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EFFECT OF ETHANOLIC ROOT EXTRACT OF *APOROSA LINDLEYANA* ON LIPID PEROXIDATION, ENZYMATIC STATUS OF INH-RIF INDUCED TOXICITY ON MALE ALBINO WISTAR RATS

Ramakrishnan S.[1]* and Venkataraman R.[2]

[1]Department of Biotechnology, Sri Paramakalyani College, Alwarkurichi 627 412.

[2]PG & Research Department of Chemistry, Sri Paramakalyani College, Alwarkurichi 627 412.

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***Corresponding Author**
Dr. Ramakrishnan S.
Department of Biotechnology, Sri Paramakalyani College, Alwarkurichi 627 412.

ABSTRACT

In this present study aimed to explore the hepato and renal protective effect of Ethanolic Root Extract of *Aporosa lindleyana* (EREAL) on INH-RIF induced toxicity on rats. INH-RIF administered rats showed increased oxidative stress not only by increasing the lipid peroxidation but also by decreasing the antioxidant capacity in plasma, tissues of liver and kidney. Administration of EREAL effectively controlled the lipid peroxidation process in INH-RIF rats, which were manifested by the decreased levels of lipid peroxidative markers such as Thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides in the plasma and tissues. EREAL also improved the antioxidant status of INH-RIF rats by increasing the activities of superoxide dismutase(SOD), catalase(CAT) and glutathione peroxidise(GPx).

KEYWORDS: EREAL, INH-RIF, Lipid peroxidation, SOD, CAT, GPx.

INTRODUCTION

INH-RIF (Isoniazid – Rifampicin) administration causes a number of changes in vital organs and systems. The liver is one of the target organ affected by INH-RIF.[1] Rifampicin is widely used in most antitubercular chemotherapeutic regimens.[2] However, this drug is also well known as hepatotoxic agent at doses.[3] In the present study, following an overdose of INH-RIF, the rise in liver marker enzymes level has been attributed to damaged structural integrity of the liver. When the liver plasma membrane is damaged, a variety of enzymes located in

cytosol are released into the blood stream and their estimation in the serum is a quantitative marker for the extent and type of hepatocellular damage.[4] As the liver has a wide variety of functional capabilities, no one single test can provide an accurate assessment of its function. It possesses hepatoprotective effect[5] and antioxidant activity[6] and also showed antihyperglycemic effect[7], and ethanolic extract of Aporosa lindleyana leaves showed anti-inflammatory activity.[8]

Objective of the Study

In this present study, Effect of EREAL evaluated on Isonizid – Rifampicin induced toxicity in albino wistar rats by measurement of lipid peroxidation markers such as TBARS and lipid hydroperoxides and the enzymatic antioxidants of Superoxide dismutase, Catalase and Glutathione peroxidase.

MATERIALS AND METHODS

Collection and processing of plant material

The roots of *Aporosa lindleyana* was collected from Keeriparai, Kanyakumari District, Tamilnadu. The specimen was identified by Dr. V. Chelladurai, Taxonomist, Department of Ayurvedic Sciences, Tirunelveli District. The roots were cleansed and shade dried for a weak and grounded into uniform powder. 1g of plant material was added to 20 ml of aqueous ethanol (20% v/v) for 18 h at room temperature. The extracts was filtered and used for the estimation of total phenols and antioxidant activity. The chemicals were purchased from SD Fine Chemicals Ltd., Mumbai. India.

EXPERIMENTAL ANIMALS

Healthy adult male albino Wistar rats, bred and reared in Central Animal House, Department of Pharmacology, K.M College of Pharmacy, Uthangudi, Madurai, were used for the experiment. Weight matched animals (180-200g) were selected and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 h light/12 h dark). The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India). Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC Registration Number: 661/02/c/CPCSEA) and animals were cared in accordance with the "Guide for the care and use of laboratory animals" and "Committee for the purpose of control and supervision on experimental animals" (CPCSEA).

EXPERIMENTAL DESIGN

The animals were randomly divided into five groups of six animals each. EREAL (200 mg/kg BW) and silymarin (70 mg/kg BW) was suspended in 2 mL of 1% CMC (vehicle solution) and fed by intragastric tube daily for 21 days. After 21 days of treatment, the animals were fasted for 12 h, and sacrificed by cervical dislocation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of various biochemical parameters. Tissue (liver and kidney) were surgically removed, washed with cold physiological saline, cleared off adherent lipids and immediately transferred to ice-cold containers. Erythrocytes were also prepared for the estimation of various biochemical preparations.

PROCESSING OF BLOOD AND TISSUE SAMPLES

Serum preparation

Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 minutes.

Plasma preparation

The blood, collected in a heparinized centrifuge tube, was centrifuged at 2000 rpm for 10 minutes and the plasma was separated by aspiration.

Erythrocyte preparation

After the separation of plasma, the buffy coat, enriched in white cells, was removed and the remaining erythrocytes were washed three times with physiological saline. A known volume of erythrocyte was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2500 rpm for 10 minutes and the supernatant was used for the estimation of enzymic antioxidants.

Tissue homogenate preparation

Liver and kidney tissues (250 mg) were sliced into pieces and homogenised in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 minutes at 0 C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

LIPID PEROXIDATION PRODUCTS

ESTIMATION OF THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)[9]

The concentration of TBARS in the plasma, erythrocytes and tissues were estimated. In this method, malondialdehyde and other thiobarbituric acid reactive substances (TBARS) react with thiobarbituric acid in an acidic condition to generate a pink colour chromophore which was read at 535 nm.

ESTIMATION OF LIPID HYDROPEROXIDES[10]

Lipid hydroperoxide in the plasma, erythrocytes and tissues were estimated Oxidation of ferrous ion (Fe^{2+}) under acidic conditions in the presence of xylenol orange leads to the formation of a chromophore with an absorbance maximum at 560 nm.

ENZYMATIC ANTIOXIDANTS

ASSAY OF SUPEROXIDE DISMUTASE (SOD, EC 1.15.1.1)[11]

Superoxide dismutase in the erythrocytes and tissues was assayed. The assay is based on the inhibition of the formation of NADH- phenazinemethosulphate, nitroblue tetrazolium formazon. The reaction was initiated by the addition of NADH. After incubation for 90 sec. adding glacial acetic acid stops the reaction. The color developed at the end of the reaction was extracted into n- butanol layer and measured in a Spectrophotometer at 520 nm.

ESTIMATION OF CATALASE (CAT, EC 1.11.1.6)*[12]*

The activity of catalase in the erythrocytes and tissues was determined. Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm. The catalase preparation was allowed to split H_2O_2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H_2O_2 as chromic acetate was determined colorimetrically.

ESTIMATION OF GLUTATHIONE PEROXIDASE (EC 1.11.1.19)[13]

The activity of GPx in the erythrocytes and tissues was measured. A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of GSH for a specified time period. Then the remaining GSH content was measured.

STATISTICAL ANALYSIS

All quantitative measurements were expressed as means ± SD for control and experimental

animals. The data were analyzed using one way analysis of variance (ANOVA) on SPSS/PC (statistical package for social sciences, personal computer) and the group means were compared by Duncan"s Multiple Range Test (DMRT). The results were considered statistically significant if the *P* value is less than 0.05.

RESULTS AND DISCUSSION

EFFECT OF EREAL ON LIPID PEROXIDATION AND ANTIOXIDANT STATUS

Table 1 represents the measurement of lipid peroxidation by the concentrations (TBARS and lipid hydroperoxides) in the plasma and tissues (liver and kidney) of INH-RIF hepatotoxic and control rats. Increased levels of TBARS and lipid hydroperoxides were observed in the plasma and tissues of INH-RIF administered group. Treatment with EREAL (200mg/kg BW) significantly decreased the levels of TBARS and lipid hydroperoxides.

Lipid peroxidation has been postulated as being a destructive process in liver injury caused by INH-RIF administration.[14] In the present study, elevations in the levels of end products of lipid peroxidation in liver of rat treated with INH-RIF were observed. The increase in TBARS and LOOH level in liver suggests provoked lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. In accordance with our present study, the treatment with *EREAL* significantly improved the condition by increasing enzymatic antioxidants, thereby reducing lipid peroxidation status. Hence it may be possible that the mechanism of hepato and renal protection of *EREAL* is due to its antioxidant effect.

All tissues have the capacity to neutralize oxygen radicals to some extent by using cellular antioxidants. Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. The cells have enzymatic and non-enzymatic systems to neutralize free radicals. The enzymes superoxide dismutase, catalase and glutathione peroxidase as well as the non-enzymic antioxidants such as reduced glutathione, α-tocopherol and ascorbic acid are the main antioxidants.[15]

EFFECT OF EREAL ON ENZYMATIC MARKERS AND ANTIOXIDANT STATUS

The activities of SOD, CAT and GPx in the erythrocyte and tissues of liver and kidney were given in Tables 2,3, and 4 respectively. The activities of these antioxidant enzymes were remarkably decreased in erythrocyte and tissues of tissues of liver and kidney in INH-RIF

administered rats. In response to EREAL and silymarin treatment, the activities of these enzymatic antioxidants were significantly increased to near normal values.

The body has evolved a complex defense strategy to minimize the damaging effects of various oxidants. Central to this defense, are the antioxidant enzymes SOD, CAT, GPx which act in concert to protect an organism from oxidative damage. Increase in the serum activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury.[16,17] SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide, hence diminishing the toxic effect caused by this radical. Previous report suggested that the methanolic extract of roots of *Aporosa lindleyana* known to increase the levels of catalase and SOD erythrocyte and tissues of liver and kidney on CCl_4 induced hepatocellular and renal damage.[18] In this study, observed results showed that EREAL significantly increased the hepatic SOD and catalase activity of the INH-RIF induced liver and renal damage in rats. This indicates that the EREAL can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic and renal antioxidant enzymes.

Catalase (CAT) is a key component of the antioxidant defense system, widely distributed in tissue and the highest activity is found in the red cells and in liver. CAT decomposes hydrogen peroxide (H_2O_2) and protects the tissue from highly reactive hydroxyl radical. The reduction in the activity of CAT may result in a number of deleterious effects due to the accumulation of H_2O_2. Administration of EREAL increases the activity CAT in INH-RIF induced liver damage rats which prevent the accumulation of excessive free radicals and protects the liver from INH-RIF intoxication. Selenium containing enzyme GPx detoxifies H_2O_2 by utilizing GSH and H_2O_2 as substrate to yield, H_2O and oxidized glutathione.[19] Decrease in the activity of GPx has been associated with a provoked lipid peroxidation in INH-RIF induced hepatotoxic rats. Administration of EREAL significantly increased the level of glutathione in a dose dependent manner.

Table 1: Effect of EREAL on TBARS and lipid hydroperoxides in the plasma and tissues of liver and kidney.

Groups	Plasma (mmoles/dL)		Liver (mmoles/100 g wet tissue)		Kidney (mmoles/100 g wet tissue)	
	TBARS	Lipid hydroperoxides	TBARS	Lipid hydroperoxides	TBARS	Lipid hydroperoxides
Control rats received 1% CMC only	0.13 ± 0.11^{a}	7.39 ± 1.20^{a}	0.72 ± 0.43^{a}	96.21 ± 5.57^{a}	1.45 ± 0.09^{a}	86.89 ± 6.05^{a}
Control + EREAL (200mg/kg BW)	0.11 ± 0.51^{a}	7.95 ± 0.72^{a}	0.69 ± 0.62^{a}	93.66 ± 7.02^{a}	1.42 ± 0.07^{a}	82.73 ± 7.28^{a}
INH-RIF - EREAL (50 mg/kg BW)	0.45 ± 0.41^{b}	16.43 ± 1.02^{b}	1.84 ± 0.58^{b}	152.20 ± 9.45^{b}	2.82 ± 0.03^{b}	160.33 ± 11.12^{b}
INH-RIF - EREAL (200 mg/kg BW)	0.26 ± 0.11^{c}	11.84 ± 0.92^{c}	1.30 ± 0.20^{c}	121.95 ± 6.08^{c}	2.16 ± 0.07^{c}	128.55 ± 5.23^{c}
INH-RIF +Silymarin (70 mg/kg BW)	0.18 ± 0.01^{d}	10.12 ± 0.53^{c}	0.83 ± 0.07^{d}	106.44 ± 8.17^{d}	1.23 ± 0.14^{d}	99.20 ± 8.37^{d}

Values are given as means ± SD for six rats in each group. Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 2: Effect of EREAL on the activities of SOD, CAT and GPx in the erythrocyte.

Groups	Erythrocyte		
	SOD (U*/mg Hb)	CAT ($U^{\#}$/mg Hb)	GPx ($U^{\$}$/mg Hb)
Control rats received 1% CMC only	7.60 ± 0.28^{a}	172.61 ± 10.71^{a}	13.85 ± 0.75^{a}
Control + EREAL (200mg/kg BW)	7.32 ± 0.35^{a}	175.33 ± 13.28^{a}	13.06 ± 0.48^{a}
INH-RIF - EREAL (50 mg/kg BW)	3.58 ± 0.50^{b}	124.70 ± 8.08^{b}	6.40 ± 0.36^{b}
INH-RIF + EREAL (200 mg/kg BW)	5.64 ± 0.57^{c}	156.40 ± 8.55^{c}	8.40 ± 0.39^{c}
INH-RIF + Silymarin (70 mg/kg BW)	6.14 ± 0.35^{c}	161.19 ± 8.53^{d}	10.01 ± 0.95^{d}

U* = enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.

U# = µmole of H_2O_2 consumed/minute.

U$ = µg of GSH utilized/minute.

Values are given as means ±SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$(DMRT).

Table 3: Effect of EREAL on the activities of SOD, CAT and GPx in the liver.

Groups	Liver		
	SOD (U*/mg protein)	CAT(U#mg)protein)	GPx (U$/mg protein)
Control rats received 1% CMC only	8.25 ± 0.55[a]	77.42 ± 5.58[a]	8.91 ± 0.29[a]
Control + EREAL (200mg/kg BW)	8.41 ± 0.56[a]	79.65 ± 5.45[a]	9.73 ± 0.53[a]
INH-RIF – EREAL (50 mg/kg BW)	4.59 ± 1.12[b]	47.37 ± 4.08[b]	4.93 ± 0.49[b]
INH-RIF + EREAL (200 mg/kg BW)	6.25 ± 0.56[c]	61.86 ± 4.77[c]	7.81 ± 0.82[c]
INH-RIF + Silymarin (70 mg/kg BW)	6.97 ± 0.52[c]	71.94 ± 5.06[d]	8.13 ± 0.69[c]

U* = enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.

U# = µmole of H2O2 consumed/minute.

U$ = µg of GSH utilized/minute.

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 4: Effect of EREAL on the activities of SOD, CAT and GPx in the kidney.

Groups	Kidney		
	SOD (U*/mg protein)	CAT (U# /mg protein)	GPx (U$/mg protein)
Control rats received 1% CMC only	13.89 ± 0.71[a]	35.15 ± 2.51[a]	7.92 ± 0.49[a]
Control + EREAL (200mg/kg BW)	13.65 ± 0.75[a]	38.14 ± 2.23[a]	7.58 ± 0.76[a]
INH-RIF - EREAL (50 mg/kg BW)	6.43 ± 0.45[b]	23.49 ± 1.54[b]	4.24 ± 0.61[b]
INH-RIF + EREAL (200 mg/kg BW)	12.03 ± 0.94[c]	30.37 ± 2.68[c]	5.53 ± 0.16[c]
INH-RIF + Silymarin (70 mg/kg BW)	12.18 ± 0.95[c]	36.23 ± 2.17[a]	6.86 ± 0.21[d]

U* = enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.

U# = µmole of H2O2 consumed/minute.

U$ = µg of GSH utilized/minute.

Values are given as means ±SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

CONCLUSION

The observed results suggested that the EREAL have potential clinical applications. Antioxidative action has been reported to play an important role in the liver protective ability of many compounds. EREAL is a strong antioxidant compound or a free radical scavenger as it reduces lipid peroxidation and induces enzymatic antioxidants. Further studies on the plant which reveals other potential effects on modern medicine.

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