Journal of Global Biosciences
ISSN 2320-1355
Volume 5, Number 5, 2016, pp. 4082-4089
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

DEFENSIVE EFFECT OF EUGENOL ON ISONIAZID AND RIFAMPICIN-INDUCED HEPATOTOXICITY IN ALBINO RATS

Sangamithira, S.P.[1] and Sheik Abdullah[2]

[1] Research scholar,
Research and Development centre,
Bharathiyar University,
Coimbatore, Tamilnadu, India
[2]Biochemistry Faculty,
Department of Chemistry and Biosciences,
Sastra University, Kumbakonam, Tamilnadu
India.

Abstract

Antituberculosis drug induced hepatotoxicity is a significant problem for treatment of tuberculosis. The present study was designed to prove the hepatoprotective effect of eugenol (6mg/kg bw and 12mg/kg bw) on isoniazid (50mg/kg bw) and rifampicin (100mg/kg bw) induced hepatotoxicity. The hepatotoxicity was manifested by a significant increase in triglycerides, phospholipids and cholesterol in serum and liver. The level of HDL decreased and LDL and VLDL levels increased in hepatotoxic rats. Protein level decreased, bilirubin level and prothrombin time increased in the rats treated with antituberculosis drug. Treatment with eugenol altered the parameters to near normal range. The biochemical observations were supplemented by histopathological examination of liver sections. Results of this study proved that eugenol has protective effect against antituberculosis drug induced hepatotoxicity.

Key words: eugenol, isoniazid, rifampicin, hepatotoxicity, albino rats.

INTRODUCTION

Tuberculosis remains one of the most serious infectious diseases and a major cause of disability and death worldwide, despite noteworthy socioeconomic development and advances in medical sciences [1]. Toxic reactive metabolites generated during hepatic biotransformation of some of the antitubercular drugs are thought to covalently bind with cellular macromolecules to generate free radicals, which in turn bring about cellular injury. Isoniazid (INH) metabolites, acetylhydrazine and hydrazine, have been implicated as the causative hepatotoxins. Oxidative activation of these metabolites in liver by cytochrome 450 monooxigenase system generates electrophilic intermediates and free radicals which are capable of causing liver injury in animals. Rifampicin (RIF), a powerful inducer of mixed function oxidase, contributes to hepatotoxicity of isoniazid by enhancing the production of toxic metabolites [2]. Phytochemicals are of great

importance due their curative properties and in healthcare. Search for heptoprotective agents is on the increase because of the important role in that liver plays in detoxicification, excretion of internally and externally generated toxin. Modern medicine does not so far have fully effective cure particularly for hepatitis [3]. Eugenol, a naturally occurring allyl benzene and an active principle of clove, osmium, cinnamon and nutmeg. It has been used as a flavouring agent in a variety of food and pharmaceutical products and as analgesic in dental material. It has been reported that oral administration of eugenol increased the activities of liver detoxifying phase II biotransformation enzymes in a dose dependent manner. As intestine is the first target for any drug by oral administration, through which it is absorbed and enter into the blood circulation to produce its desirable effect [4]. In recent years, the pharmacodynamics of eugenol has been developed to central nervous regulation, cardiovascular system, digestive system and hepatoprotection. Hence the present study was designed to manifest the hepatoprotective effect of eugenol on isoniazid and rifampicin induced hepatotoxic rats.

MATERIALS AND METHODS

Source of chemicals

Eugenol was purchased from Sigma Chemicals Co., and other chemicals purchased from Himedia Laboratories Pvt. Ltd., Mumbai. All the chemicals used were of analytical grade.

Animals

Adult male Albino rats of Wistar strain (140-160g) were used for this study. They were housed in well-ventilated rooms (temperature 23±2°C), humidity 65-70% and 12hr (light/dark cycle) at animal house of Srimad Andavan College, Thiruchirappalli and was approved by the Institutional Ethical Committee (SAC/IAEC/BC/2015/Ph.D-004). Animals were fed with standard pellet diet and water *ad libitum*. They were divided into six groups, each group comprised of five rats.

Experimental design

Group I : Normal control
Group II : Disease control (Isoniazid50mg/kg bw and Rifampicin 100mg/kg bw)
Group III: Hepatotoxic rats treated with eugenol (6mg/kg bw)
Group IV: Hepatotoxic rats treated with eugenol (12mg/kg bw)
Group V : Normal rats with eugenol (12mg/kg bw)
Group VI: Hepatotoxic rats with silymarin (25mg/kg bw)

At the end of 21days, rats were fasted overnight and sacrificed by cervical decapitation. Blood samples were collected in a heparinised tube and centrifuged at 1600×g for 10 min at 20°C. The blood samples were separated and stored at -20°C for assay. The organs liver and kidney were carefully collected, weighed accurately and stored at -80°C and used for biochemical and histopathological studies.

Preparation of tissue

Immediately after killing, the liver tissues were homogenized with 1M phosphate buffer, P^H 7.2, centrifuged at low speed (3000 rpm) and the supernatant was used for the biochemical estimation.

Biochemical Assays

Plasma lipids were extracted by the method of Folch et al., [5], cholesterol, triglycerides, phospholipids were estimated by the methods of Parekh and Jung [6], Foster and Dunn [7], Barlette [8] . HDL was estimated by the method of Friedewald [9].

Statistical analysis

Results are presented as mean **±**SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a statistical software package. P- values <0.05 were considered to be statistically significant.

RESULTS

Table 1 shows the levels of triglycerides, phospholipids and cholesterol in serum and liver tissue. Administration of isoniazid and rifampicin caused abnormal levels of lipid profile in all rats. Though, the rats treated with eugenol and antituberculosis drugs showed significantly reduced levels of triglycerides, phospholipids and cholesterol.

Table 2 shows the effect of eugenol on lipoproteins. Increased levels of VLDL and LDL seen in antituberculosis drug induced rats. The level of HDL decreased in hetatotoxic rats. Pretreatment of rats with eugenol reversed these altered parameters to near normal.

Table 3 shows the levels of protein, bilirubin and prothrombin time in normal and hepatotoxic rats. Increased bilirubin content seen in rats treated with antituberculosis drugs. Protein level decreased in hepatotoxic rats. Treatment of eugenol afforded a significant protection against isoniazid and rifampicin induced hepatotoxicity by maintaining the levels to near normal.

Table 1 Effect of Eugenol on Triglycerdes, Phospholipids and Total Cholesterol in liver tissue and serum of hepatotoxic rats.

Groups	Serum Triglycerides (mg/dl)	Liver tissue Triglycerides (mg/g)	Serum Phospholipid (mg/dl)	Liver tissue Phospholipid (mg/g)	Serum Cholesterol (mg/dl)	Liver tissue Cholesterol (mg/g)
Group I (normal)	140.09±1.11 [a]	37.45±0.05 [a]	85.82±0.30 [a]	23.55±0.45 [a]	164.27±1.34[a]	76.19±0.04 [a]
Group II (INH 50mg+ RIF 100mg)	295.14±0.95 [b]	72.45±0.02 [b]	152.19±0.72 [b]	62.76±1.17 [b]	357.18±2.55[b]	173.26±0.07[b]
Group III(hepatotoxic rats +eugenol 6mg)	211.64±1.40 [a]	51.15±0.04 [a]	132.17±0.46 [a]	45.60±0.52 [a]	255.46±4.70[a]	130.15±0.04[a]
GroupIV (hepatotoxic rats+eugenol 12mg)	147.41±0.61 [a]	39.00±0.03 [a]	98.01±0.23 [a]	27.89±0.44 [a]	184.91±1.47[a]	97.37±0.05 [a]
Group V(normal rats + eugenol 12mg)	145.32±0.24 [a]	38.11±0.03 [a]	92.37±0.48 [a]	25.92±0.33 [a]	179.50±0.93[a]	88.04±0.96 [a]
GroupVI (hepatotoxic rats+silymarin)	143.75±0.71 [a]	37.81±0.04 [a]	88.60±0.49 [a]	24.07±0.12 [a]	177.52±0.58[a]	81.30±2.55 [a]

Values are means **±**SD of 5 rats from each group.

Values not sharing common alphabets as superscript are significantly different from each other at the level of P<0.05(ANOVA followed by DMRT).

Table 2 Effect of Eugenol on HDL, VLDL and LDL in normal and hepatotoxic rats

Groups	HDL (mg/dl)	VLDL(mg/dl)	LDL(mg/dl)
Group I (normal)	65.69±0.16 [a]	28.02±0.22 [a]	70.56±0.16 [a]
Group II (INH 50mg+ RIF 100mg)	23.69±0.18 [b]	59.03±0.19 [b]	274.47±0.16 [b]
Group III(hepatotoxic rats +eugenol 6mg)	37.19±0.14 [a]	42.33±0.28 [a]	175.94±0.16 [a]
GroupIV (hepatotoxic rats+eugenol 12mg)	54.94±0.21 [a]	29.48±0.12 [a]	100.48±0.16 [a]
Group V(normal rats + eugenol 12mg)	62.46±0.18 [a]	29.06±0.05 [a]	87.98±0.16 [a]
GroupVI (hepatotoxic rats + silymarin)	58.58±0.47 [a]	28.75±0.14 [a]	90.19±0.16 [a]

Values are means **±**SD of 5 rats from each group.
Values not sharing common alphabets as superscript are significantly different from each other at the level of P<0.05(ANOVA followed by DMRT).

Table 3 Levels of bilirubin, protein and prothrombin time in normal and hepatotoxic rats

Groups	Total Bilirubin(mg/dl)	Total Proteins (g/dl)	Prothrombin Time (Sec)
Group I (normal)	0.35±0.03 [a]	7.13±0.163 [a]	12.50±0.43 [a]
Group II (INH 50mg+ RIF 100mg)	1.5±0.26 [b]	3.48±0.098 [b]	22.50±0.43 [b]
Group III(hepatotoxic rats +eugenol 6mg)	0.92±0.05 [a]	4.55±0.083 [a]	20.17±0.31 [a]
GroupIV (hepatotoxic rats+eugenol 12mg)	0.72±0.03 [a]	6.43±0.081 [a]	16.50±0.43 [a]
Group V(normal rats + eugenol 12mg)	0.37±0.03 [a]	6.81±0.325 [a]	14.83±0.31 [a]
GroupVI (hepatotoxic rats + silymarin)	0.36±0.03 [a]	6.53±0.051 [a]	14.33±0.42 [a]

Values are means **±**SD of 5 rats from each group.
Values not sharing common alphabets as superscript are significantly different from each other at the level of P<0.05(ANOVA followed by DMRT).

HISTOPATHOLOGICAL OBSERVATION

Liver tissues from control group did not show any abnormalities. Animals received isoniazid and rifampicin showed liver with congestion, foci of haemorrhage and feathery degeneration of hepatocytes(fig 1). Fig 2 shows hepatocytes with adjacent areas of minimal congestion and devoid of inflammation. In fig 3 photomicrograph of hepatotoxic rats with eugenol(12mg) shows liver with foci of micro congestion and absence of inflammation. Regenerative changes are also evidented.

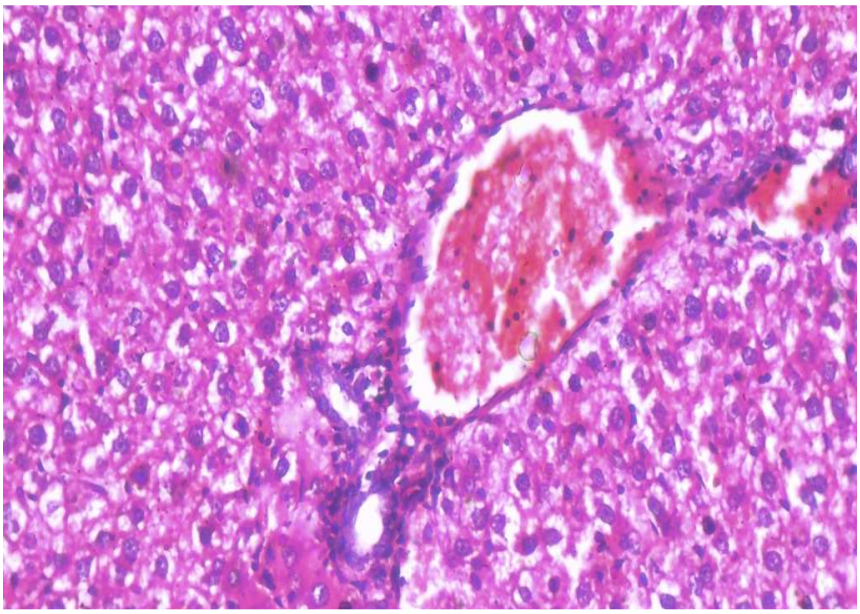

FIG 1: PHOTOMICROGRAPH OF ISONIAZID- RIFAMPICIN INDUCED RAT LIVER (GROUP II)

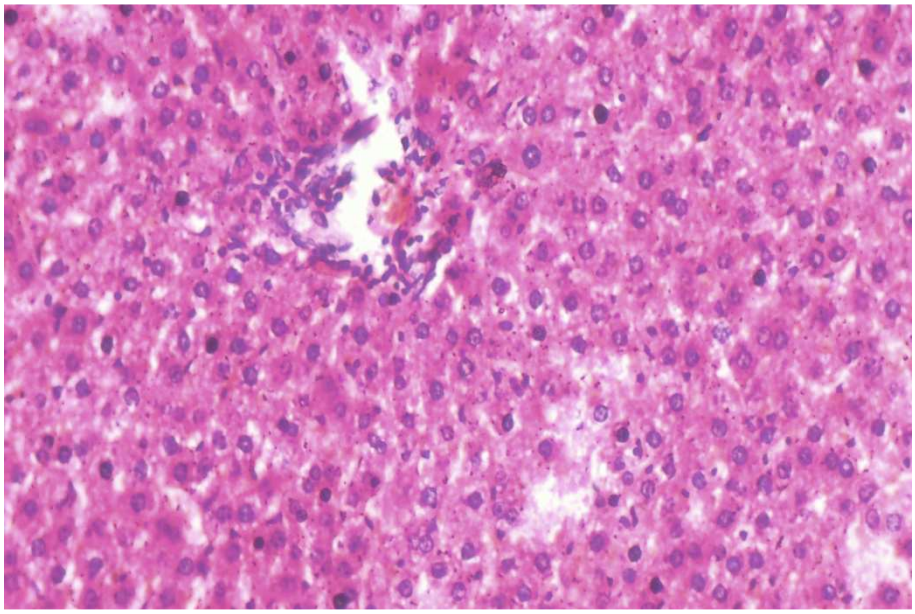

FIG 2: PHOTOMICROGRAPH OF ISONIAZID- RIFAMPICIN INDUCED RAT LIVER TREATED WITH EUGENOL (6mg **) (GROUP III)**

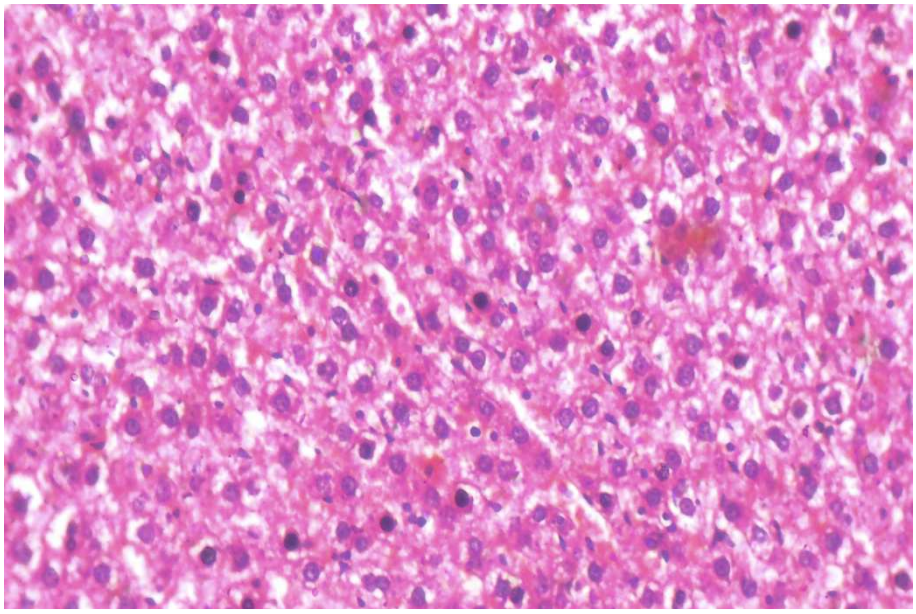

FIG 3: PHOTOMICROGRAPH OF ISONIAZID- RIFAMPICIN INDUCED RAT LIVER TREATED WITH EUGENOL (12mg **) (GROUP IV)**

DISCUSSION

The development of Drug Induced Hepatotoxicity during chemotherapy for TB is the most common reason leading to interruption of therapy. Isoniazid and rifampicin given together produce hepatotoxicity more frequently than either drug alone [10]. Drug-related hepatotoxicity cannot be viewed as a single disease. Many different mechanisms lead to hepatotoxicity, including disruption of cell membrane and cell death. Administration of isoniazid and rifampicin produces different types of metabolic and morphologic aberrations in the liver because the liver is the main site for detoxifying these anti tubercular drugs [11].

In recent years, eugenol has attracted the attention of many researchers because of its anti- inflammatory and chemopreventive effects, as well as its superior antioxidant activity due to the presence of its broad range of pharmacological and biological activities, studies on eugenol and clove products still remains a research priority [12].

In the present study, administration of isoniazid and rifampicin are reported to induce hepatotoxicity in rats judged by elevated levels of triglycerides, phospholipids and cholesterol in serum and liver tissue. Administration of eugenol with antitubercular drugs decrease the elevated level of lipid profile to near normal. It is confirmed that eugenol can ameliorate the hepatotoxicity induced by anti tubercular drugs.

Any liver disease will show an increased blood cholesterol level[13]. The significant increase of cholesterol noted in this study might have been due to the inability of the diseased liver to remove cholesterol from circulation.

In the present study, increased levels of VLDL and LDL seen in antituberculosis drug induced rats. The level of HDL decreased in hetatotoxic rats. Pretreatment of rats with eugenol reversed these altered parameters to near normal. Thus eugenol has protective effect against antitubercular drug induced hepatotoxicity. Serum bilirubin is a well known indicator of liver tissue damage by toxic substances [14]. Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function [15]. Increased bilirubin content seen

in rats treated with antituberculosis drugs. Protein level decreased in hepatotoxic rats. Treatment of eugenol provided a significant protection against isoniazid and rifampicin induced hepatotoxicity by maintaining the levels to near normal.
In the present study, histopathological patterns clearly showed that the antitubercular drugs caused congestion, haemorrhage and feathery degenation of hepatocytes. Eugenol protect the liver against hepatic inflammations in rats after treatment with isoniazid and rifampicin.

In this study, the results depicted the defensive effect of eugenol against isoniazid and rifampicin induced hepatotoxicity by lowered the levels of triglycerides, phospholipids and cholesterol, LDL and VLDL.

ACKNOWLEDGEMENT
Authors are thankful to the professors, lecturersand lab technicians of Srimad Andavan College, Tiruchirapalli, Tamilnadu for providing all laboratory facilities and technical assistance.

REFERENCES

1. Khushboo Ambreen, Rolec Sharma, Kaleshwar P. Singh and Sudhir Kumar,2014, Antituberculosis drug Induced hepatotoxicity: A Review, International Journal of Advanced Biotechnology and Research, Vol.5, issue 3,PP 423-437.
2. VandanaTayal, Bhupinder Singh Kalra, SaritaAgarwal, Nita Khurana, Usha Gupta, 2007, Hepatoprotective effect of tocopherol against isoniazid and rifampicin induced hepatotoxicity in albino rabbits, Indian Journal of experimental Biology, Vol.45, 1031-1036.
3. Ansari J.A., M.Sayyad and F.Sayyad, 2011, Management of non-alcoholic fatty liver disease and their complications. Int. J. Pharmacol, vol 7; 579-588.
4. N.Vidhya and N.NiranjaliDevaraj, 1999,Antioxidant effect of eugenol in rat intestine, Indian Journal of experimental biology, Vol.37, 1192-1195.
5. Folch J, Lees M and Stanley GHS, 1970, A simple method for isolation and purification of total lipids from animal tissues. *J.Biol Chem*. 226: 497-509.
6. Parekh AC and Jung DH. 1970. Determination of Cholesterol with ferric acetate, Uranium Acetate and sulphuric acid-ferrous sulphate reagents. *Anal Chem.* 42: 1523-1529.
7. Foster LB, and Dunn RT. 1973. Stable reagents for determination of Serum triglycerides by a colorimetric Hantzsch condensation method. *Clin Chem*. 196: 338-340.
8. Bartlette, GR. 1959, Phosphorus assay in column chromatography. J.Biol Chem. 234: 466-468.
9. Friedewald WT, Levy RI and Fredrickson DS. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of proparative centrifuge. *J Clin Chem*. 18: 499.
10. Rohit Singla, Surendra K. Sharma, Alladi Mohan, Govind Makharia†, V. Sreenivas‡, Brajesh Jha, Sanjeev Kumar, Pawan Sarda & Sarman Singh, 2010,Evaluation of risk factors for antituberculosis treatment induced hepatotoxicity, Indian J Med Res 132, 81-86.
11. Gulala Ibrahim Qader, RoshanaShawkat Aziz, ZheenAorahman, ZanaFaeq Abdullah, SaadAbdulrahmanHussain, 2014, protective effects of Quercetin against

isoniazid and rifampicin induced hepatotoxicity in rats, American Journal of Pharmacological Science, Vol. 2, No.3, 56-60.

12. Guy. P.Kamatou, IlzeVVermaak and Alvaro M.Viljoen, 2012, Eugenol -from the remote Maluku Islands to the International market place: A review of a remarkable and versatile molecule, Molecules, 17, 6953-6981.
13. Vilvanathan Ravikumar, Kanchi Subramanian Shivashankari, Thiruvengadam Devaki, 2005, Hepatoprotective activity of *Tridax procumbens* against D-galactosamine/lipopolysaccharide- induced hepatitis in rats, Journal of ethanopharmacology 101, 55-60.
14. Rubin E, Rottenberge H, 1982, Ethanol induced injury and adaptation in biological membranes, fed proc, vol 41; 2465-2471.
15. Martin,P., Friedman,L.S., 1992, Assessment of liver function and diagnostic studies, Handbook of liver disease. Churchill Livingstone, Philadelphia, 33-71.