

## SCREENING OF ETHANOLIC EXTRACT OF *OCIMUM TENUIFLORUM* FOR RECOVERY OF ATORVASTATIN INDUCED HEPATOTOXICITY

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### ABSTRACT

**Objective:** To investigate the hepatoprotective potential of *Ocimum tenuiflorum* in statin induced hepatotoxic experimental rats.

**Method:** Wistar albino rats were divided into 6 groups: Group I served as control; Group II served as hepatotoxic (Atorvastatin (AT) 80 mg/kg treated) group; Group III, IV and V served as (50, 150 and 300 mg/kg b.w.) ethanolic extract of leaves of *Ocimum tenuiflorum* (EEOT) treated groups. Liver marker enzymes serum glutamate oxaloacetic transaminase, serum glutamic pyruvic transaminase, Alkaline phosphate, Total bilirubin were measured and compared along with histopathological studies.

**Results:** Obtained results show that the treatment with EEOT significantly ( $P < 0.05$ ) and dose-dependently reduced AT induced elevated serum level of hepatic enzymes, which was confirmed by the histopathological studies.

**Conclusion:** The results of this study indicate the protective effect of EEOT against AT induced acute liver toxicity in rats and thereby scientifically support its traditional use.

**Keywords:** Atorvastatin (AT), Ethanolic extract of leaves of *Ocimum tenuiflorum* (EEOT)

### INTRODUCTION

Liver diseases are amongst the most serious health problems in the world today and their prevention and treatment options still remain limited despite tremendous advances in modern medicine. The pathogenesis of hepatic diseases as well as the role of oxidative stress and inflammation is well established [1, 2] and accordingly blocking or retarding the chain reactions of oxidation and inflammation process could be promising therapeutic strategies for prevention and treatment of liver injury. The liver, unique in its capacity for regeneration following injury, may give rise to malignancies commonly associated with the inflammatory state of advanced fibrosis, or cirrhosis.

Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. *Ocimum tenuiflorum* also known as *Ocimum sanctum*, *tulasi*, or *tulasi*, is an aromatic plant in the family Lamiaceae which is native throughout the Eastern World tropics and widespread as a cultivated plant and an escaped weed [3]. It is an erect, much branched subshrub, 30–60 cm tall with hairy stems and simple, opposite, green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed. The flowers are purplish in elongate racemes in close whorls [4].

*Ocimum tenuiflorum* contains active principle oleanolic acid, eugenol, carvacrol, linalool,  $\beta$ -caryophyllene (about 8%),  $\beta$ -elemene (c. 11.0%), germacrene D (about 2%) [5]. It has COX-2 inhibitor [6], reduce blood glucose levels in type 2 diabetics [7], protection from radiation poisoning and cataracts [8] anti-oxidant properties [9], antihyperlipidemic and cardioprotective effects [10]. The possible modulating effect of *Ocimum tenuiflorum*, extract in the presence of statins has not been yet investigated. Hence, we aimed in the current investigation to evaluate the possible protection of ethanolic extract of leaves of *Ocimum tenuiflorum*, against atorvastatin-induced hepatic injury in rats.

### MATERIALS AND METHODS

#### Drugs and chemicals

AT was a kind gift from Darvin formulation Pvt. Vijayawada, India. All other chemicals were of analytical grade and were obtained from commercial sources.

#### Collection and extraction of plant material

Fresh leaves of *Ocimum tenuiflorum* were collected from botanical garden, Shri Vishnu college of Pharmacy, Bhimavaram. The air dried coarse powder of the leaves of *Ocimum tenuiflorum* was extracted successively with ethanol by maceration for 7 days. The extract were filtered and concentrated under reduced pressure in the rotary evaporator (IKA HB-10-Digital), dried and kept in the desiccators.

#### Animals

Wistar rats weighing 180–200 g were purchased from MKM, Hyderabad. Animals were used after acclimatization for a period of 1 week to animal house conditions and had free access to food and water. The experiments were conducted according to Institutional Animal Ethics Committee guidelines for the care and use of laboratory animals (439/PO/01/a/CPCSEA).

#### Experimental design

According to the [11] method, animals were divided into five groups as follow:

- First group was treated with Tween 80 and kept as control.
- Second group was treated with AT (80 mg/kg/day, orally).
- Third group was treated with EEOT (50 mg/kg/day, orally) + AT (80 mg/kg/day, orally).
- Fourth group was treated with EEOT (150 mg/kg/day, orally) + AT (80 mg/kg/day, orally).
- Fifth group was treated with EEOT (300 mg/kg/day, orally) + AT (80 mg/kg/day, orally).

After 28 days of treatment, blood was collected by retro orbital puncture, allowed to clot and centrifuged at 1000×g for 15–20 min to separate sera using cooling centrifuger (REMI C24 BL). The sera were used for the determination of the following biochemical parameters: SGOT, SGPT, alkaline phosphatase (ALP), Total bilirubin by using Autoanalyzer (Coralyzers 100). Samples of the liver from all animals were fixed in 10% neutral formalin and paraffin embedded. Sections (5m thickness) were stained with hematoxylin and eosin (H&E) for the histological examination.

#### Statistical analysis

Data are expressed as mean  $\pm$  S.E.M., with a value of  $P < 0.05$  considered statistically significant. Statistical evaluation was

performed by one way analysis of variance (ANOVA) followed by the Dunnett's for multiple comparisons. All analysis was made with the statistical software Graph pad prism 5.

**RESULTS**

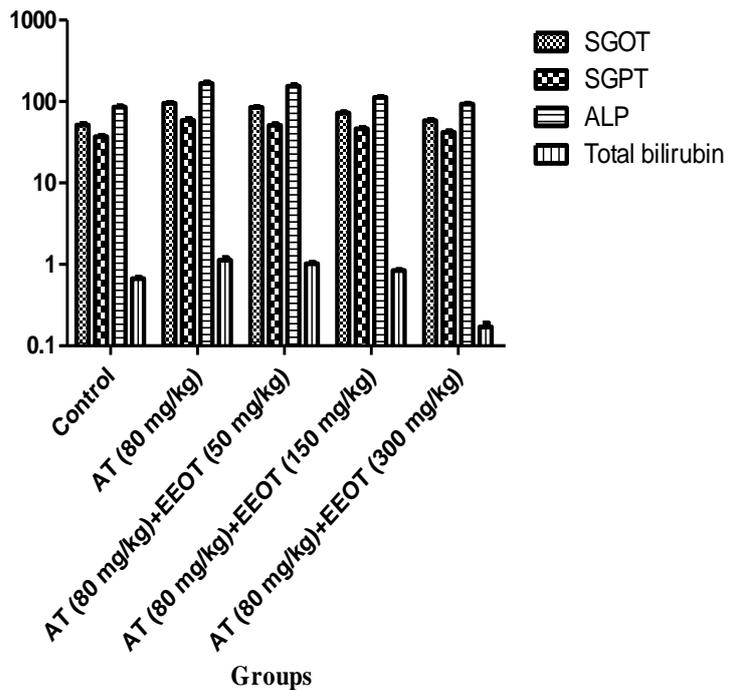
In AT-treated groups, the serum biochemical levels of were markedly raised compared to control group (Table 1). Simultaneous administration of EEOT with AT (80 mg/kg) reduced the serum levels of SGOT SGPT, ALP, and Total bilirubin compared to AT-treated groups.

The histological examination of liver sections in the control rats showed the normal hepatocytes architecture and the central vein (Fig. 2 a). Liver sections of animals treated with AT showed vacuolation, necrosis and mononuclear inflammatory cells dissecting the parenchyma) as well as extensive cell necrosis, vacuolar degeneration, mononuclear cellular infiltration pyknotic nuclei and apoptotic bodies (Fig 2 b) Rats were treated with different doses of EEOT plus AT shown decreased in damaged area around the central vein (fig 2 c, d and e).

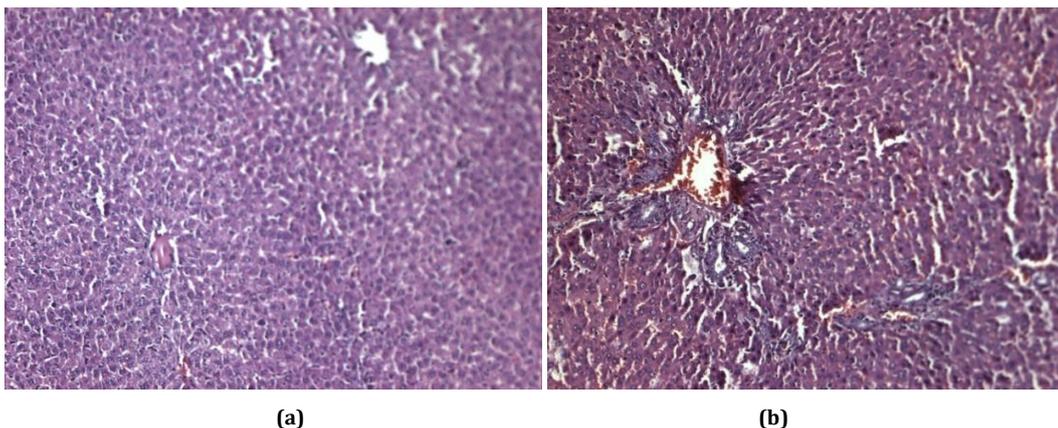
**Table 1: Influence of EEOT on biochemical sera parameter on Atorvastatin induced hepatotoxic rats.**

Groups	SGOT	SGPT	ALP	Total bilirubin
Control	*51.67±2.08	*37.00±1.19	*85.42±2.43	*0.67±0.02
AT (80mg/kg)	95.17±2.2	58.50±3.62	167.00±5.80	1.13±0.08
A AT(80mg/kg)+ EEOT(50mg/kg)	84.67±1.75	51.17±1.87	154.33±6.38	1.02±0.03
AT(80mg/kg)+EEOT(150mg/kg)	72.33±1.86	46.12±1.90	112.5±2.17	0.84±0.02
AT(80mg/kg)+EEOT(300mg/kg)	*58.17±1.79	*41.93±1.48	*93.00±1.90	*0.71±0.02

Values are Mean ± S.E.M; n = 6. All changes significant at \*p < 0.05 compared to Atorvastatin (80mg/kg) exposed animals.

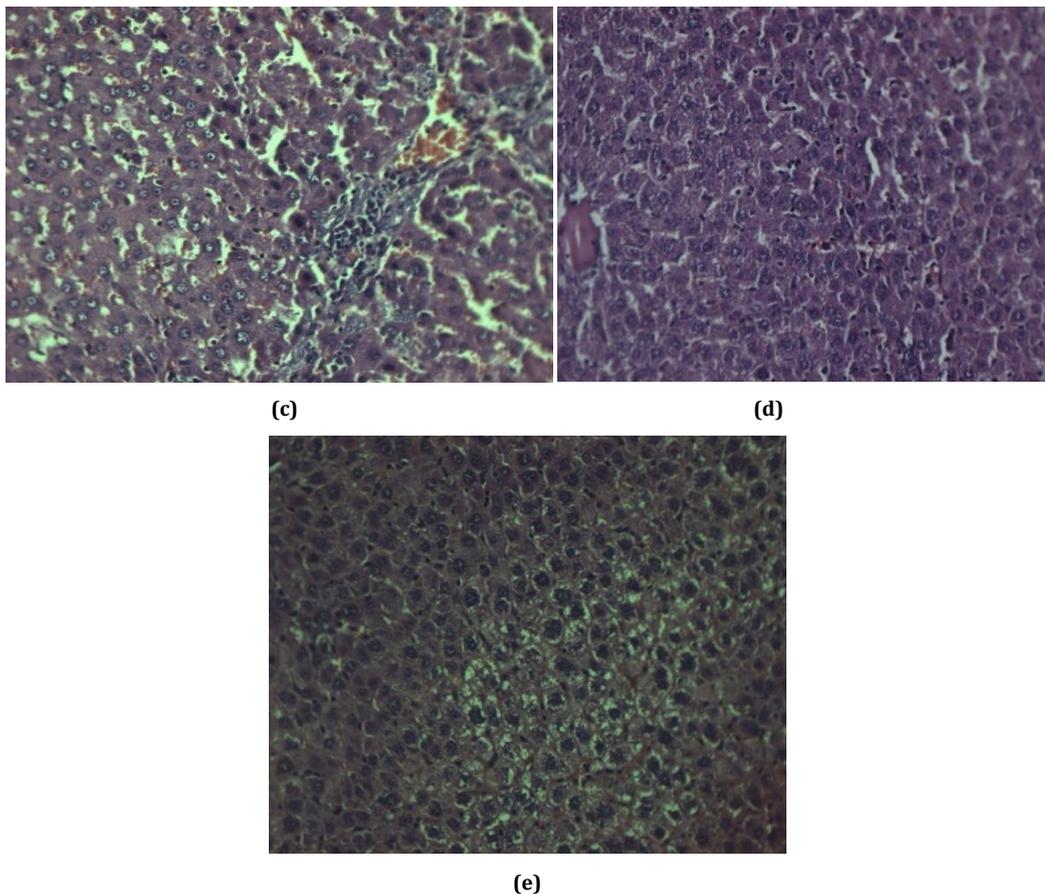


**Fig. 1: Effect of EEOT on Atorvastatin induced hepatotoxic rats.**



(a)

(b)



**Fig. 2: Light micrograph of liver sections in all studied groups. (a) Control (b) AT (80 mg/kg) showing distorted architecture, centrilobular necrosis, cloudy swelling and ballooning degeneration in the hepatocytes. (c, d, e) AT (80) + EEOT (50 mg/kg, 150 mg/kg, 300 mg/kg) showing mild cloudy swelling, hydropic degeneration in the hepatocytes.**

## DISCUSSION

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) are group of drugs that have been recognized as the most efficient drugs for the treatment of hyperlipidemia [12]. Animal studies and pre-marketing clinical trials point to statin-induced significant liver problems, primarily elevations in serum aminotransferases levels. The frequency of persistent transaminases elevation is consistent with all commercialized statins, and is dose dependent [13]. Although the usual doses of lovastatin did not cause significant liver injury, when given in very high doses they caused hepatocellular necrosis in rabbits [14]. Similarly, high doses of simvastatin caused hepatocellular necrosis in guinea pigs [15]. It has been speculated that elevation of serum aminotransferases in AT-treated rats can be attributed to alteration of the hepatocyte cellular membrane with enzyme leakage rather than direct liver injury [16]. Mechanism suggest that lipophilic statins like simvastatin, lovastatin, cerivastatin, fluvastatin and atorvastatin cause an apoptotic injury in human hepatocytes by stimulating caspase-3 subsequent to the activation of caspase-9 and caspase-8, in which the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase may be involved [17].

Many previous studies investigated the hepatoprotective effects of *Ocimum tenuiflorum* against liver toxicity induced by heavy metals [18], antitubercular drugs [19], carbon tetrachloride [20] and acetaminophen [21] with significant decrease in the levels of ALT and AST.

Our study also confirms earlier reported antioxidant activity of *O. tenuiflorum* in vitro and in vivo [22,23]. *O.tenuiflorum* contains potent antioxidants flavanoids (orientin, vicenin), phenolic compounds (eugenol, cirsilinoleol, apigenin) [24], triterpenes (ursolic

acid) and anthocyanins [25]. In accordance with these studies, concurrent administration of EEOT and AT significantly reduced serum SGOT, SGPT, ALP, Total bilirubin, compared to AT-treated groups (Table 1, Fig1 and Fig 2). Regarding the histopathological changes following administration of AT for 4 weeks, severe hepatic changes were observed with high dose AT (80 mg/kg). In addition, ballooning, cloudy swelling, hydropic degeneration and hepatic necrosis were the most predominant lesions. Such lesions were induced via the formation of free radicals, especially the reactive oxygen species. In consistent with the present findings, the hepatotoxic effect of AT was ameliorated with partial disappearance of hepatic damage after treatment EEOT. Presence of antioxidant profile EEOT play a key role in the attenuation of hepatic injury, and then preserve the structural integrity of the hepatocellular membrane, supporting the biochemical findings are reported.

## CONCLUSION

In our study, all of the biochemical parameter changes that were induced by AT (80 mg/kg) treatment were at least partially normalized given together with EEOT.

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