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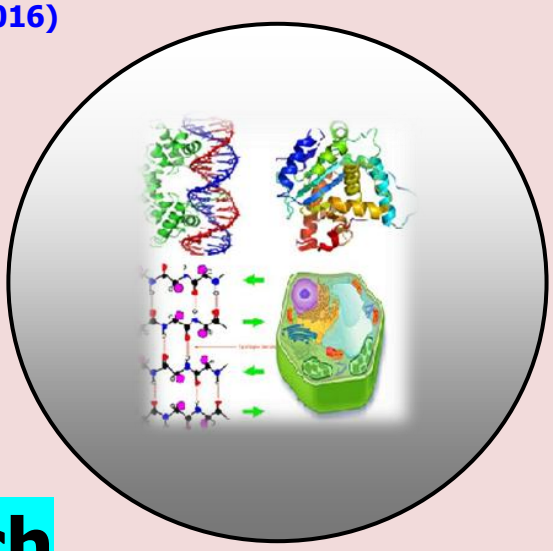

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RESEARCH PAPER

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Protection of the Resveratrol on the 7, 12 DMBA Damage Over the Rat Liver by Oxidant/Antioxidant Stress

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ABSTRACT

Trans-Resveratrol (3,5,4'-trihydroxy-trans-stilbene) has been showed to limited interview of chemoprotection against 7,12-dimethylbenz[a]anthracene (DMBA)-induced liver carcinogenicity in vivo and in vitro. We studied; marker's of oxidant/antioxidant and liver degeneration with GSH, MDA, NO, TGF Beta, TNF Alpha, ALT, AST, GGT paremeter's in 7,12 DMBA induced rats (n=7) , Resveratrol (n=7), DMBA+R (n=7) and control (sham). Our results show that GSH and TGF Beta higher in Resveratrol groups compared to 7,12 DMBA induced groups (p<0,005). MDA, NO, TNF Alpha, ALT, AST, GGT were increased in 7,12 DMBA induced groups compared to sham (p<0,005). This results show that Resveratrol has an antioxidant effect on the DMBA induced damage to liver. The effect of Resveratrol on the DMBA induced rat liver very limited study on the literature screening.We believe that this study will be a light for future work for the cancer prevention with resveratrol.

Key words: 7,12 DMBA, Resveratrol, Glutathione, malondialdehyde, Nitric Oxide, Tumor Necrosis factor Alpha and Tumor Growth Factor Beta.

INTRODUCTION

Primary liver cancer (PLC) is the fifth most common world wide and is stil associated with malignancy (Maruyama). The changes of rat liver oxidant and antioxidant marker's with liver enzymes were reported more reproducible and reliable during 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced liver carcinoma. [2] Reactive oxygen species generate (ROS) can diffuse from the site of generation to targets within the cells in the course of DMBA metabolism [3].

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is a natural polyphenolic compound situated in various red colored plant species, including *Polygonum* and *Vitaceae* (Chang et al., 2001). This naturally occurring compound is also ingested by humans through the consumption of grapes and juice (Chang et al., 2001).

Recent studies have exhibited that pterostilbene and resveratrol possess anti-inflammatory, antioxidant and anticarcinogenic properties which may be responsible for their cancer chemopreventive potency (Agca et al., 2012). The effect of Resveratrol on the DMBA induced rat liver very limited study on the literature screening.

In this purpose, the present article designed to study the changes in GSH, MDA, NO, TNF Alpha, TGF Beta, ALT, AST, GGT, 5' NT enzyme activities/levels were measured in the liver because it is known that this marker's can provide much more sensitive indicator.

MATERIAL AND METHODS

Groups were designed as following; 7,12 DMBA was solved in corn oil (20 mg/kg (ip) twice a week; for 3 weeks DMBA) induced rats (n=7), Resveratrol (20 mg/kg in DMSO) (n=7), DMBA+R (n=7) and control (sham: DMSO and corn oil) . Animals were observed daily, and all the necessary data were recorded. The experiment was terminated at the end of 3 Wk and all animals were sacrificed by cervical dislocation after an overnight fast. Blood was collected and normal, and suspicious lesions were rapidly removed, measured, and rinsed in physiological saline. Liver tissue samples for histological evaluation were prepared in 10% buffered formalin and later embedded in paraffin. The sections were stained with hematoxylin and eosin (HE). Fresh tissues were used for each experimental process. Blood samples were centrifuged at 3,000 × g for 10 min, and the serum was carefully removed and stored at –80◦C until further analysis. We studied GSH, MDA, NO, TGF Beta, TNF Alpha, ALT, AST, GGT levels in the groups. All chemicals were obtained from Sigma; except that TGF Beta and TNF Alpha kits were purchased from Assaypro (ET3102-1; Missouri, USA) and eBioscience (BMS622; Austria) ; respectively as ELISA kits (Enzyme-Linked Immunosorbent Assay Technique) by ELISA analyzer (Brio, Seac, Radim, Italy). ALT, AST analysis were carried out using an autoanalyser (Olympus AU 600, Japan) and commercial kits from Olympus. MDA in plasma was determined by thiobarbituric acid-reactive substances of lipid peroxidation method (Uchiyama, 1978). Results are given as nmol/gwettisue In the determination of glutation, Fairbanks and Klee method was used. This is also, based upon the reaction of sulfidril groups with Elman marker's,. Results were calculated as nmol /gwet tissue. NO was determined by Cortas and Wakid method, which is based on the spectrophometric measurement of the coloured complex produced by the interaction of NO formed by NOS activity in the environment with Griess reactive (Karabulut et al., 2010, Kirimlioglu et al., 2008). For the statistically analysis: Data are described median as minimum and maximum values,. Kruskal-Wallis test was used for group comparisons. Kruskal-Wallis test after multiple comparisons were made by the method of Conover. 0,001 and 0,05 level of significance for all tests was considered.

RESULTS

Our study results show that increased activity ALT, AST, GGT, 5' NT, MDA, NO, TNF α, levels in 7,12 DMBA group than control ($p<0.001$). In the contrary, GSH and TGF β levels decreased in DMBA group than control. But the decreased activity were found that ALT, AST, GGT, 5' NT, MDA, NO, TNF α in the DMBA and Resveratrol group compared to Resveratrol ($p<0.001$). (Table 1-2) Histopatologically parellely to our study, showed increased damage in DMBA was decreased by resveratrol.

Table. 1 Biochemicalliverenzymeactivity in allgroups.

	ALT	AST	GGT	5'Nucleotidase
Control (sham)	44.14±3.13	52.71 ±3.73	4.53±0.54	5.74±0.1
7,12 DMBA (n=7)	66.29±3.30[a]	112.57±5.09[a,b]	41.43±4.12[a,b]	9.06±0.44[a,b]
Resveratrol (n=7)	53.43±3.41	59.43±3.87	7.16±2.2	5.82±0.78
DMBA+R (n=7)	54.00±3.74	80.57±8.4[c]	25.86±4.1[c]	6.02±0.56[c]

Table 2. Oxidant and antioxidant marker's of allratgroups.

	GSH	MDA	NO	TGF-B1	TNF alpha (pg/gwettisue)
Control (sham)	84.27±7.28	29.98±3.7	27.84±1.97	611.27±54.98	1136.55±84.81
7,12 DMBA (n=7)	45.41±6.93[a]	92.78±7.72[a]	52.56±18.33[a]	412.25±174.76[a]	1635.13±181.4[a]
Resveratrol (n=7)	93.77±1.95	31.68±8.88	33.87±12.59	906.67±61.88[a]	1466.24±150.59[a]
DMBA+R (n=7)	72.48±3.67[b]	47.33±6.57	31.03±11.22	679.36±106.38[b]	1643.61±426.6[a,b]

a= p<0.05comparedtoconrol
b=p<0.05comparedtoresveratrol

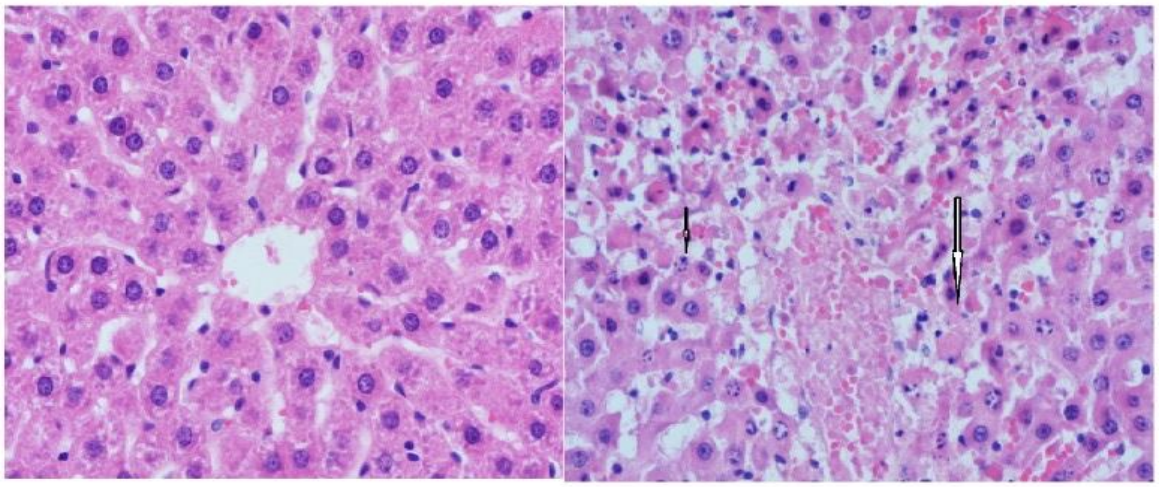

Fig 1.(a) control liver mukoza (HEx200)

Fig 1 (b). Mitosis and pericentral necrosis with pleomorphism was observed in the group treated with DMBA

DISCUSSION

It is essential to provide a suitable range of antioxidants to supress the ROS before they could diffuse and induce serious damage to DNA, protein and lipids, primary to degenerative diseases like cancer [5]. DMBA treatments generate ROS in affected area of organism and ultimately lead to carcinogenesis (Das et al., 2010). Many studies have demonstrated the protective properties of polyphenolic flavonoids such as Resveratrol is effective antioxidants may provide protection against cancer (Arulkumaran et al., 2007). *Trans*-Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) has been reported to interview chemoprotection against 7, 12-dimethylbenz[*a*]anthracene (DMBA)-induced carcinogenicity in a rat model. [2]. Leung and Yung, studied the comet assay in the DMBA introduced DNA damage to cells, and co-treatment of resveratrol at 5 or 10 microm could alleviate the damage (Leung et al., 2009). Roy and Kalra et al 2009 was reported resveratrol's chemopreventive features were reverberate with tardiness in beginning of tumorigenesis, decreased number of tumors, and reduction in tumor volume. Results of their western blott showed that resveratrol treatment decreased the expression of Bcl-2 and Survivin though increased with the DMBA suppressed p53 and Bax. Further, resveratrol supplementation resulted in caspases activation and increase in mechanism of apoptosis induction (Roy et al., 2009) In this perpective we studied apoptosis marker's as TNF alpha and TGF Beta parellel to their results as increased activity with DMBA decreasaed by resveratrol for the TNF alpha. TGF beta results show that decreased levels in DMBA increased with resveratrol. These results show that resveratrol can be used a regeneration of liver (Lin et al., 2012). Our previous studies reported that dietary administration of resveratrol can be protective against the formation of azoxymethane (AOM)- induced liver in rats (Simsek et al., 2012). Parellel to our study; Lycopene supplementation attenutaed the hepatic response to DMBA by altering the oxidative stress biomarkers. Protein oxidation and lipid peroxidation were significantly increased in the DMBA group than in the control group (Flesher and Myers, 1985). This is consistent with previous studies that DMBA induces critical oxidative damage in the liver in vivo. Resveratrol has been defined as an important modulator of cell phenotype with a complex and pleiotropic way of action (Borriello et al., 2014). Extensive literature regarding its activity, mainly employing cellular models, suggests that this compound handling of cell proliferation, situmulates differentiation, and activates apoptosis.

CONCLUSION

Although there is a few studies for DMBA and resveratrol effect on the liver oxidative stres paremeters, resveratrol can be protective of DMBA induced rat liver by the oxidative stress and apoptosis. Resveratrol can be a protective for DMBA induced liver carcinogenesis but it needs some dose evaluating study in the future.

List of abbreviations

DMBA : 7,12-dimethylbenz[a]anthracene; R: Resveratrol; GSH; Glutathione, MDA; malondialdehyde, NO: Nitric Oxide; TNF : Tumor Necrosis Factor, TGF: Transforming growth factor.

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