Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/14228
DOI URL: http://dx.doi.org/10.21474/IJAR01/14228

RESEARCH ARTICLE

RESVERATROL MODULATES THE 7, 12-DIMETHYLBENZ[A]ANTHRACENE INDUCED OXIDATIVE STRESS IN SKIN CARCINOGENESIS

Sonu Singhal[1], Shreshtha Gaur[1], Rakesh Mishra[2] and Surabhi Bajpai[*1]

1. Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali-304022, Rajasthan, India.
2. Getwell Polyclinic and Hospital, JLN Marg, Jaipur- 302004, Rajasthan, India.

Manuscript Info

Manuscript History
Received: 10 December 2021
Final Accepted: 13 January 2022
Published: February 2022

Key words:-
Skin Carcinogenesis, Resveratrol, Reactive Oxygen Species, Histopathology, Corrugated Epidermis

Abstract

Resveratrol is a natural polyphenol present in the Japanese knotweed. It exhibits variety of chemopreventive and anti-proliferative effects against the different cancer cell lines. During skin carcinogenesis, the radical scavenging activity gets altered which are important to prevent the deleterious effect of free radicals. The present study targeted to elucidate the anti-cancer potential of resveratrol against the 7, 12-dimethylbenz[a]anthracene induced skin carcinogenesis in swiss albino mice. Intraperitoneal administration of resveratrol (5mg/kg and 10mg/kg of the body weight) revealed a significant reduction ($p<0.001$) in the tumor incidence, tumor burden and the mean tumor volume as compared to the control group. Also, towards the end of 10 and 22 weeks, resveratrol restored the activities of the antioxidant enzymes significantly in the treated groups. Histopathological studies revealed thickened corrugated and hyper-proliferative epidermis in skin tumor of 7, 12-dimethylbenz[a]anthracene induced mice and the similar results were found to be at significantly lesser extent post resveratrol treatment. The cumulative results of this study indicate that the resveratrol has potentials to modulate the 7, 12-dimethylbenz[a]anthracene induced histopathological and biochemical alterations during skin melanoma in mice.

Introduction:-

Skin act as a crucial environmental interface allocating a protective envelope that is important for first line of defense system in body(Samarasinghe and Madan, 2012). Skin cancer develops and affects mainly the epidermis and it is primarily of three type's basal cell carcinoma, squamous cell carcinoma and melanoma (Eisemann et al., 2014). It affects the normal functioning of skin and pathogenesis involves development of lesions and thickened corrugated epidermis, hyperkeratosis and hyper-proliferative epidermis majorly. Nowadays, it becomes a major growing public health problem(Samarasinghe and Madan, 2012). Non- melanoma skin cancers (NMSCs) comprise of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) (Eisemann et al., 2014). Polycyclic aromatic hydrocarbons (PAHs), occupational and environmental exposures to arsenic are the other common cause of NMSCs (Surdu et al., 2013). Current knowledge about carcinogenesis accompanied to the development of various preventive approaches such as the prohibiting of known carcinogens (Umar et al., 2012). Chemopreventive conspiracies affecting initiation, promotion or progression play an important role in decelerating or inhibiting the carcinogenesis process. The chemopreventive actions of compounds are key components of the process of carcinogenesis. They

Corresponding Author:- Dr. Surabhi Bajpai
Address:- Department of Biosciences & Biotechnology, Banasthali Vidyapith,Banasthali-304022, Rajasthan, India.

include the accumulation of DNA-reactive compounds, the stimulation of DNA repair mechanism, and the scavenging of free radicals (Eisemann et al., 2014).To achieve the inhibition of angiogenesis and proliferation, various anti-progressive and anti-promoting strategies have adopted (Bilecová-Rabajdováet al., 2013). Some of these include suppressing the carcinogenesis process and promoting the use of natural plant compounds (Cragg and Newman, 2005).

Resveratrol (3, 5, 4'-trihydroxy-tans-stilbene), a polyphenol is extracted from the berries, grapes, peanuts and red wine (Kim and Kim, 2018). Resveratrol (RV) is a powerful antioxidant that has anti-proliferative, anti-hyperalgesic effects (Ko et al., 2017)and is known to improve the survival rate of various cancer patients (Aziz et al., 2006; Singhet al., 2013). It is a potent antioxidant with robust anti-proliferative and anti-inflammatory affects.

It is naturally present in Polygonum cuspidatum, an ancient Chinese medicinal plant(Kim and Kim, 2018). Skin cancer may be caused by the oxidative stress and may lead to DNA damage in the skin that includes DNA single and double strands break, DNA base damage and cross-linking between DNA and proteins (van Berlo et al., 2010). The 7,12dimethybenz(a)anthracene (DMBA) is the most commonly used chemical to induce the skin cancer model.

This study aims to analyse potential of resveratrol in amelioration of skin cancer in DMBA induced skin cancer model. Different parameters like the tumor burden, total number of lesions, tumor incidence and tumor volume were used to measure the chemotherapeutic potential of RV. To govern the mechanism of action of RV in chemically induced skin carcinogenesis the histopathological alterations and the antioxidant defense system were also studied.

Material and Methods:-

Animal model

Healthy male swiss albino mice (around 20-25g) were obtained from the Lala Lajpat Rai University of Veterinary & Animal Science, Hisar (Haryana, India). Mice were housed in polypropylene cages bedded with sterilized rice husk and were provided water ad libitum and standard pellet diet. All animals were kept in cages at a 24 ± 1 °C temperature and of 55 ± 5%, humidity with 12 hours light-dark cycle. Two days prior to the experimentation, the dorsal side of mice skin was shaved.

Experimental design

Mice were randomly segregated into 6 groups with 6 mice in each group: Control (NC^1), RV (5mg/kg) (NC^2), RV (10mg/kg) (NC^3), DMBA administered (C^1), DMBA + RV (5mg/kg) (C^2) and DMBA + RV (10mg/kg) (C^3) treated group. The total 48 animals are used in all experiments. In histopathology study every group has 2 mice each. The sample size was designed according to the prior studies done on carcinogenesis. From the third week of the treatment period, the DMBA administration was initiated, as for the first two weeks mice were acclimatized. DMBA was applied topically at a dose of 25nmol in 0.2ml of acetone twice a week for the treatment period and during this period every mouse was caged singly (1 mice/cage). The RV treatment started after confirmation of skin cancer development using morphological studies. The RV treated group mice were administered intraperitoneally (i.p.) with RV at a dose of 5 mg/kg and 10mg/kg of the body weight using DMSO as its vehicle thrice a week throughout the treatment period(Kaur et al., 2009). In the RV + DMBA group mice, RV was directed in a similar manner (at a dose of 5 mg/kg and 10mg/kg of the body weight using DMSO, 200µl) as that for the RV treated group but this time mice were already confirmed for melanoma due to DMBA administration. The control group was administered with DMSO (200 µl). The animal treatment schedule followed in current study is depicted in Fig 1. Throughout the treatment period, the water consumption, body weight and diet were observed every week in all the groups. The mice were sacrificed by cervical dislocation after the development of evident skin tumorigenesis lesions.

Tumor assessment

The skin /skin tumors were excised at the end of the treatment period (22 weeks), and analyzed for:
(a) Tumor incidence percentage (number of animals showing carcinogenic response / total number of animals in the group × 100) (Beauchamp and Fridovich, 1971).
(b) Mean tumor volume was measured by using the formula i.e. $4/3\ \pi r^3$ where r is mean tumor radius in mm. Tumor radius was analysed using vernier calipers manually.
(c) Mean tumor burden was calculated by multiplying the mean tumor volume and mean number of tumors.
(d) Total number of lesions.

Effect of RV on biochemical markers
Investigation of the effect of RV administration on the extent status of oxidative stress at pre-initiation, post-initiation and promotion stages of skin carcinogenesis was done by sacrificing the animals at the end of 2 (before cancer induction), 10 and 22 weeks (after DMBA treatment) respectively.

Estimation of Superoxide Dismutase (SOD) Activity
25µl tissue supernatant was added to reaction mixture containing 5mM phosphate buffer, 1mM EDTA, 100mM methionine,450mM nitro blue tetrazolium (NBT) and 10mM riboflavin. The samples were incubated in light for 30minutes.The blue color was observed and absorbance was taken at 560 nm (Aebi, 1984).

Estimation of Catalase (CAT)
The tissue supernatant (equivalent to 50ng protein) was added to a 1 ml of a reaction mixture containing 8.8 mM H_2O_2 of 3%, 0.5 mM sodium-phosphate buffer (Na_3PO_4) having a pH 7.0. The absorbance was taken at 240 nm (Mokrasch and Teschke, 1984).

Assessment of Glutathione Reductase (GSH) level
Formic acid (0.1 M) was added to supernatant of samples and centrifuged for 10 minutes at 10,000 rpm. The tissue supernatant was added to the tubes containing buffered formaldehyde (1:4 (v/v) 36% formalin: 0.5 M Na_2HPO_4). Each tube was filled with Na_3PO_4 (0.1 M, pH 8.0), then o-phthalaldehyde (OPA) (100 µg/ml) was added. The absorbance was recorded at excitation wavelength which is 345 nm and at emission wavelength which is 425 nm after 45 minutes of incubation at room temperature (Carlberg and Mannervik ,1985).

Estimation of Glutathione Peroxidase (GPx) Activity
The supernatant (0.4 mg) was added to a reaction mixture of Na_3PO_4 (0.1 M, pH 7.2) containing 20 mM oxidized glutathione, 2 mM EDTA and 3 mM NADPH. The absorbance was taken at 340 nm (Ruiz-Larrea et al., 1994).

Determination of Lipid Peroxidation (LPO)
The supernatant (0.2 ml) was mixed with 1.2 ml of 20% acetic acid solution changed to pH 3.4 with help of NaOH, 1.2 ml of 0.9% TBA and 0.1 ml of 8.1% SDS. Thereafter, the 4ml distilled water was added to volume of reaction mixture. The reaction mixture was heated for 60 minutes at 95°C. Further, the reaction mixture was cooled at room temperature and centrifuged at 10,000 rpm. The absorbance was taken at 532 nm (Williams and Arscott, 1971; Ohkawaet al., 1979).

Determination of Glutathione Reductase (GR) activity
The GR activity was based on the NADPH dependent reduction of GSSG to GSH. The GR reduces GSSG to GSH which further reacts with the DTNB to produce the yellow colour compound. The absorbance was taken at 340 nm (Koulet al., 2006).

Histopathological Studies
Skin tumor tissues from the control as well as the RV treated mice were extracted using standard ethical protocol on 10 and 22 week post treatment and utilized for histopathological investigations. The paraffin-wax embedded 6µm thick sections were prepared by fixing the tissue in formalin and embedded in paraffin. The sections made from each tissue were stained with hematoxylin and eosin. The slides were examined under the light microscope for the morphometric tissue changes (Sharma et al., 2010).

Statistical Analysis
The results of each experimental group were represented as mean ± SD. The results of controls were compared with the treated groups. The results were analyzed by using two-way ANOVA followed by a SNK (Student Newman-Keuls) test by Sigma at 3.5 software. The data with $p \leq 0.001$ and $p \leq 0.01$ were considered statistically significant.

Results:-

Body weight and dietary intake
The body weight of the entire mice group showed no subsequent change during the period of experimental time. The average body weight of the animal was tabulated very week and it was represented as a function of time in weeks as shown in Fig. 2.

Tumor statistics

At the end of the 22 week, the chemopreventive potency of RV against DMBA induced skin tumorigenesis was measured using tumor volume, tumor burden and tumor incidence parameters. Papilloma's first appeared in the DMBA group i.e. C^1 group during the sixth week of treatment whereas, in case of the RV (5mg/kg) +DMBA (C^2) and RV (10mg/kg) +DMBA group (C^3), their first appearance was retarded by one week (i.e. 7^{th} and 8^{th} week). The tumor incidence in the DMBA group was 82.0% whereas 63% in the RV(5mg/kg) +DMBA and 59% in the RV (10mg/kg) +DMBA group with a precautionary effect of about 19% and 23% respectively (Fig 3A). The mean tumor volume of the DMBA group was found to 135.9± 2.53mm^3 whereas on the other hand the mean tumor volume of RV (5mg/kg) +DMBA and RV (10mg/kg) +DMBA group was 63.85± 2.12mm^3 and 30.9± 1.56mm^3 respectively which showed a marked decrease as compared to the DMBA (Table 1). Likewise, the mean tumor burden was significantly reduced in the RV (10mg/kg) +DMBA (43.56± 1.41mm^3) and RV (5mg/kg) +DMBA (105.99± 2.32mm^3) as compared to the DMBA administered control group (486.52± 1.89 mm^3) (Table 1). In the DMBA group the total numbers of lesions were found to be 43 whereas in RV (5mg/kg) +DMBA and RV (10mg/kg) +DMBA lesions were found to be 20 and 17 respectively (Fig 3B).

Biochemical Parameters

Pre-initiation stage of skin carcinogenesis:

After pre-initiation (2 weeks) treatment period, the antioxidant activities were evaluated from given biochemical parameters as recorded in Table 2. No significant difference was observed in the activities of SOD, CAT, GR, GPx, GSH level and LPO level of the RV 5mg/kg (NC^2) and RV 10mg/kg (NC^3) as compared to control.

Post-initiation stage of skin carcinogenesis

Effect of RV on antioxidant parameters

After post-initiation (10 weeks) of treatment period, the SOD, GSH, CAT, GPx, GR activities and LPO level were measured and represented graphically in Fig. 4. The SOD, GPx, GR, GSH and CAT activities in C^1 DMBA administered mice was found to be significantly decreased ($p<0.001$) in comparison with NC^1 control mice. However, the SOD, GPx, GR, GSH and CAT activities displayed a highly significant ($p<0.001$) improvement in C^3 (RV (10mg/kg) + DMBA) group as compared C^1 control group. In case of LPO, C^1 the DMBA administered mice displayed a significant increase ($p<0.001$) in LPO level but when treated with RV (10mg/kg) the LPO levels exhibited significant reduction ($p<0.01$) in comparison with C^1 control mice.

Promotion Stage of skin carcinogenesis

Effect of RV on antioxidant parameters

The LPO level and SOD, GPx, GR, GSH and CAT activities were measured after 22 weeks of treatment and depicted graphically in Fig. 5. The C^1 group exhibit a significant increase ($p\leq0.001$) in the level of LPO as well as the significant reduction in the activities of GPx ($p< 0.001$), SOD ($p< 0.001$), GR ($p< 0.001$), GSH ($p< 0.001$) and CAT ($p< 0.001$) was observed compared to NC^1 control mice. Treatment with RV 5mg/kg and 10mg/kg the LPO level exhibited highly significant reduction ($p< 0.001$) whereas activity of SOD, GPx, GR, GSH and CAT statistically displayed a significant increase ($p< 0.001$) in their activities as compared to the DMBA controls.

Histopathology

At the end of 10 weeks and 22 weeks the histopathological analysis of the skin/skin tumors were carried out and the results are shown in Fig.6 and Fig.7respectively. A well defined epidermis (E), underlying dermis (D) and the subcutaneous (SC) tissue was displayed by the skin of the control mice. Post 10 and 22 weeks treatment, the skin of RV (5mg/kg and 10mg/kg) administered mice were similar to that of the control mice. At the end of the 10 weeks, there was epidermal proliferation with the invasion of epidermal cells towards the dermis in the depilated skin of the DMBA administered mice. The epidermal thickening occurs more in RV (10mg/kg) intervened mice as compared to RV (5mg/kg) intervened mice as shown in Fig.6D and Fig.6F.

At the end of promotion stage (22 weeks) the DMBA induction resulted in well-developed SCC with thickened corrugated epidermis, hyperchromatia and hyper keratinization as a remarkable feature (Fig.7). The RV (5mg/kg) treatment in DMBA induced skin tumor exhibited empty spaces with considerably more keratinization around the epidermis as compared to the RV (10mg/kg). However, the RV(5mg/kg) administration in DMBA induced skin tumors showed less keratinization as compared to DMBA control group as shown on Fig.7 (B,D,F).

Discussion:-

Malignancy is a group of disease that can happen in all living cells in the body. Epidemiological examinations have shown that 70–90 % of all diseases are natural. Lifestyle related components are the most significant and preventable among the natural openings (Hanahan and Weinberg, 2000). Carcinogenesis is brought about by physical, chemical, or viral systems. It is a multistage process in which epigenetic and genetic changes regulate the alterations of normal cells to transformed cells (Aroraet al., 2011). The origin of cancer lies in the genetic variations in DNA repair genes, oncogene and/or tumor suppressor gene. One of the best ways to study neoplastic transformation is the mouse skin carcinogenesis model; it also helps to study the multistage nature of tumor development (Agrawalet al., 2010). In cancer chemoprevention, the prevention of tumor promotion is an effective approach. Upon the induction of tumor by the tumor promoter agents such as DMBA, there are many key events has been recognized such as hyperplasia, oxidative stress, proliferation and inflammation (Fürstenberger et al., 1981). DMBA known as tumor promoter, activate the protein kinase C (PKC) pathway by mimicking the diacylglycerol (DAG) and thereby stimulating the cell progression(Wong et al., 2017).

In experimental animals, the chemical carcinogens are engaged to initiate and promote the neoplastic transformation. Therefore, DMBA is the most commonly used chemical carcinogen for inducing experimental skin carcinogenesis (De and Villegas, 2007).

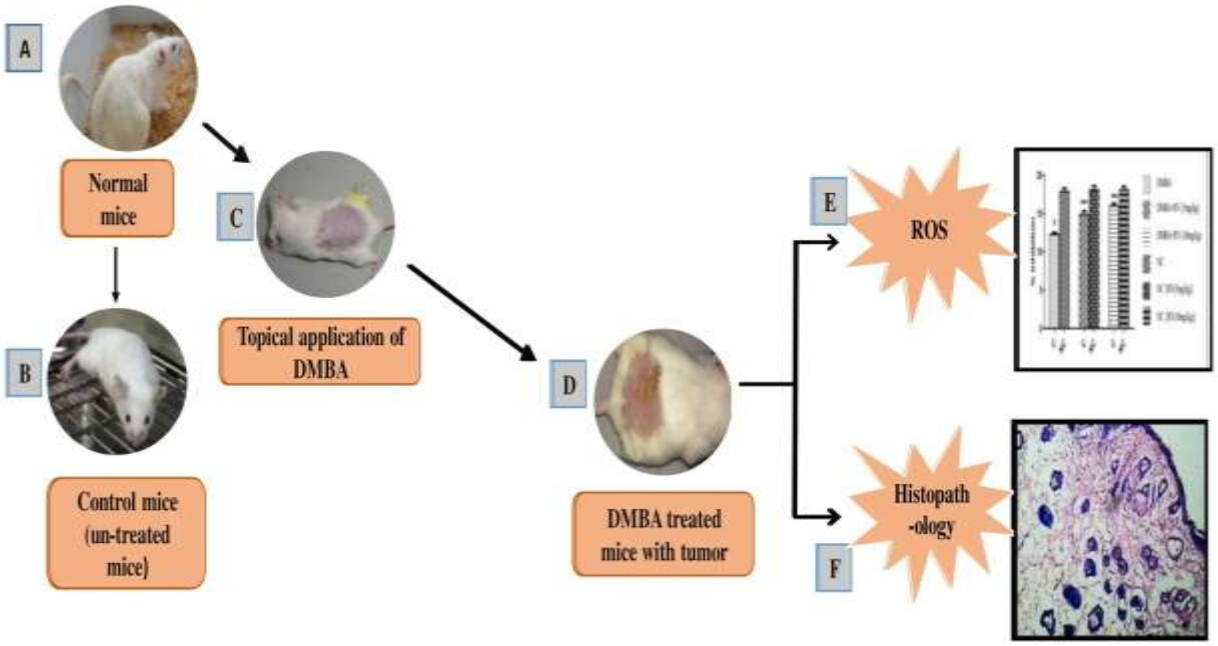
The ROS induced by carcinogen is one of the causative key factor of the tumor promotion (Balmainet al., 1991). The species of swiss albino mice are known for their interference on cellular activities, such as the dysregulation of transcription factors, interference of protein kinase and unusual modifications in the membrane lipid bilayer (Bickers and Athar, 2006). Under certain conditions, when there is off- balanced between the effects exerted by the antioxidants and oxidants , which results in the oxidative stress ultimate leads to the generation of disease such as cancer (Ning et al., 2022).In the present study, the anti-oxidative potential of RV was analyzed against the carcinogenic action of DMBA on the mouse skin. RV is a phytoalexin present in berries, grapes, red wine and peanuts. It provides the cancer chemopreventive effects as it inhibits the events associated with tumor initiation, promotion and progression (Bhaskaraet al., 2020). Across the chemical structure of RV, there is a phenolichydroxyl group and potential for electron delocalization due to which it can act as a potent antioxidant(Janget al., 1997). The RV has ability to promote the activities of variety of antioxidant enzymes due to which it becomes potent antioxidant and free radical scavenger (Holme and Pervaiz, 2007); similar finding have been corroborating in our studies. It inhibits the signal transduction through the phosphoinositide-3-kinase (PI3K) (Meng et al., 2021), NF-ƙB, mitogen activated protein kinase (MAPK) pathways which might be beneficial in cancer prevention (Paliwal et al., 2011).

The present results show that by the application of DMBA in swiss albino mice, there is a significant decrease in the body weight, increase in MDA level with a significant decline in antioxidant levels. After the administration of RV the lipid peroxidation and antioxidant level gets restored in the DMBA treated mice, which suggested the free radical scavenging efficacy of RV. It was reported that that the low level of ROS leads to the cell proliferation and survival (Parolaet al., 1999). Similar observations had been reported earlier by researchers where they showed that in skin carcinogenesis, an increased level of ROS affects the antioxidant defense system (including antioxidant enzymes) resulting in up-regulation of lipid peroxidation and decline in other antioxidant enzyme levels (Roduan et al., 2017; Khalaf et al., 2021). There are several reports which state that the RV significantly restored the optimum the levels of antioxidant enzymes restoring the balance between the ROS and its scavenging machinery in the cells affected by DMBA(Sharma et al., 2019). It has been also reported that RV exhibits pro-apoptotic effect against various cell lines by the activation of various downstream signaling pathways which leads to the death of tumor cells (Vervandier-Fasseur and Latruffe, 2019).

As shown in Fig. 6B and 7B, the skin of DMBA administered mice exhibited the hyper-proliferative epidermis and hyper keratinization. This effect is associated with the overexpression of cyclooxygenase- 2 and NF-ƙB activity through the modulation of GSH level (Mongaet al., 2014; Nagapanet al., 2018). The elevated level of GSH was evaluated after the administration of RV on DMBA induced mice. The same results were also observed in the development of curcumin as the chemopreventive drug. This leads to the inhibition of NF- ƙB activation (Biswas et al., 2005).Despite to this it was stated by many researchers that RV restored the murine epidermal hyperplasia by the declining the expression pattern of various protein such as COX-2, Bcl-2, p21 (Kowalczyket al., 2010; Iqbal et al., 2019).

Concurrently, in present study we noticed that the oxidative stress caused the massive disturbance in endogenous levels of oxidative markersin DMBA induced cancerous mice. In view of the above findings, resveratrol seems to be a potent chemopreventive agent as it exhibit modulatory effects during skin carcinogenesis. Although we have to still study further that how RV affects these histopathological changes remarkably.

Figures:-



Graphical Abstract

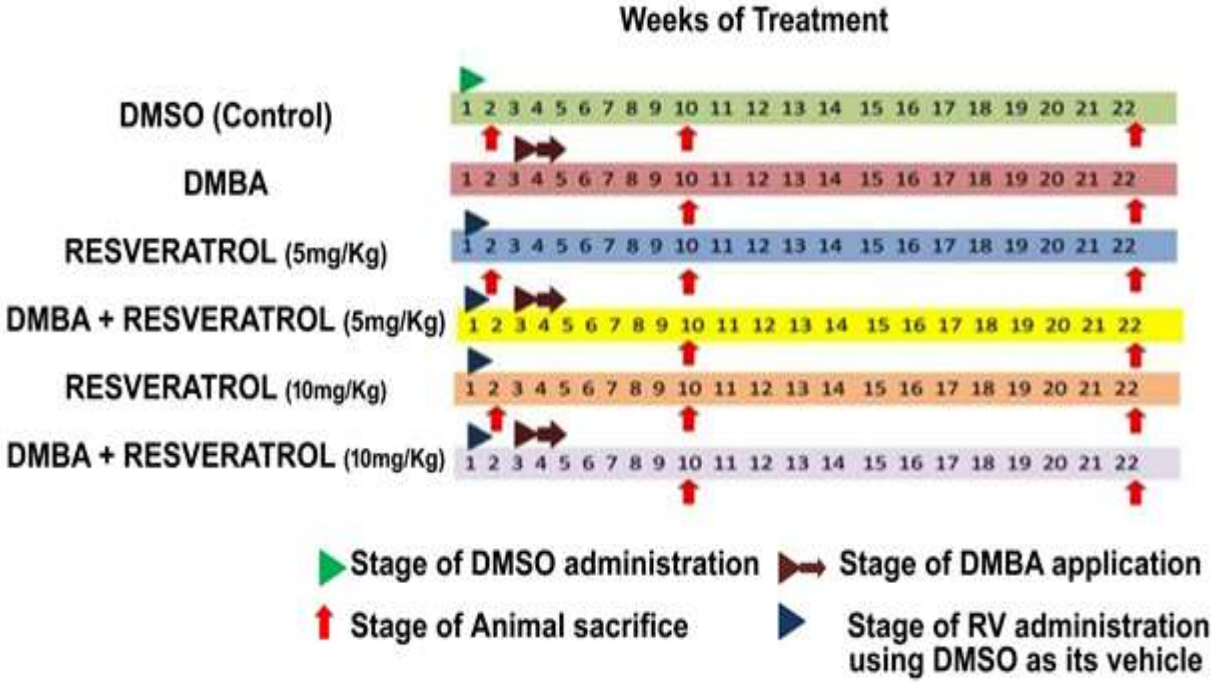


Fig.1:-The schedule of animal treatment to study the consequence of Resveratrol (RV) on the DMBA incited skin carcinogenesis.

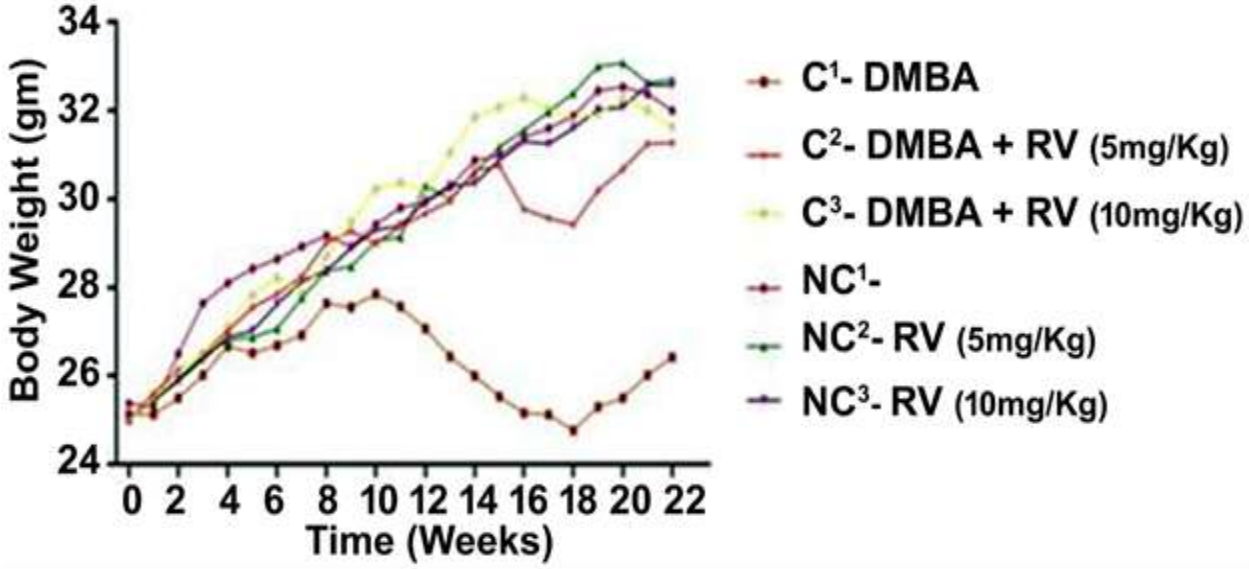


Fig.2:-Average body weight of the animals in different groups. Measurements were done at the end of each week till the end of experiment (22nd weeks).

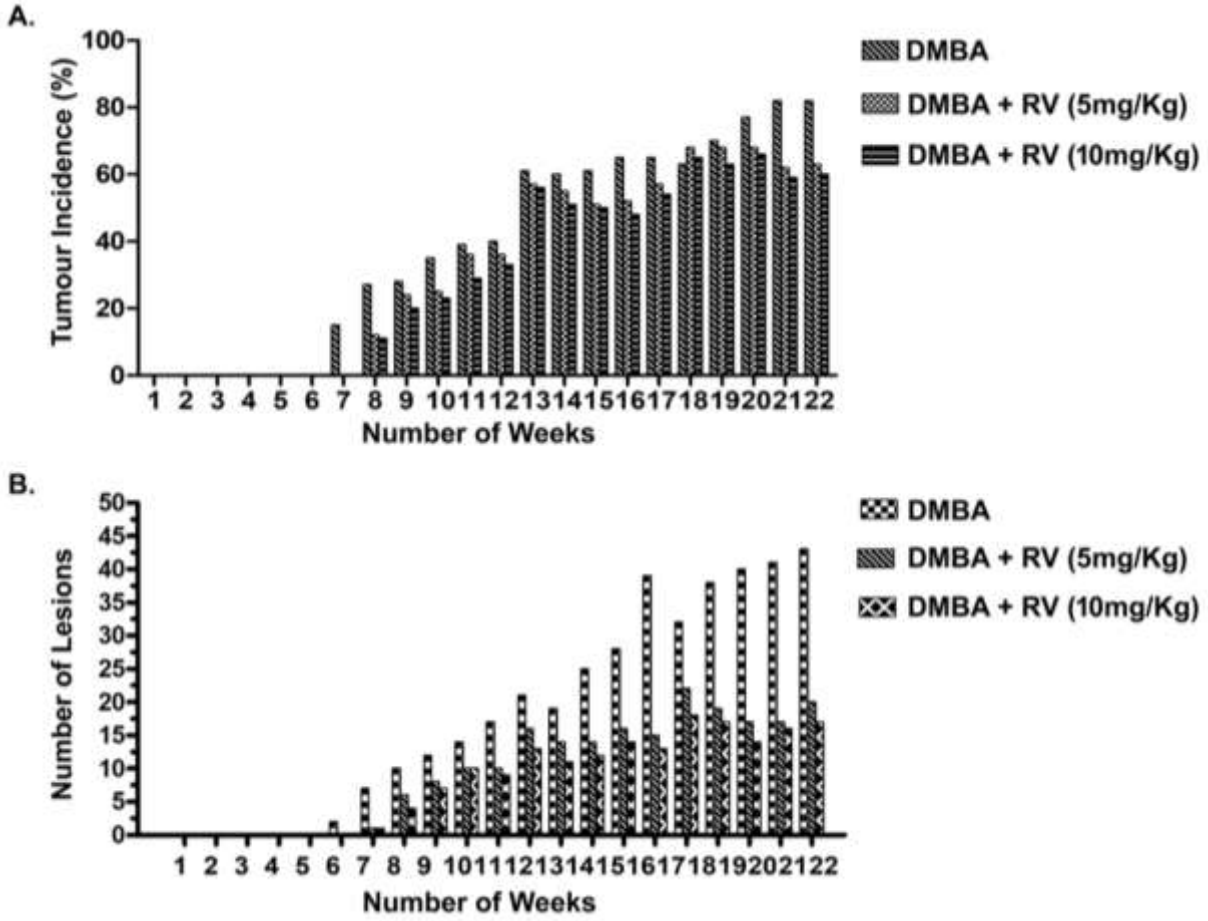


Fig. 3:-Tumor incidence in percentage (A) and the total number of lesions (B) in the DMBA incited, RV (5mg/kg) +DMBA and RV (10mg/kg) + DMBA administered mice groups were marked with respect to the number of weeks of treatment (n= 6 mice/group).

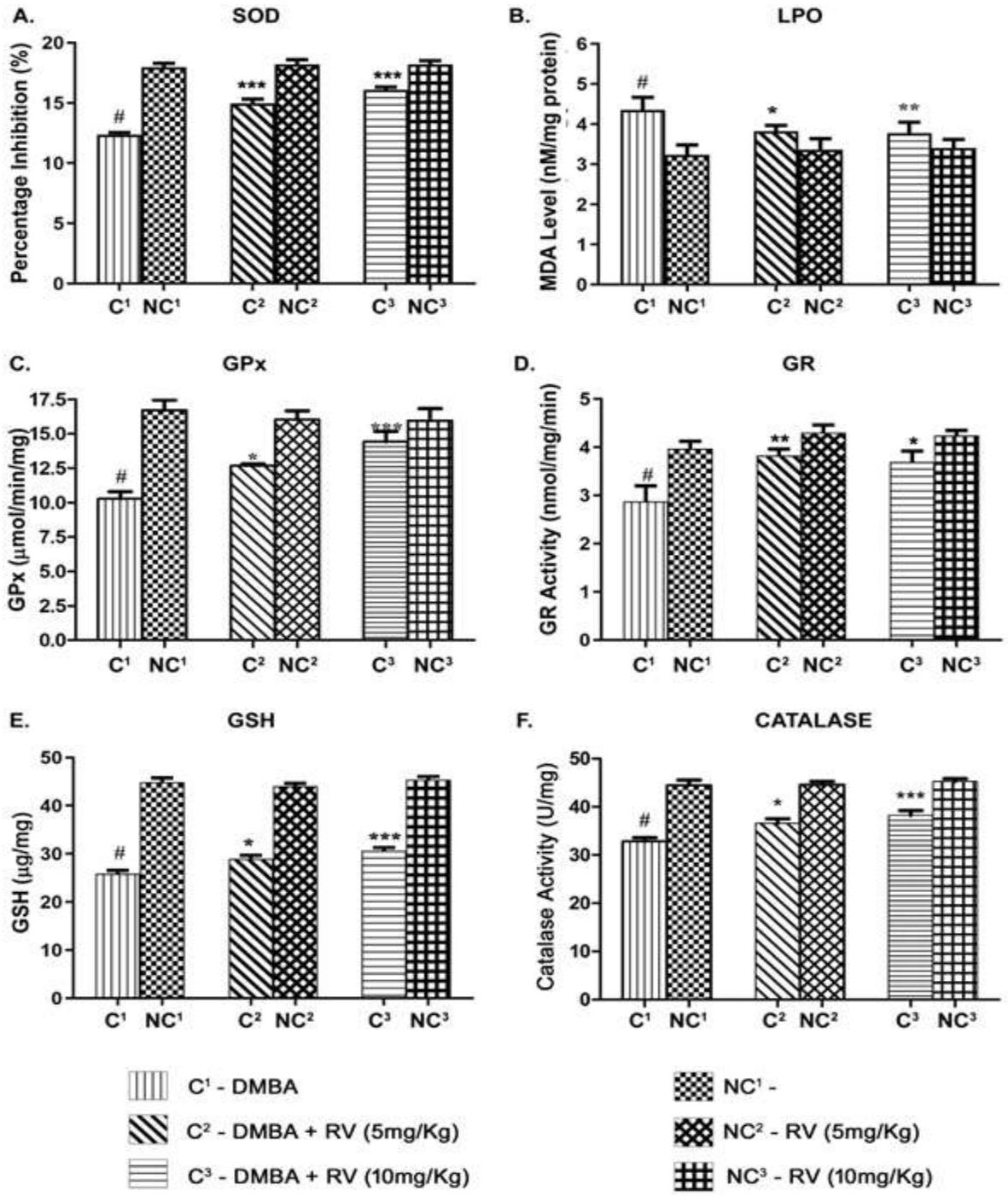


Fig.4:-The Post-initiation antioxidant activity of Resveratrol (5mg/Kg) and Resveratrol (10mg/Kg). Graphs showing SOD activity (A), LPO activity (B), GPx activity (C), GR activity (D),GSH activity (E) and Catalase activity (F). Values are represented as mean ± SD, (n=6). The data with ***p≤0.001 and **p≤0.01 were statistically significant.

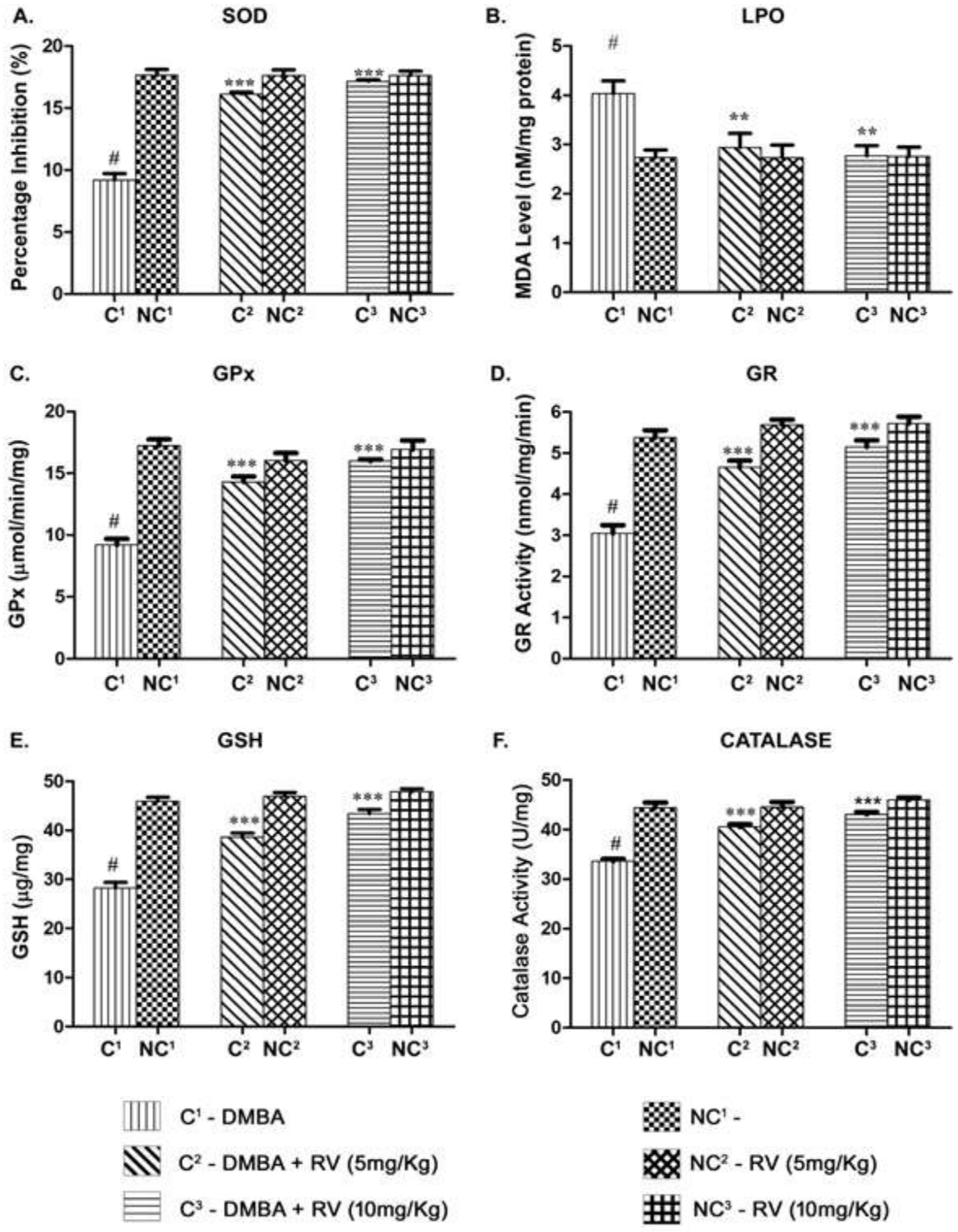


Fig.5:-Antioxidant activity of Resveratrol (5mg/kg) and Resveratrol (10mg/kg) at the promotion stage. Graphs showing SOD activity (A), LPO activity (B), GPx activity (C), GR activity (D), GSH activity (E), and Catalase activity (F). Values are represented as mean ± SD (n=6). The data with ***p≤0.001 and **p≤0.01 were considered statistically significant.

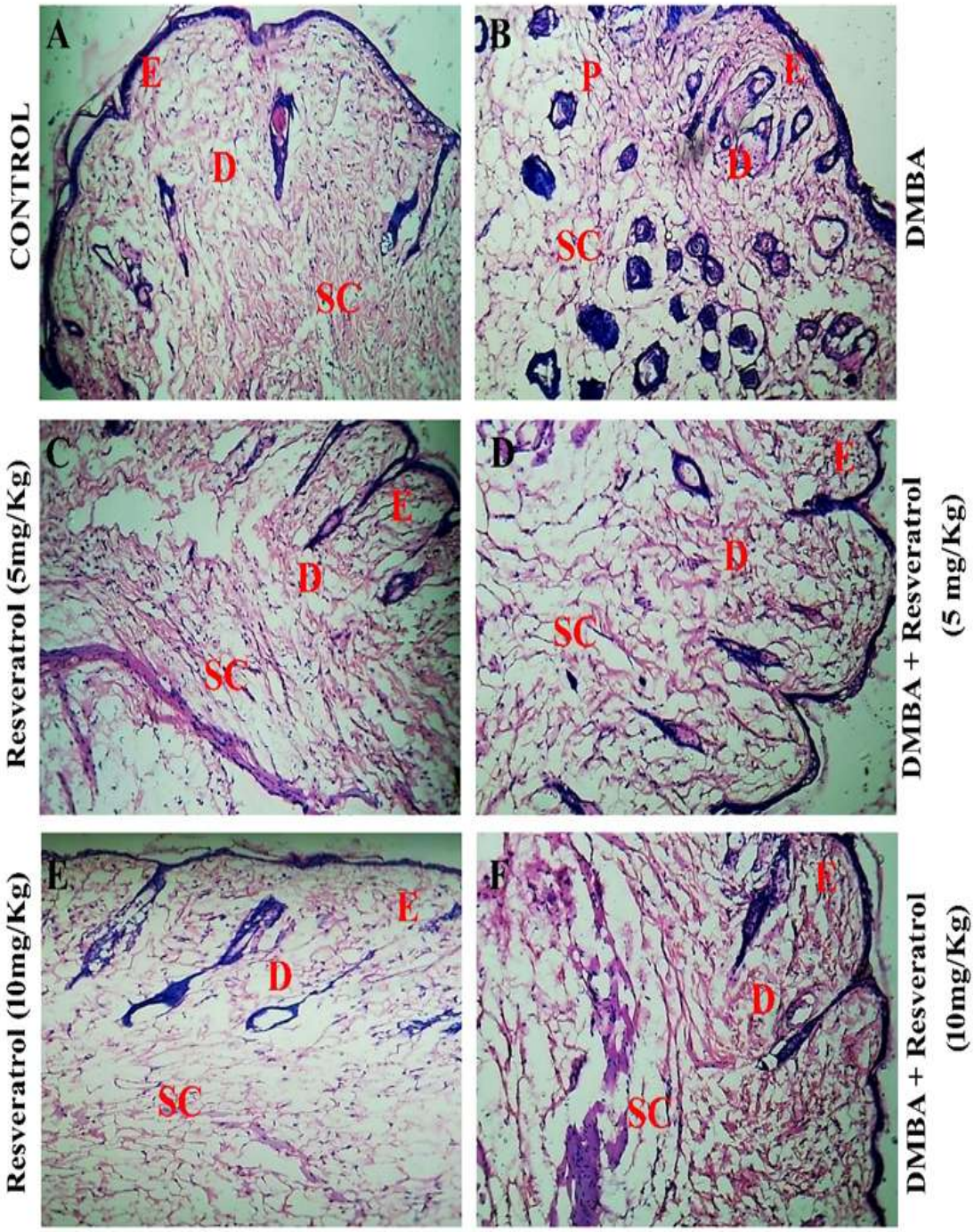


Fig.6:- (A) Represents the histopathology of normal skin from the control groups showing well defined epidermis (E), dermis (D) and subcutaneous tissue (SC); **(B)** Represents histopathology of DMBA treated groups having Papilloma's denoted as P; **(C)** Represent histopathology of Resveratrol (5mg/kg) control group; **(D)** Represents the histopathology of Resveratrol (5mg/kg)+DMBA treated group; **(E)** Represent histopathology of Resveratrol (10mg/kg) control group; **(F)** Represents the histopathology of Resveratrol (10mg/kg)+DMBA treated group; the double headed arrow and single headed arrow indicates the proliferation of epidermis and the infiltration of epidermal cells towards dermis respectively.

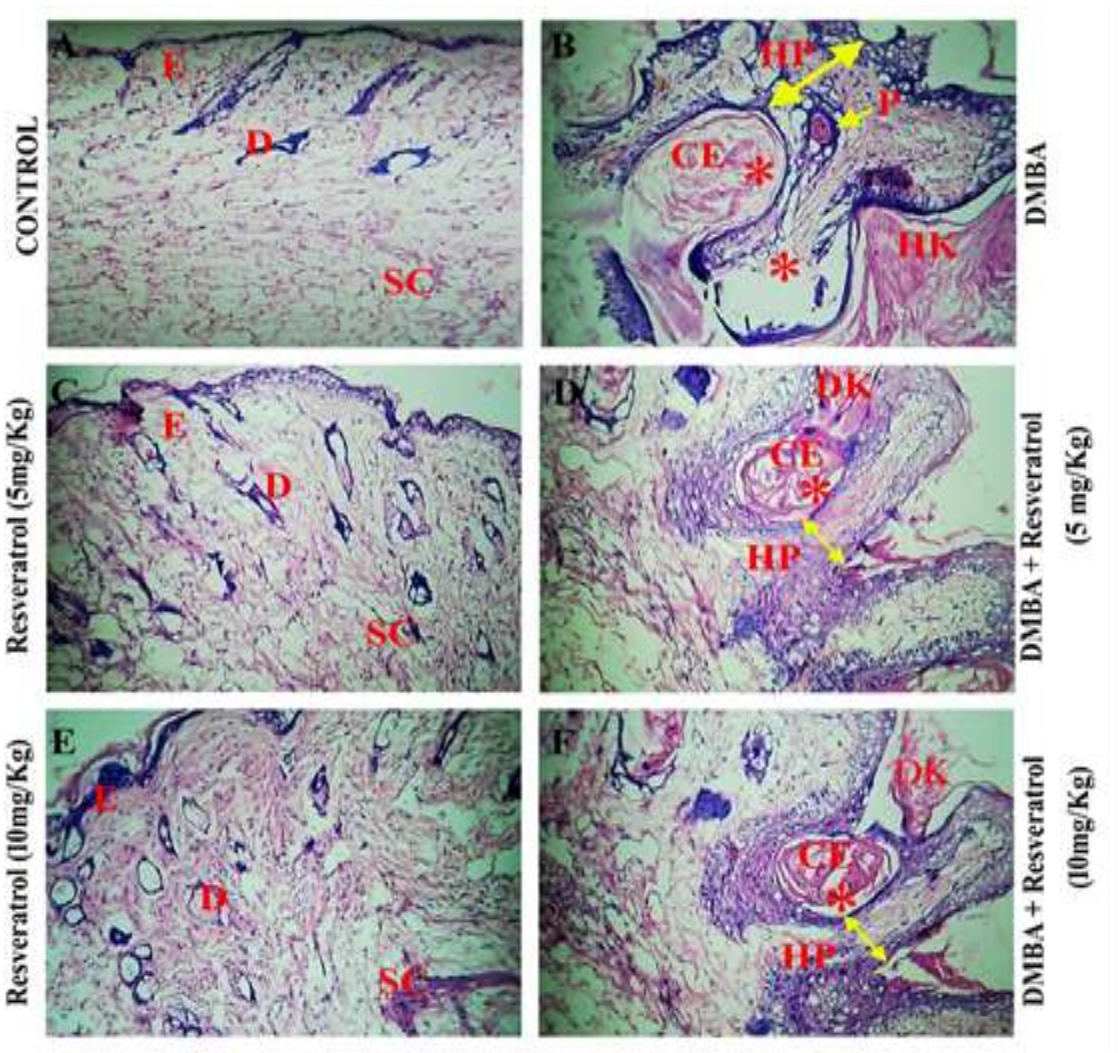


Fig.7:- (A) Represents normal skin histopathology from the control displaying well defined epidermis (E), dermis (D) and subcutaneous tissue (SC); **(B)** represents anomalous histopathology exhibiting hyper proliferative epidermis (HP) showed by double headed arrow, corrugated epidermis (CE) showed by asterisk, hyper keratinization (HK) and well developed papilloma (P) from the DMBA administered group; **(C)** Represents histopathology of Resveratrol (5mg/kg) control group; **(D)** Represents aberrant histopathology of Resveratrol (5mg/kg)+ DMBA treated group displaying prominent effect of hyper proliferative epidermis (HP) with decreased keratinization (DK); **(E)** Represents histoarchitecture of Resveratrol (10mg/kg) control group; **(F)** Represents divergent histopathology of Resveratrol (10mg/kg)+ DMBA treated group revealing the prominent effect of hyper proliferative epidermis (HP) with decreased keratinization (DK) as compared to Fig.7 (D).

Table:-

Table 1:-Modulatory influence of resveratrol (RV) on DMBA induced skin carcinogenesis mice.

Parameters	**Test groups**					
	NC^1	**C^1**	**NC^2**	**C^2**	**NC^3**	**C^3**
	Control	DMBA	RV (5mg/kg)	DMBA + RV (5mg/kg)	RV (10 mg/kg)	DMBA + RV (10 mg/kg)
Tumor incidence (%)	0%	82.00%	0%	63.00%	0%	59%
Mean tumor Volume (mm^3)	Nil	135.9± 2.53	Nil	63.85± 2.12	Nil	30.9± 1.56
Mean tumor	Nil	486.52±1.89	Nil	105.99±2.32	Nil	43.56± 1.41

burden (mm^3)						

Conclusions:-

In conclusion, we have obtained significant evidence highlighting that, Resveratrol has potentials to modulate the 7, 12-dimethylbenz[a]anthracene induced histopathological and biochemical alterations during skin melanoma in mice. Therefore, Resveratrol seems to be potential candidate as chemopreventive drug for skin cancer

Acknowledgements:-

The author SB acknowledges Department of Science and Technology (DST), Inspire (IFA-12, LSBM-32) for the financial support and Department of Biosciences and Biotechnology, Banasthali Vidyapith, Rajasthan. The author SS acknowledges Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan.

Competing Interest

Author Sonu Singhal, Shreshtha Gaur, Rakesh Mishra and Surabhi Bajpai, declare no conflict of interest.

Ethics approval and consent to participate

All the experiments were conducted strictly in accordance with guidelines of the Institute Ethical Committee regulated by the Committee for the purpose of control and supervision of Experiments on Animals (CPCSEA). The experimental protocol was approved by the institutional animal ethical committee (BV/IAEC/March 2019/1).

References:-

1. Aebi H. (1984) [13] Catalase in vitro. Meth. Enzymol 105:121-6. doi: 10.1016/S0076-6879(84)05016-3
2. Agrawal A, Verma P, Goyal PK (2010) Chemomodulatory effects of Aegle marmelos against DMBA-induced skin tumorigenesis in Swiss albino mice. Asian Pac J Cancer Prev 11(5):1311-4
3. Arora N, Koul A, Bansal MP (2011) Chemopreventive activity of Azadirachta indica on two-stage skin carcinogenesis in murine model. Phytother Res 25(3):408-16. doi: 10.1002/ptr.3280
4. Aziz MH, Nihal M, Fu VX, Jarrard DF, Ahmad N (2006) Resveratrol-caused apoptosis of human prostate carcinoma LNCaP cells is mediated via modulation of phosphatidylinositol 3′-kinase/Akt pathway and Bcl-2 family proteins. Mol Cancer Ther. 5(5):1335-41. doi: 10.1158/1535-7163.MCT-05-0526
5. Balmain A, Kemp CJ, Burns PA, Stoler AB, Fowlis DJ, Akhurst RJ (1991) Functional loss of tumour suppressor genes in multistage chemical carcinogenesis. In Princess Takamatsu Symposia 22:97-108
6. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44(1):276-87.doi:10.1016/0003-2697(71)90370-8
7. Bhaskara VK, Mittal B, Mysorekar VV, Amaresh N, Simal-Gandara J. (2020) Resveratrol, cancer and cancer stem cells: A review on past to future. Curr Res Nutr Food Sci 3: 284-295. doi:10.1016/j.crfs.2020.10.004
8. Bickers DR, Athar M (2006) Oxidative stress in the pathogenesis of skin disease. J Invest Dermatol 126(12):2565-75. doi:10.1038/sj.jid.5700340.
9. Bilecová-Rabajdová M, Birková A, Urban P, Gregová K, Durovcová E, Mareková M (2013) Naturally occurring substances and their role in chemo-protective effects. Cent Eur J Public Health 21(4):213. doi: 10.21101/cejph.a3886
10. Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I (2005) Curcumin induces glutathione biosynthesis and inhibits NF-κB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. Antioxid Redox Signal 7(1-2):32-41. doi:10.1089/ars.2005.7.32
11. Carlberg I, Mannervik B (1985) [59] Glutathione reductase. Meth. Enzymol 113:484-90. doi: 10.1016/S0076-6879(85)13062-4
12. Carter LG, D'Orazio JA, Pearson KJ (2014) Resveratrol and cancer: focus on in vivo evidence. Endocr Relat Cancer 21(3):R209-25. doi:10.1530/ERC-13-0171
13. Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. J Ethnopharmacol. 100(1-2):72-9. doi: 10.1016/j.jep.2005.05.011
14. De La Lastra CA, Villegas I (2007) Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. Biochem Soc Trans 35(5):1156-60. doi: 10.1042/BST0351156
15. Eisemann N, Waldmann A, Geller AC, Weinstock MA, Volkmer B, Greinert R, Breitbart EW, Katalinic A (2014) Non-melanoma skin cancer incidence and impact of skin cancer screening on incidence. J Invest Dermatol 134(1):43-50.doi: 10.1038/jid.2013.304

16. Fürstenberger G, Berry DL, Sorg B, Marks F. (1981) Skin tumor promotion by phorbol esters is a two-stage process. Proc Natl Acad Sci 78(12):7722-6. doi:10.1073/pnas.78.12.7722
17. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell, 100. Transpl Immunol 5: 179-83. doi: 10.1016/S0092-8674(00)81683-9
18. Holme AL, Pervaiz S (2007) Resveratrol in cell fate decisions. J Bioenerg Biomembr 39(1):59-63. doi: 10.1007/s10863-006-9053-y
19. Iqbal J, Abbasi BA, Ahmad R, Batool R, Mahmood T, Ali B, Khalil AT, Kanwal S, Shah SA, Alam MM, Bashir S (2019) Potential phytochemicals in the fight against skin cancer: Current landscape and future perspectives. Biomed Pharmacother 109:1381-93. doi:10.1016/j.biopha.2018.10.107
20. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275(5297):218-20. doi:10.1126/science.275.5297.218
21. Kaur M, Velmurugan B, Tyagi A, Deep G, Katiyar S, Agarwal C, Agarwal R (2009) Silibinin suppresses growth and induces apoptotic death of human colorectal carcinoma LoVo cells in culture and tumor xenograft. Mol Cancer Ther 8(8):2366-74. doi: 10.1158/1535-7163.MCT-09-0304
22. Khalaf AA, Elhady MA, Hassanen EI, Azouz AA, Ibrahim MA, Galal MK, Noshy PA, Azouz RA (2021) Antioxidant role of carvacrol against hepatotoxicity and nephrotoxicity induced by propiconazole in rats. Rev bras farmacogn 31(1):67-74. https://doi.org/10.1007/s43450-021-00127-8
23. Kim SM, Kim SZ (2018) Biological activities of resveratrol against cancer. JPhys Chem Biophys 7: 267. doi: 10.4172/2161-0398.1000267
24. Ko JH, Sethi G, Um JY, Shanmugam MK, Arfuso F, Kumar AP, Bishayee A, Ahn KS (2017) The role of resveratrol in cancer therapy. Int J Mol Sci 18(12):2589. doi:10.3390/ijms18122589
25. Koul A, Mukherjee N, Gangar SC (2006) Inhibitory effects of Azadirachta indica on DMBA-induced skin carcinogenesis in Balb/c mice. Mol Cell Biochem 283(1):47-55. doi:10.1007/s11010-006-2269-7
26. Kowalczyk MC, Kowalczyk P, Tolstykh O, Hanausek M, Walaszek Z, Slaga TJ (2010) Synergistic effects of combined phytochemicals and skin cancer prevention in SENCAR mice. Cancer Prev Res 3(2):170-8. doi: 10.1158/1940-6207.CAPR-09-0196
27. Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J (2021) Anti-inflammatory action and mechanisms of resveratrol. Molecules 26(1):229. doi:10.3390/molecules26010229
28. Mokrasch LC, Teschke EJ (1984) Glutathione content of cultured cells and rodent brain regions: a specific fluorometric assay. Anal Biochem 140(2):506-9. doi: 10.1016/0003-2697(84)90201-X
29. Monga J, Aggarwal V, Suthar SK, Nongalleima K, Sharma M (2014) Topical (+)-catechin emulsified gel prevents DMBA/TPA-induced squamous cell carcinoma of the skin by modulating antioxidants and inflammatory biomarkers in BALB/c mice. Food Funct 5(12):3197-207
30. Nagapan TS, Ghazali AR, Basri DF, Lim WN (2018) Oral administration of resveratrol ameliorates epidermal hyperplasia in ultraviolet B irradiated BALB/c mice. J App Pharm Sci 8(10):047-52. doi: 10.7324/JAPS.2018.81007
31. Ning R, Deng X, Wang Q, GeY (2022) Carnosic Acid Protects Against Myocardial Infarction by Controlling Oxidative Stress and Inflammation in Rats. Rev bras farmacogn 1-11. https://doi.org/10.1007/s43450-021-00216-8.
32. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2):351-8. doi:10.1016/0003-2697(79)90738-3
33. Paliwal R, Sharma V, Pracheta SS (2011) Hepatoprotective and antioxidant potential of Moringa oleifera pods against DMBA-induced hepatocarcinogenesis in male mice. Int J Drug Dev Res 3(2):128-38
34. Parola M, Bellomo G, Robino G, Barrera G, Dianzani MU (1999) 4-Hydroxynonenal as a biological signal: molecular basis and pathophysiological implications. Antioxid Redox Signal 1(3):255-84. doi:10.1089/ars.1999.1.3-255
35. Roduan MR, Abd Hamid R, Sulaiman H, Mohtarrudin N (2017) Annona muricata leaves extracts prevent DMBA/TPA-induced skin tumorigenesis via modulating antioxidants enzymes system in ICR mice. Biomed Pharmacother 94:481-8. doi: 10.1016/j.biopha.2017.07.133
36. Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H (1994) Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steroid 59(6):383-8. doi: 10.1016/0039-128X(94)90006-X
37. Samarasinghe V, Madan V (2012) Nonmelanoma skin cancer. J Cutan Aesthet Surg 5(1):3. doi: 10.4103/0974-2077.94323

38. Sharma B, Iqbal B, Kumar S, Ali J, Baboota S (2019) Resveratrol-loaded nanoemulsion gel system to ameliorate UV-induced oxidative skin damage: from in vitro to in vivo investigation of antioxidant activity enhancement. Arch Dermatol Res 311(10):773-93. doi:10.1007/s00403-019-01964-3
39. Sharma P, Parmar J, Verma P, Goyal PK (2010) Phyllanthus niruri in swiss albino mice. Int J Biol Med Res 1(4):158-64
40. Singh CK, George J, Ahmad N (2013) Resveratrol-based combinatorial strategies for cancer management. Ann N Y Acad Sci 1290(1):113. doi: 10.1111%2Fnyas.12160
41. Surdu S, Fitzgerald EF, Bloom MS, Boscoe FP, Carpenter DO, Haase RF, Gurzau E, Rudnai P, Koppova K, Févotte J, Leonardi G (2013) Occupational exposure to ultraviolet radiation and risk of non-melanoma skin cancer in a multinational European study. PloSone 8(4):e62359. doi: 10.1371/journal.pone.0062359
42. Umar A, Dunn BK, Greenwald P (2012) Future directions in cancer prevention. Nat Rev Cancer 12(12):835-48. doi:10.1038/nrc3397
43. van Berlo D, Wessels A, Boots AW, Wilhelmi V, Scherbart AM, Gerloff K, van Schooten FJ, Albrecht C, Schins RP (2010) Neutrophil-derived ROS contribute to oxidative DNA damage induction by quartz particles. Free Radic Biol Med 49(11):1685-93. doi: 10.1016/j.freeradbiomed.2010.08.031
44. Vervandier-Fasseur D, Latruffe N (2019) The potential use of resveratrol for cancer prevention. Molecules 24(24):4506. doi:10.3390/molecules24244506
45. Williams Jr CH, Arscott LD (1971) [203] Glutathione reductase (Escherichia coli). Methods Enzymol 1750: 3-509. Academic Press. doi:10.1016/0076-6879(71)17089-9
46. Wong P, Laxton V, Srivastava S, Chan YW, Tse G. (2017) The role of gap junctions in inflammatory and neoplastic disorders. Int. J Mol Med 39(3):498-506. doi:10.3892/ijmm.2017.2859.