thymifolia* and *E. hirta* leaves were powdered mechanically and then extracted using ethanol as the solvent by hot continuous percolation method using Soxhlet apparatus for 24 h. The extracts were filtered and evaporated on a water bath followed by drying in a vacuum. The prepared extracts were stored under the refrigerator until further analysis. All other chemicals mentioned in this study were analytical grades obtained from Hi-Media (Mumbai, India).

## 2.2. Animal housing and experimental design

The eight weeks old Albino Wistar rats (150-165 g) were procured from the Biogen Laboratory Animal Facility, Bengaluru, India. The same was maintained at  $25 \pm 1^\circ\text{C}$  with a 12 h light/dark cycle. The animals were fed with a standard pellet diet which is obtained from Amrut Laboratory Animal Feed, Bangalore, India. The diet consists of 22.21 per cent of protein, 3.32 per cent of fat, 3.11 per cent fiber, which is balanced with 67% of carbohydrates, minerals and vitamins as well as water *ad libitum*. The study and experimental procedures were approved by the Ethical Committee of the Srimad Andavan College of Arts and Science (Registration Number: 790/03/ac/CPCSEA), Tiruchirappalli, India. The rats have cared as per the guidelines provided by the board for control and supervision of experimental animals (CPCSEA, 2004).

Ethanol leaf extract of *E. thymifolia* and *E. hirta* (300 mg/kg b.w.) were freshly suspended in sterile water and the same was administered to the animals orally by intubation early morning every day till the end of the study period. The rats were arbitrarily divided into 7 groups with 6 rats in each group and the same were housed individually in the ventilated cages. Animal groups were categorized as control (basal diet, G1) were given saline water,  $\text{CCl}_4$ -induced single cardiac dose (1.5 mL/kg, b.w., i.p.) as negative control (G2), G1 were given 300 mg/kg b.w., of ethanol extract of *E. thymifolia* (G3) and *E. hirta* (G4), G2 supplemented with 300 mg/kg b.w., of ethanol extract of *E. thymifolia* (G5), *E. hirta* (G6), and 25 mg/kg b.w., of silymarin (G7) for the period of 21 days.

## 2.3. Biochemical evaluation

All the rats were sacrificed by decapitation at the end of the study period and the blood was taken from the jugular vein. It has been centrifuged at 3000 rpm for 20 min and the serum samples were stored under a refrigerator until used. The following parameters were analyzed for the ATPase including calcium  $\text{Ca}^{2+}$ -ATPase and sodium/potassium ( $\text{Na}^+/\text{K}^+$ )-ATPase (Hjerten and Pan, 1983), magnesium ( $\text{Mg}^{2+}$ )-ATPase (Ohnishi et al., 1982), and mitochondrial enzymes like ICH (isocitrate dehydrogenase) (Bell & Baron, 1960), KDH ( $\alpha$ -ketoglutarate dehydrogenase) (Reed & Mukherjee, 1969), SDH (succinate dehydrogenase) (Slater & Bonner, 1952), MDH (malate dehydrogenase) (Mehler et al., 1948), cytochrome-C-oxidase (Pearl et al., 1963) and nicotinamide adenine dinucleotide dehydrogenase (NADPH) (Minakami et al., 1962). The lipid profiles including TC (total cholesterol) (Allain et al., 1974), TG (triglycerides) (Mcgowan et al., 1983), PL (phospholipids) (Zilversmit & Davis, 1950), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and very-low-density lipoprotein (VLDL) cholesterol by the calculating method described by Friedewald et al. (1972). All the biochemical parameters are done using commercial available kits and was determined by fully automated biochemical analyzer (Turbo Chem 100, CPC Diagnostics).

## 2.4. Statistical analysis

The data were analyzed using the commercial statistical software (SPSS for Windows, with version 17.0, Chicago, USA) for one-way analysis of variance (ANOVA) which is followed by Duncan's multiple range tests (DMRT) analysis. The results were expressed as means  $\pm$  SD (standard deviation). In the present study, P values with  $<0.05$  were considered significant.

## 3. RESULTS

The different membrane-bound enzymes such as  $\text{Na}^+/\text{K}^+$ ATPase,  $\text{Mg}^{2+}$ ATPase and  $\text{Ca}^{2+}$ ATPase of the control and treatment groups were seen in Table 1. These enzyme activities were significantly reduced at  $P < 0.05$  when the administration of  $\text{CCl}_4$ . After, the treatment of silymarin and ethanolic extracts of leaves of the *E. thymifolia* and *E. hirta* significantly ( $P < 0.05$ ) restored the levels of the altered enzyme when compared with the control group. There was no adverse effect was observed in plant extracts alone groups of both plants. Table 2 depicts the mitochondrial enzymes (ICH, KDH, SDH, MDH, cytochrome-C-oxidase and NADPH dehydrogenase) in the control and treated groups. Toxication with  $\text{CCl}_4$  reduced the levels of the mitochondrial enzymes significantly ( $P < 0.05$ ) when compared to the control group. The altered activities of the above enzymes were restored normalcy significantly ( $P < 0.05$ ) when the administration of ethanolic extract of both plant extracts and silymarin. Table 3 showed that the serum lipid profile of TC, TG, HDL, LDL, VLDL and PL in the normal control group and experimental groups. The administration of  $\text{CCl}_4$  disturbed the lipid metabolism. The ethanolic extract of *E. thymifolia* and *E. hirta* (300 mg/kg) and silymarin (25 mg/kg) normalize the altered lipid profile level significantly at  $P < 0.05$ . In the present study, the plant extracts group did not show any side effect in all the studied parameters.

## 4. DISCUSSION

The ATPase is related to the cell membrane and it helps for the translocation of ions which includes magnesium, calcium, sodium and potassium, and. It requires energy for the translocation process. The ATPase regulates the concentration of cellular electrolytes and transmembrane electrolytes. In hepatotoxic conditions, the subcellular metabolism and structural alterations are formed in the cell membrane (Premalatha & Sachidanandam, 1998), which are in agreement with the same in the present  $\text{CCl}_4$ -induced hepatotoxic animals. Among the ATPase, the  $\text{Na}^+/\text{K}^+$ ATPase regulates the concentration of intracellular  $\text{Na}^+$  at a low level and it also maintains the water content in the cell (Chandramohan et al., 1996). Inhibition of this  $\text{Na}^+/\text{K}^+$ ATPase has minimized the cellular metabolism. This ATPase is inhibited by increasing the concentration of cholesterol (Yeagle, 1983). The statement has been proved in this study, because the increasing concentration of cholesterol may be there as on for the decreasing concentration of the  $\text{Na}^+/\text{K}^+$ ATPase level are noted in the present findings.

**Table 1**Activities of ethanolic extract of *E. thymifolia* and *E. hirta* on the membrane-bound enzymes.

Animal Group	Na <sup>+</sup> /K <sup>+</sup> ATPase (mg/dl)	Ca <sup>2+</sup> ATPase (mg/dl)	Mg <sup>2+</sup> ATPase (mg/dl)
Group-I	1367.29±120.71 <sup>a</sup>	10.72±0.98 <sup>a</sup>	15.17±0.713 <sup>a</sup>
Group-II	693.17±104.32 <sup>b</sup>	4.03±0.63 <sup>b</sup>	5.32±0.62 <sup>b</sup>
Group-III	1398.10±110.35 <sup>a,c</sup>	11.02±0.54 <sup>a</sup>	15.01±0.59 <sup>a</sup>
Group-IV	1387.49±117.63 <sup>a,c</sup>	10.91±0.72 <sup>a</sup>	14.79±0.76 <sup>a</sup>
Group-V	1238.19±101.79 <sup>c</sup>	9.87±0.63 <sup>a,c</sup>	10.72±0.93 <sup>a</sup>
Group-VI	1245.16±110.18 <sup>c</sup>	10.17±0.59 <sup>a,c</sup>	12.76±0.53 <sup>a</sup>
Group-VII	1303.69±988.72 <sup>a</sup>	10.97±0.79 <sup>a,c</sup>	14.62±0.69 <sup>a</sup>

G1 - Control, G2 - Negative control - CCl<sub>4</sub>-induced, 1.5 mL/kg, G3 - G1 + *E. thymifolia* 300 mg/kg, G4 - G1 + *E. hirta*, 300 mg/kg, G5 - CCl<sub>4</sub> + *E. thymifolia*, 300 mg/kg, G6 - CCl<sub>4</sub> + *E. hirta*, 300 mg/kg, G7 - CCl<sub>4</sub> + silymarin, 25 mg/kg. All the values are mentioned as means ± SD, six rats in each group. The different superscripts significantly differ at p ≤ 0.05

**Table 2**Activities of ethanolic extract of *E. thymifolia* and *E. hirta* on mitochondrial enzymes.

Animal Group	ICH (U/mg protein)	KDH (U/mg protein)	SDH (U/mg Protein)	MDH (U/mg protein)	NADPH (U/mg protein)	CYT-C (U/mg protein)
Group-I	770.12±10.31 <sup>a</sup>	190.62±10.32 <sup>a</sup>	33.63±1.63 <sup>a</sup>	349.61±12.12 <sup>a</sup>	34.36±1.72 <sup>a</sup>	6.62±0.31 <sup>a</sup>
Group-II	551.36±28.12 <sup>b</sup>	140.31±9.76 <sup>b</sup>	22.43±1.32 <sup>b</sup>	220.54±12.11 <sup>b</sup>	18.61±2.01 <sup>b</sup>	3.97±0.35 <sup>b</sup>
Group-III	771.36±10.32 <sup>a,c</sup>	191.23±10.12 <sup>a</sup>	34.01±1.72 <sup>a</sup>	349.91±19.21 <sup>a</sup>	34.03±1.92 <sup>a</sup>	6.78±0.50 <sup>a</sup>
Group-IV	772.41±11.32 <sup>a,c</sup>	191.72±10.36 <sup>a</sup>	34.42±2.21 <sup>a</sup>	350.60±20.35 <sup>a</sup>	34.17±1.99 <sup>a</sup>	6.84±0.45 <sup>a</sup>
Group-V	754.32±15.67 <sup>c</sup>	180.42±14.69 <sup>a,c</sup>	32.41±2.02 <sup>a</sup>	336.44±18.14 <sup>a</sup>	32.13±2.12 <sup>a</sup>	6.01±0.39 <sup>c</sup>
Group-VI	760.03±19.38 <sup>c</sup>	181.62±12.92 <sup>a,c</sup>	32.83±1.72 <sup>a</sup>	339.91±17.65 <sup>a</sup>	32.79±1.99 <sup>a</sup>	6.09±0.37 <sup>c</sup>
Group-VII	792.61±16.42 <sup>a</sup>	184.79±16.12 <sup>a,c</sup>	33.02±2.35 <sup>a</sup>	341.72±18.32 <sup>a</sup>	33.99±2.03 <sup>a</sup>	6.12±0.36 <sup>a,c</sup>

G1 - Control, G2 - Negative control - CCl<sub>4</sub>-induced, 1.5 mL/kg, G3 - G1 + *E. thymifolia* 300 mg/kg, G4 - G1 + *E. hirta*, 300 mg/kg, G5 - CCl<sub>4</sub> + *E. thymifolia*, 300 mg/kg, G6 - CCl<sub>4</sub> + *E. hirta*, 300 mg/kg, G7 - CCl<sub>4</sub> + silymarin, 25 mg/kg. All the values are mentioned as means ± SD, six rats in each group. The different superscripts significantly differ at p ≤ 0.05

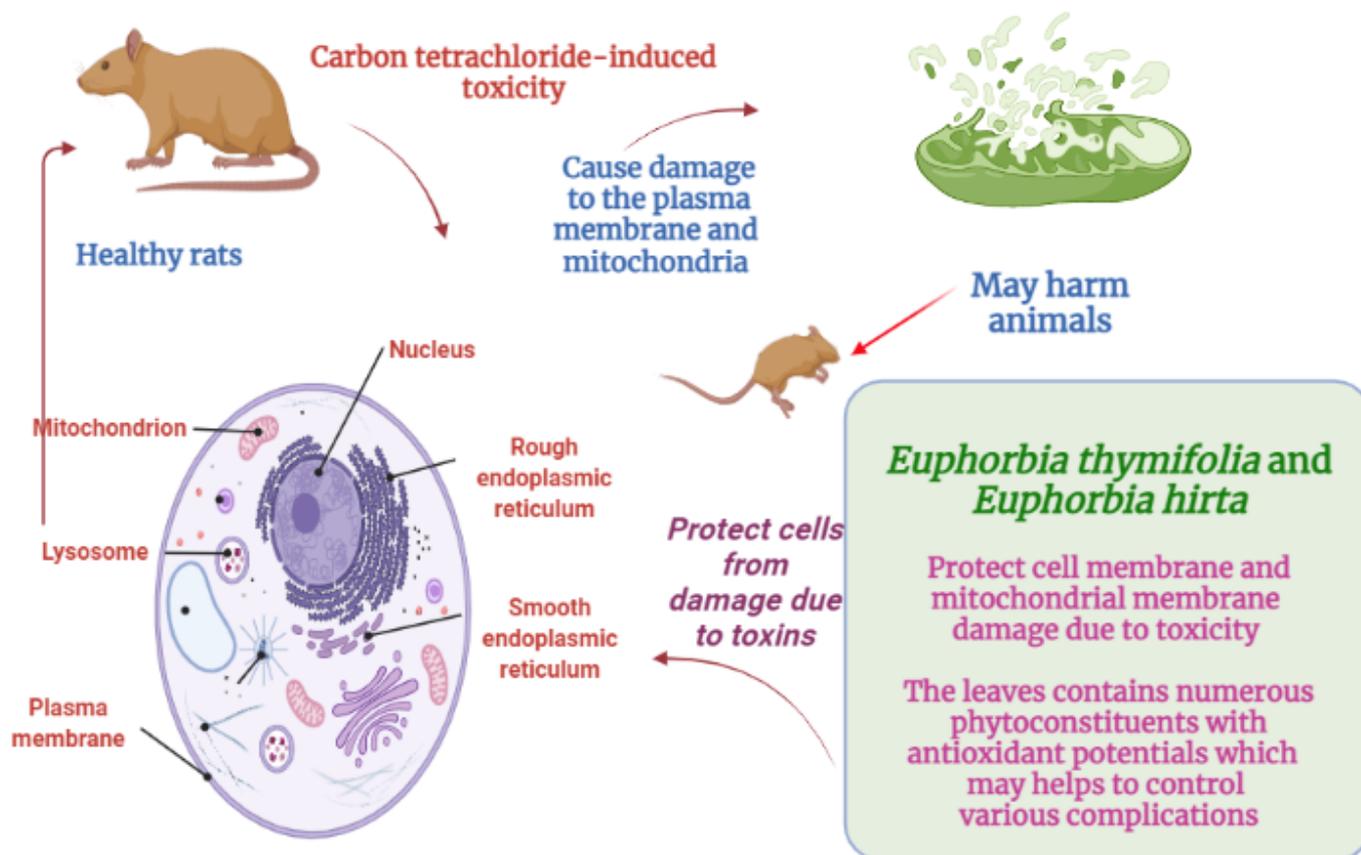
**Table 3**Effect of ethanolic extract of *E. thymifolia* and *E. hirta* on Lipid profiles.

Animal Group	TC (mg/dl)	TG (mg/dl)	PL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Group-I	75.00±6.52 <sup>a</sup>	55.86±5.31 <sup>a</sup>	116.33±7.7 <sup>a</sup>	46.03±3.51 <sup>a</sup>	11.17±0.57 <sup>a</sup>	19.20±2.11 <sup>a</sup>
Group-II	140.07±9.40 <sup>b</sup>	120.81±9.02 <sup>b</sup>	165.78±8.41 <sup>b</sup>	26.41±1.32 <sup>b</sup>	24.36±1.28 <sup>b</sup>	88.21±5.20 <sup>b</sup>
Group-III	74.07±5.02 <sup>a</sup>	54.98±4.38 <sup>a</sup>	144.42±5.68 <sup>c</sup>	45.01±2.85 <sup>a</sup>	10.92±0.65 <sup>a</sup>	18.61±1.40 <sup>a</sup>
Group-IV	73.02±4.90 <sup>a</sup>	53.71±6.38 <sup>a</sup>	112.43±4.90 <sup>a</sup>	46.72±2.51 <sup>a</sup>	11.03±0.78 <sup>a,c</sup>	18.98±2.24 <sup>a</sup>
Group-V	96.04±5.20 <sup>c</sup>	78.16±2.16 <sup>a</sup>	116.12±5.20 <sup>a</sup>	41.26±1.26 <sup>c</sup>	10.83±0.53 <sup>a</sup>	13.62±4.68 <sup>c</sup>
Group-VI	93.03±3.16 <sup>c</sup>	75.04±3.16 <sup>c</sup>	129.22±4.20 <sup>c</sup>	42.26±2.32 <sup>c,d</sup>	11.36±0.48 <sup>a,c</sup>	14.48±3.46 <sup>c</sup>
Group-VII	81.62±4.65 <sup>a,c</sup>	73.12±2.14 <sup>c</sup>	125.12±3.20 <sup>c</sup>	44.32±1.22 <sup>a,d</sup>	11.98±0.43 <sup>c</sup>	15.28±2.68 <sup>a,c</sup>

G1 - Control, G2 - Negative control - CCl<sub>4</sub>-induced, 1.5 mL/kg, G3 - G1 + *E. thymifolia* 300 mg/kg, G4 - G1 + *E. hirta*, 300 mg/kg, G5 - CCl<sub>4</sub> + *E. thymifolia*, 300 mg/kg, G6 - CCl<sub>4</sub> + *E. hirta*, 300 mg/kg, G7 - CCl<sub>4</sub> + silymarin, 25 mg/kg. All the values are mentioned as means ± SD, six rats in each group. The different superscripts significantly differ at p ≤ 0.05

The Ca<sup>2+</sup>ATPase regulate the Ca<sup>2+</sup>pump activity, and it regulates the cellular processes. Alkon and Rasmussen (1988) proves that the the Ca<sup>2+</sup>ATPase are involved in the muscle concentration and neurosecretion process. The protein depletion has been associated with the diminished level of Ca<sup>2+</sup>ATPase in CCl<sub>4</sub>-administrated animals. In CCl<sub>4</sub>-induced animals, the level of Ca<sup>2+</sup>ATPase decreased due to the H<sub>2</sub>O<sub>2</sub> present in the toxic condition. This may be evidence of the previous study (Monte et al., 1984). The increasing concentrations of lipid peroxide decrease the Ca<sup>2+</sup>ATPase level due to the inhibition of thiol oxidation. The treatment of plant extracts may increase the Ca<sup>2+</sup>ATPase level. After the treatment, the level of lipid peroxide increases and favours the thiol oxidation and increases the Ca<sup>2+</sup>ATPase level.

The Mg<sup>2+</sup>ATPase plays an important role in the electrolyte transport across the biological membrane. The administration of CCl<sub>4</sub> alters the membrane permeability, may be due to the decreasing concentrations of Mg<sup>2+</sup>ATPase. In the present findings, the level of Mg<sup>2+</sup>ATPase has been noted to be decreased in CCl<sub>4</sub>-induced animals. The restoration of the normal levels of the Mg<sup>2+</sup>ATPase is achieved may be due to the treatment of *E. thymifolia* and *E. hirta* treated animals. The decreasing concentration of lipid peroxide may protect the membrane-bound enzymes from oxidative damage and this is the reason for the reducing levels of Mg<sup>2+</sup>ATPase has noted in the plant extract-treated animals. This is evident in cold-pressed *Coriandrum sativum* oil have potential antioxidant properties (El-Hadary & Hussanien, 2016). Similarly, *Physalis*



**Figure 2.** Protective effect of *Euphorbia thymifolia* and *Euphorbia hirta* leaf on carbon tetrachloride-induced membrane damage.

*peruviana* juice acts as a modulate the apoptosis and cell cycle arrest linked to hepatocellular carcinoma.

The viability of the cell is mainly determined by the functions of the mitochondria, the cell energy obtained from the mitochondria by  $\beta$ -oxidation of fatty acids, Krebs's cycle and oxidative phosphorylation. Some of the exogenous toxins like juice act inhibit the above pathway and the events in the respiratory chain (Pessayre et al., 1999; Vijayakumar et al., 2020). In  $\text{CCl}_4$ -treatment induces oxidative stress, thereby it reduces the formation by reducing equivalents (Vijayakumar et al., 2019). The mitochondria is one of the important intracellular targets of  $\text{CCl}_4$ . Hence, Krebs's cycle enzymes and respiratory chain enzymes are easily affected by  $\text{CCl}_4$ . The ICH is an important mediator enzyme used to supply the NADPH is essential for the production of GSH, which is used to protect the mitochondria from cellular damage. In the present findings, the decline of ICH was noted in  $\text{CCl}_4$ -induced animals due to oxidative damage of the liver (Al-Assaf, 2014).

The liver is the main site for metabolic processes including lipogenesis and lipoprotein synthesis. In the present findings,  $\text{CCl}_4$  damages the liver and causes hepatic necrosis. It leads to the accumulation of fat in the liver (Becker et al., 1987). Roullier (1964), proves that the fat molecules are deposited in adipose tissue in the liver during the  $\text{CCl}_4$ -induced toxicity. In the disease condition, the production and the metabolism of

cholesterol were impaired. Due to the fat accumulation in the liver the cholesterol level is increased in the bloodstream as well as significant changes are noted TG and hepatic PL is seen in the  $\text{CCl}_4$ -induced animals in the present investigation. The elevated serum TG is an independent and important risk factor for the pathophysiology of cardiovascular disease (Anand et al., 2008). The PL, an important component in a cell membrane, and responsible for cell integrity. This compound regulates the permeability of cells because it involves signal transduction activity. In the present investigation, the PL level has been raised in the  $\text{CCl}_4$ -induced animals, due to the cell damage by the toxin. This statement has been proved in the earlier study of Kaffarnik et al. (1975). After the treatment of *E. thymifolia* and *E. hirta* decreases the PL level. This may be due to the protective activity of plant extracts on the cell membrane (Figure 2).

The present study demonstrated that the LDL and VLDL were increased and the level of HDL has decreased in the  $\text{CCl}_4$ -treated animals. The HDL plays an important role in the removal of excess cholesterol in the liver via the bile (Dietschy, 1997). The increases in cholesterol level may result from the decline of HDL level (or) the increased fatty acid synthesis in the liver as well as the accumulation of TG suppress the secretion of lysosomal acid triacylglycerol lipase activity (Gans, 1973). Due to the liver damage, the LDL receptor defect

occurs at the site of the liver and fails to perform its function, this may increase the LDL level in the blood (Bharathi et al., 2018; Vijayakumar et al., 2018). The HDL level is indirectly proportional to the concentration of LDL and VLDL. After the treatment of *E. thymifolia* and *E. hirta* alters the levels lipid profile including TC, TG, PL, LDL, HDL and VLDL to near normal. Treatment with 200 mg/kg of cold-pressed *Syzygium aromaticum* reduced the levels of lipid profile in CCl<sub>4</sub>-induced hepatotoxicity (A.E. El-Hadary & Hassanien, 2016). In the present study, there is observable change noted in the plant only treated group when compared with the control group. This was evidenced in the safety of the plants. The maximum dose of 300 mg of both plant extract values is like the standard drug silymarin treated group.

## 5. CONCLUSION

From the present findings, the extracts of *E. thymifolia* and *E. hirta* enhances the activities of mitochondrial enzymes and reduce the membrane-bound enzymes, thereby it improves the anti-oxidant mechanism of mitochondria, as well as it, restored the lipid profile levels. Hence, further molecular studies are needed for the supporting evidence and it may be used as a drug soon for various liver-related diseases.

## 6. DATA AVAILABILITY

The data set for the present study is available from the corresponding author upon request.

## CONFLICTS OF INTEREST

Given his role as Associate Editor, Balamuralikrishnan Balasubramanian has not been involved and has no access to information regarding the peer review of this article. Full responsibility for the editorial process for this article was delegated to Associate Editor Onur Bender. There is no conflict of interest.

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## AUTHOR CONTRIBUTIONS

BB, VAA - Research concept and design; DMMG, MR, SP - Collection and/or assembly of data; BB, MR, SP, AM, RLR, WL, VAA - Data analysis and interpretation; DMMG - Writing the article; BB, SP, AM, RLR, WL, VAA - Critical revision of the article. All authors approved final version of the article for publication.

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