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Evaluation and comparison of protective actions resveratrol and vitamin-e in 5-flourouracil induced hepatotoxicity

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Abstract

We investigate the protective effects of resveratrol and vitamin-E against 5-flourouracil induced hepatotoxicity in rats. Experimental rats were treated with 5-FU (20 mg/kg b.wt.) i.p. on day 1, 3, 7 to induce hepatotoxicity, evident by significant ($p < 0.05$) increase in the levels of ALT, AST, TBARS, protein carbonyls, TNF- α , while significant ($p < 0.05$) decrease in the levels of GSH, IL-10. Liver of 5-FU treated rats showed marked infiltration with inflammatory cells, moderate congestion in the central vein, mild vacuolations between hepatocytes and moderate necrosis. There is mild sinusoidal dilation, disorganized hepatocytes and periportal necrosis. Resveratrol and Vitamin-E treatment markedly reversed the changes induced by 5-FU in all parameters. These data concluded that Resveratrol and Vitamin-E had protective effects against 5-FU induced hepatotoxicity in rats.

Keywords: 5-Flourouracil, resveratrol, Vitamin-E, hepatotoxicity

Introduction

5-FU is a pyrimidine antimetabolite, has showed marked anti- cancer effects against wide range of malignancies including head, neck, stomach, colorectal cancer, skin cancer [1-3]. 5-FU acts through S phase of cell cycle. 5-FU acts by incorporating its metabolites into DNA inhibits thymidylate synthase, thus inhibiting DNA synthesis leads to imbalanced cell growth and ultimately cell death of cancer cells [4]. 5-FU also affects the growth of normal body cells and often causes side effects such as fatigue, mouth sore, hair loss, ulcers, birth defects, liver diseases and temporary drop in bone marrow function [5]. Various *in vitro* and *in vivo* studies have demonstrated that administration of 5-FU leads to generation of oxidative stress in the liver which consequently results in structural and functional disruption of hepatocytes.

Resveratrol, a natural phytoalexin present in red wine, grapes, peanuts and berries. It has numerous biological effects including anti-inflammatory, antioxidant, prevent cancer, regulate lipid peroxidation. Its antioxidant activity by scavenging and neutralization of free radicals, raised the activity of enzymes like catalase (CAT), glutathione reductase (GSH). Anti-inflammatory activity by inhibit COX-1, which is a catalyze for production of free radicles [6]. Vitamin-E can interrupt free radical chain reactions by capturing the free radicals; this imparts antioxidant properties. The free hydroxyl group on the aromatic ring is responsible for the antioxidant properties. The hydrogen from this group is donated to the free radical, resulting in a relatively stable free radical form of the vitamin. This process is important in maintaining the integrity of cell membranes [7].

Materials and Methods

All chemicals were of analytical grade and they are obtained from Qualigens Pvt. Ltd., Mumbai and SRL Pvt. Ltd., Mumbai, India.

Animals and Experimental design

A total Thirty six healthy male *Wistar* rats of 3 months of age, weighing between 150-180 g, were obtained from the Vyas labs, Hyderabad. The Experiment was conducted according to the guidelines of Institutional Animal Ethics Committee (No.3/22/C.V.Sc., Hyd. IAEC-Rats/29.02.2020). Before beginning of experiment, all animals were kept one week for acclimatization under the same environmental conditions of temperature 20-22 °C, 12 h light/dark cycle. Animals were placed on commercial standard pellet feed and provided water *ad libitum*.

Table 1: Experimental design with group wise treatment protocol

Groups	Treatments	No. of animals
1	Normal saline for 14 days (control)	6
2	5-Flourouracil control (5-FU) @ 20 mg / kg B.wt, (1, 3 and 7 days) I.P.	6
3	Resveratrol @ 200 mg/kg B.wt. (14 days) Per Oral	6
4	Vitamin E @ 200 mg/kg B.wt (14 days) Per Oral	6
5	5-Flourouracil @ 20 mg/kg (1, 3 and 7days) + Resveratrol @ 200 mg/kg B.wt. (14 days)	6
6	5-Flourouracil @ 20 mg/kg (1, 3 and 7days) + Vitamin E @ 200 mg/kg B.wt (14 days)	6

Blood collection

Blood collection was carried out at the end of the experiment for sero-biochemical analysis. Feed was withdrawn 12 h before the blood collection and blood was collected from retro-orbital plexus into serum vacutainers and centrifuged at 3000 RPM for 15 min, and serum was separated and stored at -80 °C till analysis. The sera samples were analyzed for the activity of BUN and creatinine. On the 14th day, after blood collection, rats were euthanized by carbon dioxide exposure and tissues were collected and stored at -80 °C till estimation of GSH, TBARS and protein carbonyls. Pieces of tissues from kidney were immediately kept in 10% of formalin fixative to study histological alterations.

Bio-chemical analysis**Estimation of biochemical parameters**

The levels of ALT, AST, total protein were estimated by serum autoanalyzer using kits procured from ERBA diagnostics Ltd, Surat, India.

Antioxidant markers

GSH was estimated according to the method of [8]. Malondialdehyde (MDA) was estimated by as per the method prescribed by [9]. Protein carbonyls were estimated according to the method of [10].

Cytokine profile

The assay employs a quantitative sandwich enzyme immunoassay technique to measure TNF – *alpha* and IL-10. The ELISA kit was procured from Krishgen Bio systems, Mumbai.

Histology

Tissue pieces of liver were collected from the rats that were sacrificed at the end and fixed in 10% neutral buffered

formalin (NBF) for histopathology. The small representative pieces of fixed tissues were cut and subjected to overnight washing under running tap water. The tissues were then dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin at 55-56 °C. The paraffin blocks were cut into thin sections of 5 micron thickness by microtome. The cut sections were lifted on grease free glass slides precoated with Mayer's egg albumin and were kept in incubator overnight at 37 °C for drying. The slides were stained with routine Haematoxylin and Eosin (H and E) stain and the stained sections were mounted with DPX mountant and kept ready for microscopic examination.

Statistical analysis

Data were subjected to statistical analysis by applying one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS; version 21). Differences between means were tested using Duncan's multiple comparison tests and significance was set at $P < 0.05$.

Results

A significant ($p < 0.05$) increase in the levels of ALT, AST, TBARS, protein carbonyls, TNF- α , in 5-Flourouracil treated rats when compared to group 1, 3, 4. Administration of Resveratrol (group 5) and Vit-E (group 6) significantly ($P < 0.05$) decreased values when compared to group 2. A significant ($p < 0.05$) decrease in the levels of total protein, GSH, IL-10 was observed in 5-Flourouracil treated rats when compared to group 1, 3, 4. Administration of Resveratrol (group 5) and Vit-E (group 6) significantly ($P < 0.05$) increased values when compared to group 2. The alteration value ALT, AST, total protein, GSH, TBARS, protein carbonyls, TNF- α , IL-10 in control and experimental rats are shown in Table 2 and 3.

Table 2: ALT (IU/L), AST (IU/L), TP (g/dl), GSH (nm/mg protein) in different groups of rats

Groups	ALT	AST	TP	GSH
Normal control	52.10 \pm 3.61 ^c	111.88 \pm 09.34 ^a	10.46 \pm 1.49 ^a	3.37 \pm 0.05 ^a
5-FU control	85.63 \pm 4.27 ^a	248.76 \pm 17.08 ^c	03.03 \pm 0.18 ^c	2.26 \pm 0.11 ^c
RSV	49.10 \pm 4.41 ^c	110.76 \pm 11.13 ^a	10.66 \pm 1.51 ^a	3.41 \pm 0.05 ^a
Vit-E	51.10 \pm 4.41 ^c	109.65 \pm 11.13 ^a	10.86 \pm 1.52 ^a	3.41 \pm 0.05 ^a
5-FU + RSV	73.01 \pm 4.33 ^b	160.57 \pm 15.58 ^b	06.81 \pm 0.89 ^b	3.03 \pm 0.07 ^b
5-FU+ Vit-E	71.35 \pm 4.58 ^b	154.48 \pm 10.36 ^b	06.81 \pm 0.65 ^b	3.06 \pm 0.06 ^b

Values are mean \pm SE (n=6); one way ANOVA. Means with different alphabets differ significantly ($P < 0.05$)

Table 3: Tbars (nm MDA released/mg protein), Protein carbonyls (nm/mg protein), TNF-*alpha* (pg/mg tissue) and IL-10 (pg/mg tissue) in different groups of rats

Groups	TBARS	Pc	TNF- α	IL-10
Normal control	1.02 \pm 0.12 ^c	0.62 \pm 0.05 ^c	07.40 \pm 0.34 ^c	81.23 \pm 1.18 ^a
5-FU control	3.89 \pm 0.25 ^a	1.35 \pm 0.11 ^a	17.93 \pm 0.69 ^a	52.44 \pm 2.14 ^c
RSV	0.88 \pm 0.12 ^c	0.63 \pm 0.05 ^c	07.11 \pm 0.34 ^c	80.11 \pm 1.18 ^a
Vit-E	0.96 \pm 0.12 ^c	0.61 \pm 0.05 ^c	07.30 \pm 0.34 ^c	77.66 \pm 0.55 ^a
5-FU + RSV	2.41 \pm 0.12 ^b	0.95 \pm 0.04 ^b	14.11 \pm 1.21 ^b	70.19 \pm 1.18 ^b
5-FU + Vit-E	2.17 \pm 0.12 ^b	0.91 \pm 0.05 ^b	13.11 \pm 1.21 ^b	71.27 \pm 1.18 ^b

Values are mean \pm SE (n=6); one way ANOVA. Means with different alphabets differ significantly ($P < 0.05$)

Discussion

Liver plays a central role in the detoxification process and along with kidney it faces the threat of maximum exposure to xenobiotics and their metabolic by-products. The hepatotoxic effect produced by 5-Flourouracil due to generation of highly reactive free radicals because of excessive oxidative stress and the accumulation of oxidation products in the liver that cause damage to the biological membranes and the endothelial lining of the liver leading to liver dysfunction and alteration in the permeability of liver membrane leading to their leakage into blood stream. In the present study increased the activities of ALT, AST attributed due to liver dysfunction. A similar increase in ALT, AST was observed in rats by [11-13]. Resveratrol offers hepatoprotection by influencing the levels of lipid peroxidation products and liver markers, which could be due to their free radical scavenging and antioxidant property [14]. In the present study the activities of liver enzymes (ALT, AST) reduced by Resveratrol. A similar findings were observed by [15].

In the present study total protein levels were decreased in 5-FU treated rats which might be due to the impairment of liver function resulting in oxidative stress. Reactive oxygen species can stimulate LPO and cause damage to the proteins, increase the protein expression of the proteolytic signals and decrease the capacity of liver to synthesize proteins. Decreased levels of total protein observed by [16]. In this study Resveratrol improved the levels of total protein reporting its protective effect. Similar findings were also observed in previous studies [17].

In the present study concentration of GSH was significantly decreased, while the concentration of TBARS and protein carbonyls were significantly increased in 5-FU treated rats due to free radicals induced oxidative stress in the tissues. Similar findings were observed by [18]. Resveratrol increase the levels of GSH, decreased the levels of TBARS, protein carbonyls due to its antioxidant property, ability to scavenge free radicals and inhibits lipid peroxidation. Similar findings were observed by [17].

In the present study concentration of TNF- α was increased, while the concentration of IL-10 was decreased due to oxidative stress induced by 5-FU, which triggers the expression of COX-2, catalyze the production of pro-inflammatory cytokines like TNF- α , decreased the production of anti-inflammatory cytokines like IL-10. Similar findings were observed by [19]. Resveratrol reduced the levels of TNF- α , increased the levels of IL-10 due to its anti-inflammatory properties. Similar findings were observed by [20, 21].

Histopathology

The histopathological section of the liver from the 5-FU control group showed marked infiltration with inflammatory cells, moderate congestion in the central vein, mild vacuolations between hepatocytes and moderate necrosis (Fig.2). There is mild sinusoidal dilation, disorganized hepatocytes and periportal necrosis (Fig.3). Marked central vein oedema, marked infiltration of inflammatory cells around the central vein (Fig. 4) in rats of group 2, whereas the sections in rats treated with Resveratrol (RSV) group 5 showed mild central vein oedema, mild sinusoidal congestion and mild necrosis (Fig. 7), very mild sinusoidal dilation and mild infiltration with inflammatory cells (Fig. 8). Rats treated with Vit-E showed mild sinusoidal dilation and mild infiltration with inflammatory cells (Fig. 9). The histopathological sections from rats of group 1 (Fig.1), group 3 (Fig.5), group 4 (Fig.6) showed no significant changes.

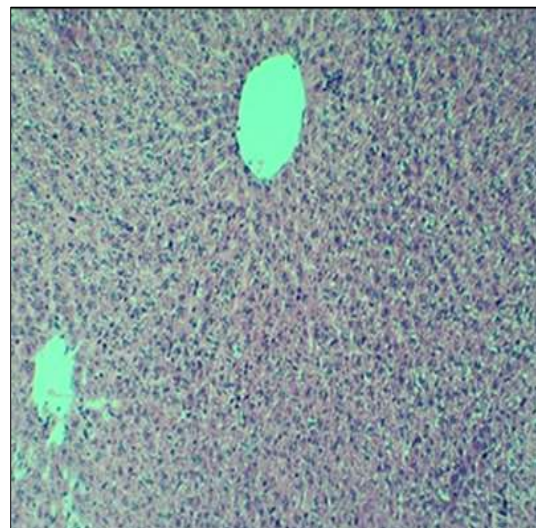


Fig 1: Photomicrograph of Liver tissue showing normal architecture. (Group1) H&E X 100

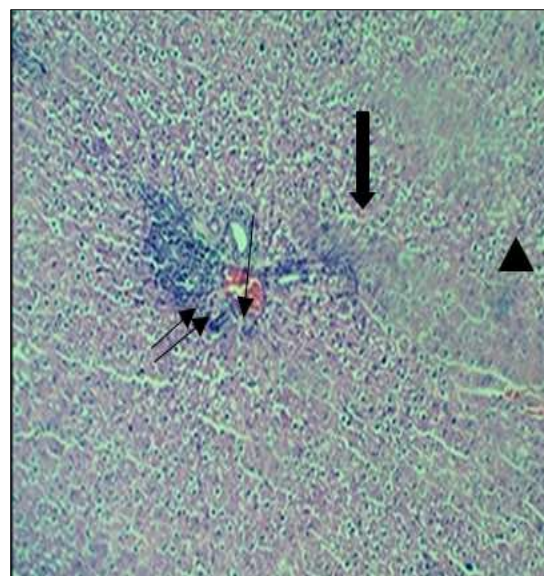


Fig 2: Photomicrograph of Liver tissue showing marked infiltration with inflammatory cells (double arrow), moderate congestion (thin arrow) in the central vein. Mild vacuolations (thick arrow) between hepatocytes and moderate necrosis (arrow head) (Group 2) H&E X100

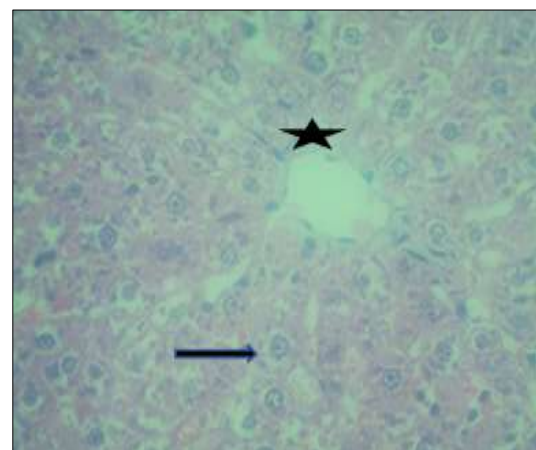


Fig 3: Photomicrograph of Liver tissue showing mild sinusoidal dilation (thick arrow) disorganized hepatocytes and periportal necrosis (star). (Group 2) H&E X400

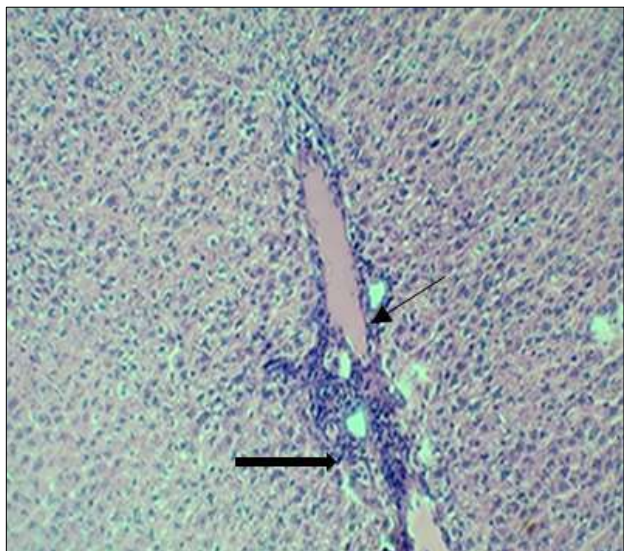


Fig 4: Photomicrograph of Liver tissue showing marked central vein oedema (thin arrow) and marked infiltration with inflammatory cells around the central vein (thick arrow). (Group 2) H&E X100

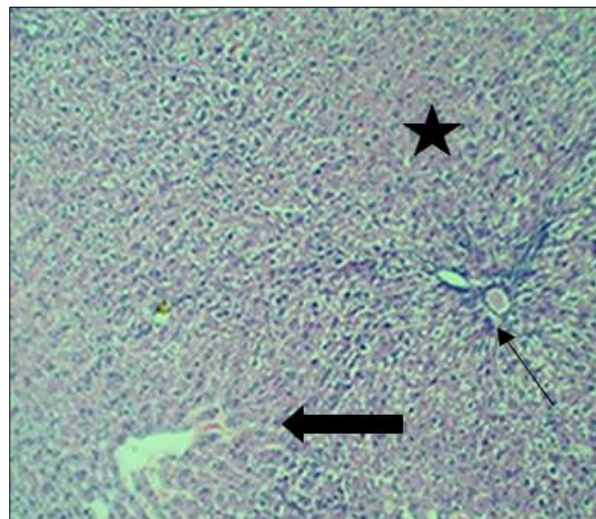


Fig 7: Photomicrograph of Liver tissue showing mild central vein oedema (thin arrow), mild sinusoidal congestion (thick arrow) and mild necrosis (star) (Group 5) H&E X100

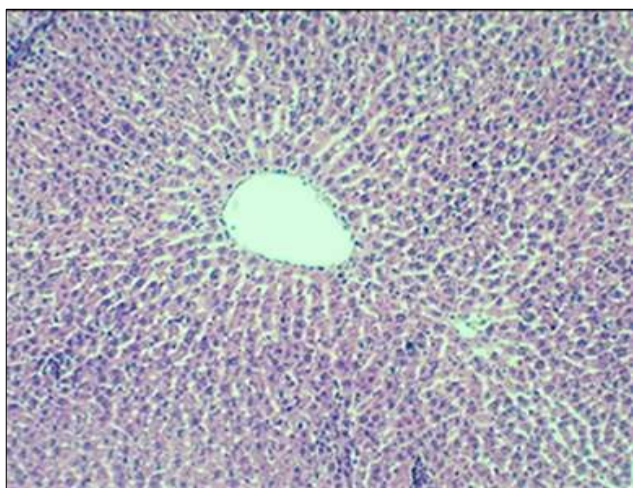


Fig 5: Photomicrograph of Liver tissue showing normal architecture of liver lobules with radiating appearance of hepatic cords. (Group 3) H&E X100

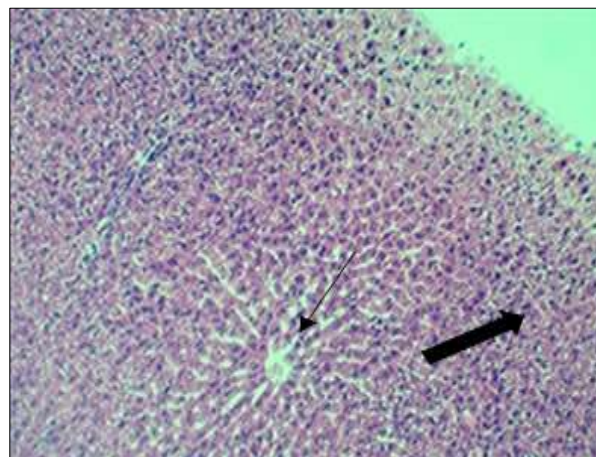


Fig 8: Photomicrograph of Liver tissue showing very mild sinusoidal dilation (thin arrow) and mild infiltration with inflammatory cells (thick arrow). (Group 5) H&E X100

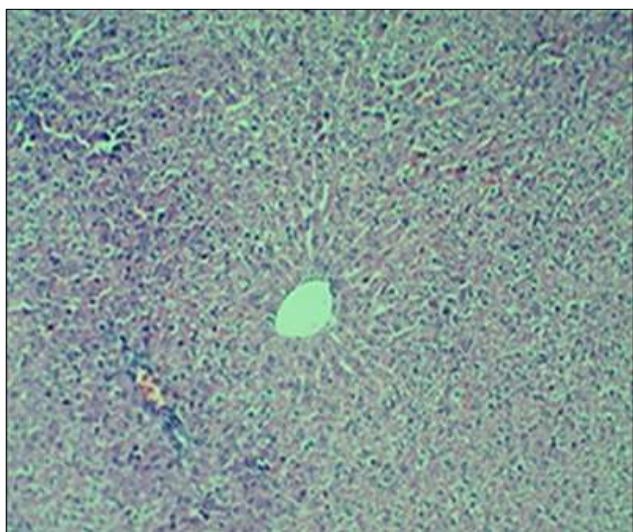


Fig 6: Photomicrograph of Liver tissue showing normal architecture of liver lobule with radiating appearance of hepatic cords. (Group 4) H&E X100

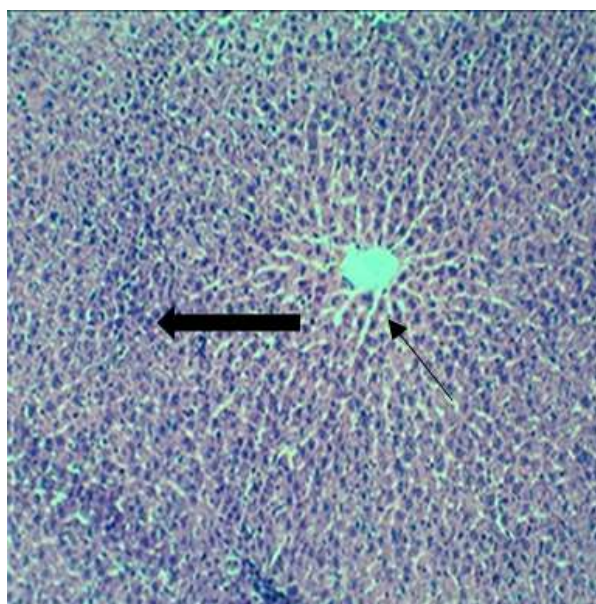


Fig 9: Photomicrograph of Liver tissue showing mild sinusoidal dilation (thin arrow) and mild infiltration with inflammatory cells (thick arrow). (Group 6) H&E X100

Conclusion

In conclusion, Resveratrol and Vitamin-E were found to possess protective actions against 5-fluorouracil (5-FU) induced hepatic toxicity, which was evident in this study by reducing the toxic markers and replenishment of membrane and restoration of antioxidant enzymes. The overall beneficial effects of Resveratrol and Vitamin-E could be attributed to their antioxidant actions.

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