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RESEARCH ARTICLE

PREVENTIVE EFFICACY OF EUGENOL ON ISONIAZID AND RIFAMPICIN INDUCED HEPATOTOXIC RATS

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

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

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ABSTRACT

Anti-tuberculosis drug induced hepatotoxicity is a major hurdle for an effective treatment of tuberculosis. The present study was aimed to assess the preventive effect of eugenol (6mg/kg bw and 12mg/kg bw) on isoniazid (50mg/kg bw) and rifampicin (100mg/kg bw) induced hepatotoxicity was manifested by a significant increase in the activities of marker enzymes (AST, ALT, ALP, LDH and GGT) level in serum. The activities of enzymic (SOD, CAT, GST and GPx) and non enzymic antioxidants (VitC, Vit E and GSH) were reduced by the antitubercular drugs. Pretreatment of rats with eugenol reversed these altered parameters to near normal values. Results of this study revealed that eugenol encounter a significant defend in the mitigation of isoniazid and rifampicin induced hepatotoxicity.

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INTRODUCTION

Anti-tubercular drug induced hepatotoxicity is an important and commonly encountered adverse effect (VandanaTayal *et al.,* 2007). Metabolic intermediates of isoniazid, are incriminated as the cause of hepatotoxicity (Yi-Shin Huang *et al.* 2003). Isoniazid is acetylated and then hydrolysed, resulting in isonicotinic acid and monoacetyl hydrazine. *In vitro* studies indicate that metabolic oxidation of acetyl hydrazine leads to a reactive acetylating species that binds covalently to microsomal protein. It is postulated that acetylhydrazine and hydrazine act as acetylating agents by binding covalently with liver cell macromolecules, causing hepatocyte injury (Bhupinder Singh Kalra *et al.,* 2007). Rifampicin is an enzyme inducer and enhances formation of reactive metabolites which impair the uptake of bilirub in and cause acute cellular necrosis. Eugenol is a naturally occurring allyl benzene and an active principle of clove, ocimum, nutmeg and cinnamon. It has been used as a flavouring agent in a variety of food and pharmaceutical products and as analgesic in dental material.

****Corresponding author: Sangamithira, S. P.***
Research and Development centre, Bharathiyar University, Coimbatore, Tamilnadu, India.

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 (Vidhya and Niranjali Devaraj, 1999). 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 determine 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) will be 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 studies.

Preparation of tissue

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

Biochemical Assays

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and LDH were estimated using the method of King (King, 1965). Gamma- glutamyl transpeptidase (GGT) was estimated using the method of (Rosalki and Rau, 1972). The activity of superoxide dismutase (SOD) was estimated by the method of (Misra and Fridovich, 1972) catalase (CAT) was estimated using the method of (Maehly, 1954) glutathione peroxidase (GPx) was estimated by the method of (Rotruck et al., 1973) GST was estimated by the method of Havig (Habig *et al.,* 1974).

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 serum hepatic markers in normal and disease control rats. Administration of isoniazid and rifampicin caused abnormal liver function in all rats. Activities of serum hepatic markers enzymes such as AST, ALT, ALP and GGT levels were significantly increased in isoniazid and rifampicin induced rats.

Table 1. Protective effect of eugenol on hepatic marker enzymes levels in isoniazid and rifampicin induced hepatotoxicrats

Groups	AST(U/L)	ALT(U/L)	ALP(U/L)	LDH(U/L)	GGT(U/L)
Group I (normal)	51.50±0.85^{a}	45.40±0.58^{a}	133.03±1.45^{a}	330.81±3.91^{a}	22.16±0.816^{a}
Group II (INH 50mg+ RIF 100mg)	91.07±0.95^{b}	94.77±0.79^{b}	251.83±2.67^{b}	937.43±13.03^{b}	83.00±0.894^{b}
GroupIII (hepatotoxic rats +eugenol 6mg)	71.14±1.09^{a}	71.06±0.95^{a}	196.03±2.22^{a}	760.44±10.17^{a}	68.83±0.816^{a}
GroupIV (hepatotoxic rats+eugenol 12mg)	58.21±0.60^{a}	51.62±2.66^{a}	164.34±1.75^{a}	654.72±8.66^{a}	41.5±0.375^{a}
Group V (normal rats + eugenol 12mg)	54.18±0.64^{a}	45.65±0.59^{a}	141.65±1.07^{a}	349.33±6.93^{a}	26.8±0.816^{a}
GroupVI(hepatotoxic rats + silymarin)	55.01±0.68^{a}	46.29±0.61^{a}	158.23±0.69^{a}	584.48±6.85^{a}	35.75±0.418^{a}

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

Table 2. Changes in the levels of enzymic antioxidants in liver and kidney of experimental rats

Group	Catalase (mM of H_2O_2 hydrolysed/g tissue)		SOD(µM of Adrenochrme formed/g tissue)		Glutathione peroxidase (µM of GSH oxidised/g tissue)		GST (U/L)
	Liver	Kidney	Liver	Kidney	Liver	Kidney	
GroupI(normal)	63.91±0.88^{a}	38.81±0.52^{a}	11.51±0.48^{a}	6.98±0.39^{a}	14.88±0.29^{a}	9.03±0.36^{a}	19.08±0.735^{a}
GroupII(INH 50mg+ RIF 100mg)	38.04±1.22^{b}	14.80±1.28^{b}	1.97±0.30^{b}	2.11±0.04^{b}	3.12±0.25^{b}	3.03±0.24^{b}	44.66±0.605^{b}
GroupIII(hepatotoxic rats +eugenol 6mg)	45.79±1.24^{a}	20.89±1.12^{a}	4.46±0.44^{a}	3.49±0.14^{a}	6.53±0.36^{a}	5.20±0.15^{a}	36.83±0.258^{a}
GroupIV (hepatotoxic rats+eugenol 12mg)	51.65±0.96^{a}	30.29±1.11^{a}	6.53±0.45^{a}	4.41±0.07^{a}	9.37±0.19^{a}	6.68±0.20^{a}	24.75±0.418^{a}
GroupV(normal rats + eugenol 12mg)	59.02±0.79^{a}	36.66±0.72^{a}	9.33±0.36^{a}	6.73±0.35^{a}	13.73±0.32^{a}	8.78±0.24^{a}	19.58±0.491^{a}
GroupVI(hepatotoxic rats + silymarin)	59.76±1.23^{a}	31.81±0.73^{a}	9.02±0.62^{a}	5.06±0.11^{a}	11.12±0.31^{a}	8.03±0.27^{a}	23.25±0.418^{a}

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

Table 3. Changes in the levels of non enzymic antioxidants in liver and kidney of experimental rats

Groups	Vitamin C		Vitamin E		GSH	
	Liver (µg/g of tissue)	Kidney (µg/g of tissue)	Liver (µg/g of tissue)	Kidney (µg/g of tissue)	Liver (µg/g of tissue)	Kidney (µg/g of tissue)
Group I (normal)	259.01±7.24^{a}	198.53±4.20^{a}	22.78±0.38^{a}	15.93±0.46^{a}	41.83±0.816^{a}	34.56±0.674^{a}
Group II (INH 50mg+ RIF 100mg)	112.88±6.64^{b}	245.01±1.03^{b}	10.82±0.43^{b}	24.38±1.05^{b}	18.75±0.418^{b}	17.6±0.536^{b}
Group III(hepatotoxic rats +eugenol 6mg)	129.48±8.53^{a}	237.72±1.51^{a}	15.73±0.30^{a}	21.25±0.53^{a}	28.41±0.584^{a}	23.53±0.484^{a}
GroupIV (hepatotoxic rats+eugenol 12mg)	182.61±11.98^{a}	223.62±3.15^{a}	19.18±0.50^{a}	18.72±0.45^{a}	35.75±0.418^{a}	30.3±0.275^{a}
Group V(normal rats + eugenol 12mg)	239.12±7.26^{a}	209.16±3.85^{a}	23.77±0.35^{a}	15.80±0.48^{a}	40.83±0.683^{a}	32.5±0.447^{a}
GroupVI (hepatotoxic rats + silymarin)	195.92±11.99^{a}	222.38±1.11^{a}	21.11±0.42^{a}	17.92±0.61^{a}	36.41±0.664^{a}	31.16±0.258^{a}

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

However, therats administered with eugenol and antitubercular drugs showed significantly decreased activities of the hepatic marker enzymes. It is confirmed that eugenol can repair the hepatic injury induced by isoniazid and rifampicin. Table 2 and 3 shows the levels of enzymic and non enzymic anti oxidants in normal and hepatotoxic rats. The levels of antioxidants significantly decreased in isoniazid and rifampicin induced hepatotoxic rats. Treatment with eugenol modulates the levels of anti oxidants in anti tubercular drug induced hepatotoxic rats.

DISCUSSION

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 (Gulala *et al.,* 2014). In recent years, eugenol has attracted the attention of many researchers because of its anti- inflammatory and chemo preventive 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 (Guy *et al.,* 2012). Anti tuberculosis medication frequently causes disturbance to liver function tests and may cause serious liver dysfunction (Thompson *et al.,* 1995). In the present study, administration of isoniazid and rifampicin are reported to induce hepatotoxicity in ratsjudged by elevated serum AST, ALT, ALP, GGT and LDH levels. Administration of eugenol in hepatotoxic rats decrease the elevated level of hepato specific markers to near normal. It is confirmed that eugenol can ameliorate the hepatotoxicity induced by anti tubercular drugs. Antioxidants play an important role in regulation and maintenance of metabolism in the body against oxidative stress. ROS generated are scavenged by the antioxidantdefence system includes enzymes such as SOD, CAT, GST, GPx (enzymic antioxidants) and Vit E, Vit C, GSH (non- enzymic antioxidants). SODis considered as the first line of defence against the deleterious effect of oxygen radicals in the cells and its scavenges ROS by catalysing the dismutation of superoxide to hydrogen peroxide (Anbu and Anuradha, 2012). There is evidence to indicate that anti tubercular drugs significantly depresses SOD activities. GPx works tandem with CAT to scavenge excess hydrogen peroxide, as well as other free radicals, in response to oxidative stress. Moreover oxidative stress is known to decrease GST activity and also lower GSH content (Bast *et al.,* 1988). Our results showed decreased activity of enzymic and non-enzymic antioxidants in isoniazid and rifampicin treated rats. It has been reported that anti tubercular drugs impairs the antioxidant system. Eugenol, a naturally occurring antioxidant would scavenge the free radicals and protect the tissues from damage. Administration of eugenol in hepatotoxic rats increased the activity of antioxidants to normalcy. It is proved that eugenol can protect liver and kidney tissues from oxidative stress.

Conclusion

In this study,the results depicted the preventive efficacy of eugenol against isoniazid and rifampicin induced hepatotoxicity by lowered serum transaminases, ALP, GGT and LDH enzyme activities and elevated levels of the enzymic and non- enzymic antioxidants.

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REFERENCES

Anbu and Anuradha, C.V., Protective effect of eugenol against alcohol- induced biochemical changes in rats, International *Journal of Research in Biotechnology and Biochemistry*, 2012, 2(2), 13-18.

Bast, A., Haenen, G.R.M.M. Interplay between lipoic acid and glutathione on the protection against microsomal lipid peroxidation, *Biochem Biophys Acta*, 1988, 963, 556- 558.

Bhupinder Singh Kalra, SaritaAgarwal, Nita Khurana, Usha Gupta, Effect of cimetidine on hepatotoxicity induced by isoniazid - rifampicin combination in rabbits, *Indian journal of Gastroenterology*, 2007, Vol.26, 18-21.

Gulala Ibrahim Qader, RoshanaShawkat Aziz, ZheenAorahman, ZanaFaeq Abdullah, Saad Abdulrahman Hussain, protective effects of Quercetin against isoniazid and rifampicin induced hepatotoxicity in rats, *American Journal of Pharmacological Science*, 2014, Vol. 2, No.3, 56-60.

Guy, P., Kamatou, IlzeVVermaak and Alvaro M.Viljoen, Eugenol-from the remote Maluku Islands to the International market place: A review of a remarkable and versatile molecule, Molecules, 2012, 17, 6953-6981.

Habig, W. H., Pabst, M. J. and Jakoby, W. B., 1974. Glutathione-S-Transferase the first enzymatic step in Mercapturic Acid Formatin.*J. BiolChem*, 249: 7130-7139

King, J. 1965. In: Practical Clinical Enzymology, Princeton MJ (Fol) Van D Nostrand Company, London, 363.

Maehly, A. C. and Chance, B. 1954. In: Methods of Biochemical Analysis, Vol. I, Glick D, editors, New York, Interscience. 357.

Misra, H. P. and Fridovich, I. 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD.*J Biol Chem*. 247: 3170-3175.

Rosalki, S. B. and Rau, D. 1972. Serum gamma glutamyltranspeptidase activity in alcoholism. *Clin Chem Acta*. 39: 41-47.

Rotruck, J. T., Pope, A. L., Ganther, H., Swanson, A. B., Hafeman, D. G. and Hoeksira, W. G. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*. 1790: 588-90.

Thompson, N.P., M.E. Caplin, M.I. Hamilton, S.H. Gillespie, S.W.Clarke, A.K.Burroughs, N.McIntyre, Antituberculosis medication and the liver dangers and recommendations in management, *European Respiratory Journal*, 1995,8, 1384-1388.

VandanaTayal, Bhupinder Singh Kalra, SaritaAgarwal, Nita Khurana, Usha Gupta, Hepatoprotective effect of tocopherol against isoniazid and rifampicin induced hepatotoxicity in albino rabbits, *Indian Journal of experimental Biology*, Vol.45, 2007, 1031-1036.

Vidhya, N. and N. Niranjali Devaraj, Antioxidant effect of eugenol in rat intestine, *Indian Journal of Experimental Biology*, Vol.37, 1999, 1192-1195.

Yi-Shin Huang, Herng-DerChern, Wei-juin Su, Jaw-Ching Wu, Shi-Chuan Chang, Chi-Huei Chiang, Full-Young Chang and Shou-Dong Lee, Cytochrome P450 2E1 Genotype and Susceptibility to Anti tuberculosis drug induced hepatitis, *Hepatology*, Vol.37, 2003, 924-930.

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