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Hepatoprotective and Antioxidant Effect of *Nyctanthes Arbor –Tristis Leaf* Fractions Against Ccl_4- Induced Liver Injury in Rats

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Abstract

The aim of the present work is to evaluate the hepatoprotective and antioxidant effect of Nyctanthes arbor –tristis leaf fractions. The petroleum ether, ethylacetate and butanolic fractions of Nyctanthes arbor –tristis leaves were studied to evaluate the hepatoprotective and antioxidant activities in CCl_4-induced hepatotoxicity in rats. Oral administration of the fractions at doses of 200 and 400 mg/kg once daily for 10 days significantly restored normalization of serum enzyme levels, viz. glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) and markers viz. total bilirubin and direct bilirubin and the results were comparable to the effects of Liv 52. The ethylacetate and butanolic extract at the dose of 400 mg/kg was found to be more potent when compared to petroleum ether extract at similar dose. The hepatoprotection is also supported by histopathology of treated animals. In regard to antioxidant activity, ethylacetate and butanolic fractions exhibited a significant effect showing increased levels of enzymatic and non-enzymatic parameters, viz. catalase, GSH, SOD and decreased level of malondialdhyde (MDA). The results of this study strongly indicate that Nyctanthes arbor –tristis leaves have potent antioxidant and hepatoprotective action against CCl_4-induced hepatic damage in rats which may be due to the presence phytoconstituents such as flavonoids.

Keywords: *Hepatoprotective, Antioxidant, Nyctanthes Arbor –Tristis, Fractions, Flavonoids.*

1. Introduction

Liver diseases are a major worldwide health problem, with high endemicity in developing countries. They are mainly caused by chemicals and some drugs when taken in very high doses. Despite advances in modern medicine, there is no effective drug available that stimulates liver function, offers protection to the liver from damage or help to regenerate hepatic cells. It is therefore necessary to search for alternative drugs to replace/supplement those in current use of doubtful efficacy and safety for the treatment of liver disease [1].

Today, human beings are exposed to certain environmental pollutants and foreign chemicals which are collectively referred to as xenobiotics, causing serious health problems. The liver is the major organ involved in the metabolism, detoxification and excretion of various endogenous and exogenous substances such as xenobiotics. The liver is an organ where many oxidative processes occur and is, therefore, an important target of Oxidative stress induced damage. Oxidative stress leads to cellular dysfunction, injury, and ultimately cell death, and the impairment of the antioxidant status in the liver contributes significantly to the pathogenesis and progression of chronic liver diseases [2]. The production of oxidative stress can be controlled by the antioxidant systems in the living organisms. Currently, many synthetic antioxidant drugs (BHT, TBHQ) have been used in drug composition. However, these

synthetic drugs can cause many side effects and lead to many potential health problems. In this connection, the herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness [3]. Management of liver diseases is still a challenge to the modern medicine. The modern medicines have little role in alleviation of hepatic ailments whereas most of the important representatives are from phytoconstituents. In Ayurveda a number of medicinal preparations have been employed for treating liver disorders and there are no rational drug therapies. The plants containing flavonoids, tannins and some phenolic components possess broad biological properties to exert beneficial effects on some liver diseases involving uncontrolled lipid peroxidation and free radical scavenging activity. Inspite of tremendous advances made in allopathic medicine, effective hepatoprotective medicine is still wanting. About 80% of world population relies on folklore medicine for curing ailments related to liver. However, only a small number of these medicinal plants as well as formulations used are scientifically evaluated for their activity. In the context of our ongoing search for new natural substances possessing hepatoprotective efficacy, the present investigation was undertaken by

utilizing the leaves of plant *Nyctanthes arbor –tristis,* a widespread large shrub belonging to the family Oleaceae is widely cultivated throughout India as a garden plant. The bitter leaves are used in Ayurvedic system of medicine for the treatment of rheumatism, sciatica, diuretic and intestinal worms. The powdered seeds are recommended for the treatment of scurvy [4, 5]. The reported phytoconstituents of the leaves are arborsides A, B, C, flavonol glycosides, astragalin and nicotiglorin. Flavonoids are large group of compounds occurring abundantly in plants. They occur as glycosides and contain several phenolic hydroxyl groups on their ring structure. Many flavonoids are found to be strong free radical scavengers and antioxidants [6]. Since leaves contain flavonoids, the present work has been to evaluate hepatoprotective and antioxidant potential of the fractions from the leaves of ***Nyctanthes arbor-tristis.***

2. Materials And Methods

Collection and Authentication

The leaves of *Nyctanthes arbor –tristis* were collected from local areas of Dharawad district and was authenticated by Dr.B.D. Huddar, Head, Department of Botany, Kadasiddheshwar Arts College and H.S. Kotambari Science Institute, Hubli.

Preparation Of Fractions

The *Nyctanthes arbor –tristis* leaves were shade dried at room temperature, pulverized into coarse powder. The leaf powder of *Nyctanthes arbor –tristis* was successively extracted by continuous hot percolation (soxhlation) with ethanol, further fractionated with Pet ether, Ethylacetate and Butanol with increasing order polarity. After the exhaustive extraction, the solvent was removed under reduced pressure (Buchi) using rotary flash evaporator then finally dried in dessicator.

Animal Selection

The experiments were carried out using Swiss albino mice weighing between 20-30 g for acute toxicity study and Wister albino rats weighing around 150-250 g for the hepatoprotective and antioxidant activity. The animals were maintained *adlibtum* at normal laboratory conditions and were given standard animal feed.

Acute Toxicity Studies

The Albino mice of either sex weighing between 20-30 g were used for the investigation. The animals were fasted overnight prior to experiment. An acute toxicity test was carried out as per OECD [7] guidelines and accordingly doses of fractions were studied. As per OECD guidelines the safest dose for all the fractions is 2000 mg/Kg body weight, hence 1/10 th and 1/5 th of the dose was taken as therapeutic dose.

Fractions Used

The petroleum ether, ethylacetate and butanolic fractions of *Nyctanthes arbor –tristis* were screened for hepatoprotective and *in-vivo* antioxidant activity. The fractions were suspended in distilled water using *Tween* 80 and were employed to assess the above activity. The dose was given orally.

Phytochemical Analysis

Phytochemical tests [8, 9] were carried out to detect the presence of phytoconstituents viz alkaloids, carbohydrates, flavonoids, tannins, triterpenoids, saponins etc.

Hepatoprotective Activity [10]

Chronic administration of carbon tetrachloride to rats induces severe disturbances of hepatic function together with histological observable liver disturbances. Hepatoprotective and i*n-vivo* antioxidant activity activity was carried out using Albino rats. The animals were divided into nine groups of six animals each as follows and maintained on standard diet and water *ad libitum.*

Group-I **:** Normal control (Vehicle treated Tween 80 (1%))

Group-II **:** Positive control (Untreated)

Group-III **:** Standard control (Liv 52)

Group-IV **:** Fraction-I (200mg/kg pet ether fraction NC)

Group-V **:** Fraction -II (200mg/kg ethylacetate fraction NC)

Group-VI **:** Fraction -III (200mg/kg butanolic fraction NC)

Group-VII **:** Fraction -I (400mg/kg pet ether fraction NC)

Group-VIII **:** Fraction -II (400/kg mg ethylacetate fraction NC)

Group-IX **:** Fraction -III (400mg/kg butanolic fraction NC)

All the groups were treated for 10 days. CCl_4 was used as a hepatotoxin to induce hepapatotoxicity to animals of groups II - IX on 3rd, 6th and 10th day by intraperitonial route. After 1 hour of the last dose of carbon tetrachloride injection, animals were sacrificed by cervical dislocation and the blood was collected from carotid artery and used for estimation of various biochemical parameters. The Biochemical parameters estimated includes serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloactetate transaminase (SGOT), serum alkaline phosphatase (SALP) and serum bilirubin with semiautoanalyser using diagnostic reagent kit.

Antioxidant Activity [10]

The isolated liver was rinsed with water and washed with ice cold saline and blotted to dry immediately. A liver homogenate was prepared with ice cold phosphate buffer. After centrifugation, the clear supernatant was used for the assay of various endogenous antioxidant parameters viz Reduced glutathione (GSH), Malondialdehyde (MDA), Superoxide dismutase (SOD) and Catalase (CAT) by standard methods.

Statistical Analysis

The results were expressed as mean $\pm$ SEM and evaluated using one way ANOVA followed by Dunnette multiple comparison test.

3. Results and Discussion

The qualitative chemical investigations of various fractions of *Nyctanthes arbor –tristis* revealed the presence of triterpenoids and steroids in Pet ether (40-60°C) extract. The ethylacetate extract was found to contain flavonoids, tannins and alkaloids, while butanolic extract contained carbohydrates, flavonoids and tannins. Further thin layer chromatographic studies were done to confirm the above phytoconstituents present in the various fractions. The results are as depicted in Table no 1.

Table 1: Qualitative chemical analysis of various solvent extracts of *Nyctanthes arbor -tristis* leaf

Phytoconstituents	Alcoholic extract	Petroleum ether (40-60°C) fraction	Ethylacetate fraction	Butanolic fraction	Aqueous fraction
Carbohydrates	+	-	-	+	+
Proteins	-	-	-	-	-
Saponin Glycosides	-	-	-	-	-
Flavonoid Glycosides	+	-	+	+	-
Steroids and Triterpenoids	+	+	-	-	-

Tannins and Phenol	+	-	+	+	+
Alkaloids	+	-	+	-	-

+ = Present, - = Absent

An attempt has been made to evaluate the hepatoprotective and *in vivo* antioxidant activity of *Nyctanthes arbor –tristis* leaf fractions by carbon tetra chloride induced hepatotoxicity model. The hepatoprotective and invivo antioxidant results of *Nyctanthes arbor –tristis* leaf fractions are reported in Table 2 and Table 3 respectively. The same results have been graphically represented in Fig.1, 2 and Fig. 4, 5 respectively.

Table 2: Effect of fractions of *Nyctanthes arbor –tristis* on biochemical parameters in carbon tetrachloride induced hepatotoxicity

Treatment	SGOT IU/L	SGPT IU/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl
Normal	62.15 ± 1.21	58.15 ± 1.48	157.8 ± 6.60	0.14 ± 0.01	0. 075 ± 0.07
Diabetes control	203.2 ± 8.73	176.6 ±1.71	380.2 ± 19.12	3.94 ± 0.94	0.498 ± 0.06
Standard (Liv-52)	73.80 ± 1.58***	73.38 ± 2.89***	174.2 ± 3.90***	1.138 ± 0.04***	0.116 ± 0.01***
Pet ether (200mg)	114.3 ± 3.60***	119.5 ± 1.87***	227.6± 4.89***	1.646 ± 0.12***	0.300 ± 0.04 ***
Ethylacetate (200mg)	102.85 ±1.83***	98.90 ± 3.31***	201.2 ± 5.25***	1.360 ± 0.15***	0.238 ± 0.02***
Butanol (200mg)	90.3 ± 2.90***	84.7 ± 2.10***	189.6 ± 6.70***	1.315 ± 0.13***	0.191 ± 0.05***
Pet ether (400mg)	100.5 ± 2.55***	100.4 ± 3.57***	201.4 ± 4.89***	1.640 ± 0.01***	0.253 ± 0.02***
Ethylacetate (400mg)	86.98 ± 6.47***	94.15 ± 5.49***	186.5 ±3.69***	1.277 ± 0.08***	0.188 ± 0.01***
Butanol (400mg)	84.32 ± 4.25***	82.48 ± 3.57***	178.1 ± 4.84***	1.213 ± 0.05***	0.125 ± 0.01***

Data were analysed by ANOVA followed by Dunnett's test.
Values are represented as mean ± S.E.M. (n=6); ns=nonsignificant, ***P < 0.001.

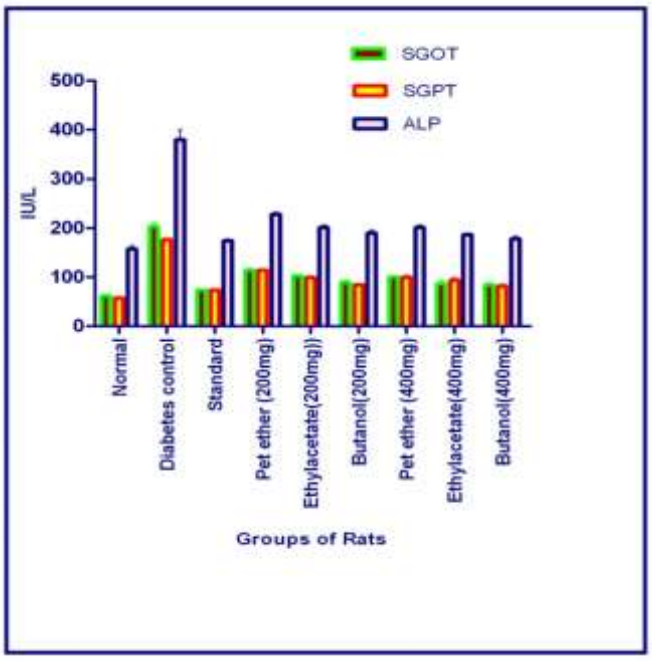

Fig.1: Graph showing effect of fractions of *Nyctanthes arbor –tristis* on

SGOT, SGPT and ALP in carbon tetrachloride induced hepatotoxicity

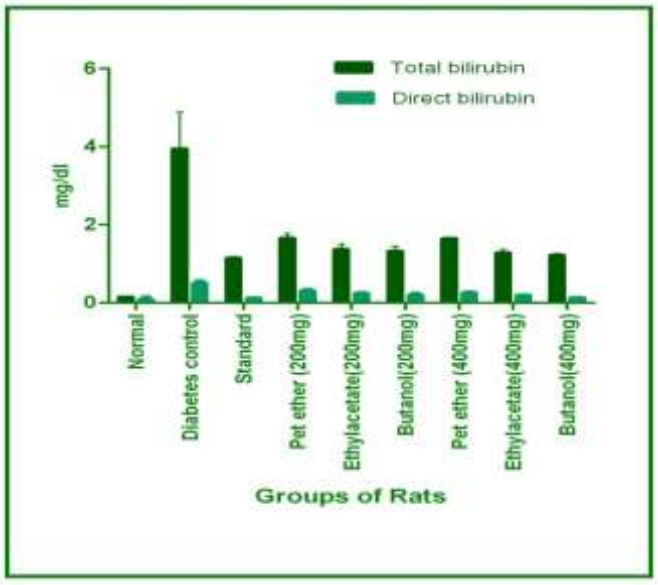

Fig. 2: Graph showing effect of fractions of *Nyctanthes arbor –tristis* on total bilirubin and direct bilirubin in carbon tetrachloride induced hepatotoxicity

Table 3: Effect of fractions of *Nyctanthes arbor –tristis* on antioxidant enzymes

Treatment	Antioxidant level			
	MDA nmol/mg wet tissue	**GSH nmol/mg wet tissue**	**SOD U/mg protein**	**CAT U/mg protein**
Normal	2.45 ± 0.11	6.45 ± 0.15	12.91± 0.24	47.07± 0.41
Diabetes control	6.40 ± 0.18	2.55 ± 0.27	4.58 ± 0.13	25.45 ± 1.80
Standard (Liv-52)	3.13 ± 0.15 ***	5.40 ± 0.40 ***	11.50 ± 0.12***	39.06 ± 0.42***
Pet ether (200mg)	4.81 ± 0.18 **	3.95 ± 0.47*	7.24 ± 0.45**	30.95 ± 1.42*
Ethylacetate (200mg)	4.22 ± 0.54**	4.03 ± 0.21*	8.01 ± 0.47***	31.85 ± 1.06**
Butanol (200mg)	4.01 ± 0.41**	4.51 ± 0.17**	9.02 ± 0.28***	33.82 ± 1.40***
Pet ether (400mg)	4.7 ± 0.36**	4.40 ± 0.39**	7.45 ± 0.41**	34.05 ± 1.21**
Ethylacetate (400mg)	3.76 ± 0.30***	5.20 ± 0.44***	9.48 ± 0.31***	36.62 ± 2.10***
Butanol (400mg)	3.66 ± 0.18***	5.30 ± 0.25***	10.78 ± 0.98***	37.58 ± 1.35***

Data were analysed by ANOVA followed by Dunnett's test.
Values are represented as mean ± S.E.M. (n=6); ns=nonsignificant, ***P < 0.001 and **P < 0.001.

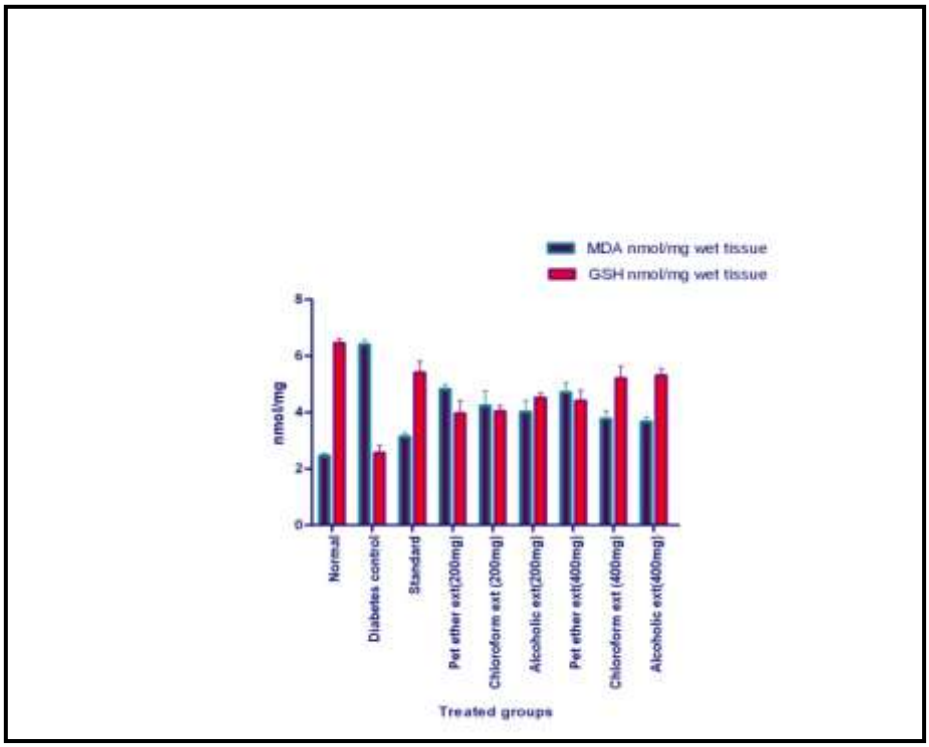

Fig. 3: Effect of fractions of *Nyctanthes arbor –tristis* on antioxidant enzymes (MDA and GSH).

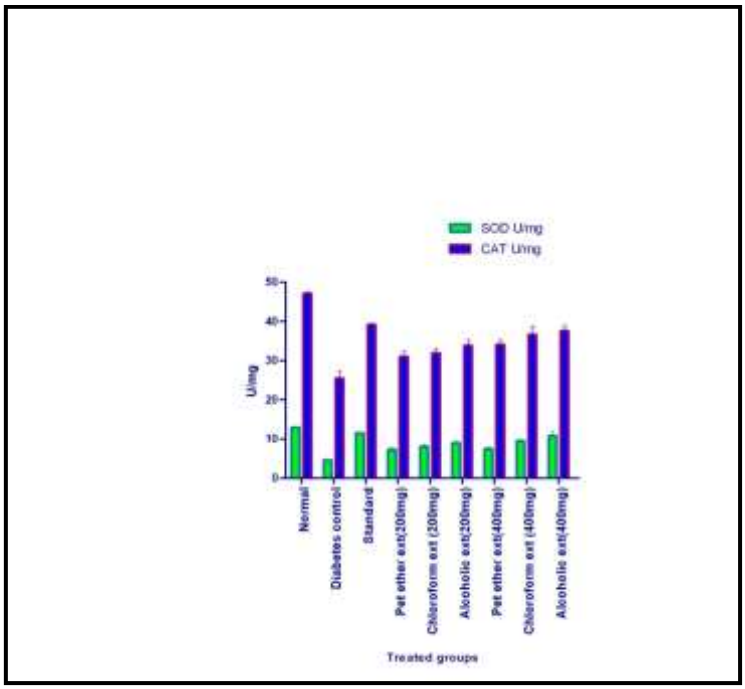

Fig. 4: Effect of fractions of *Nyctanthes arbor –tristis* on antioxidant enzymes

Liver disease is a metabolic disorder, which is the most common cause of mortality and morbidity worldwide. Hence, medicinal herbs with hepatoprotective and antioxidant properties have received considerable attention from researchers. Recently, medicinal herbs have been utilized by researchers in experiments to investigate their hepatoprotective and antioxidant properties on animals [11]. In the present study, the capability of the above fractions to protect against CCl_4 induced hepatotoxicity and oxidative stress was investigated. Hepatotoxicity induced by CCl4 is the one of the most commonly used models for testing the hepatoprotective activity, as CCl4 pathologic injuries in animals are closely similar to the symptoms of human liver disease. Carbon tetrachloride is metabolized to the CCl_3 radical in the hepatocytes which is further converted to the trichloromethylperoxy radical, a highly reactive species by cytochrome P450 enzyme Covalent binding of the trichloromethylperoxy radical to the macromolecules results in the peroxidative degradation of the membrane. This changes the permeability of plasma membranes, membrane of endoplasmic reticulum, and mitochondria, causing the loss of calcium homeostasis contributing to hepatocytes death through necrosis [12]. The elevated serum enzyme levels of enzymes viz., SGPT, SGOT and SALP and bilirubin have been attributed to the damaged

structural integrity of the liver because they are cytoplasmic in origin and are released into the blood after hepatic damage[13]. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release.[14] In present study, pre-treatment with *Nyctanthes arbor-tristis* fractions caused a decrease in the activities of the above enzymes when compared with CCl_4 treatment groups, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue. Similar observations have been reported by Pal *et al* [15]

In the present study, an elevation in the levels of MDA in liver of animals treated with carbon tetrachloride was observed. The increased in MDA in liver suggests provoked lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanism to prevent formation of excessive free radicals. In the present study treatment with ethylacetate and butanolic fractions of *Nyctanthes arbor – tristis* play an important role in reducing the free radicals which resulted in the subsequent decrease in the membrane damage and MDA level.

Hence it may be possible that the mechanism of the hepatoprotective by the above fractions is due to its antioxidant effect indicating the free radical scavenging activity under in vivo conditions.

The non-enzymic antioxidant, glutathione is one of the most abundantly naturally occurring tripeptides present in the liver [16]. its functions are mainly concerned with the removal of free radical species such as hydrogen peroxide, superoxide radical, alkoxy radical and maintenance of membrane protein thiols and as substrates for glutathione peroxidase and GSH. The results in the study indicate that the decrease level of GSH has been associated with an enhanced lipid peroxidation in CCl_4 treated rats. Administration of above fractions significantly increased the level of glutathione in dose-dependent manner.

Decrease in enzyme activity of SOD is sensitive index in hepatocellular damage and it's the most sensitive enzymatic index in liver injury. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defence system [17]. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. The above fractions showed significant increase in hepatic SOD activity and thus reducing free radical induced oxidative damage in liver.

CAT is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Administration of above fractions increases the level of CAT in induced liver damage in rats to prevent the accumulation of excessive free radical and protected the liver from CCl_4 intoxication.

4. Conclusion

From the above studies the ethylacetate and butanolic fractions of *Nyctanthes arbor –tristis* showed significant hepatoprotective activity by decreasing the elevated levels of serum enzymes and significant antioxidant activity by increasing the decreased levels of antioxidant enzymes such as superoxide dismutase, catalase and reduced glutathione and decreasing the lipid peroxidation state. These parameters were also comparable with that of the standard. These results can thus be concluded that possible mechanism of hepatoprotection of leaves may be due to its antioxidant action.

The above significant activity of ethylacetate and butanolic fractions of *Nyctanthes arbor –tristis* may be attributed due to presence of the flavonoid [18] which is known to counteract free radical mediated injuries and is also known to exhibit protection against CCl_4 induced liver injuries and attenuate ethanol-induced oxidative stress [19].

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