Int.J.Curr.Microbiol.App.Sci (2021) *10*(06): 344-355

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 06 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1006.037

Chemopreventive Efficacy of *Hesperidin* against *N-methyl-N-nitrosourea* Induced Mammary Tumors in SD Rats

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Keywords

N-methly-N-nitrosourea, Hesperidin, Mammary tumors, Histopathology, Immunohistochemistry, PCNA, Ki-67, VEGF

Article Info

Accepted:
12 May 2021
Available Online:
10 June 2021

A B S T R A C T

The experiment was conducted in order to investigate the antitumor, antiproliferative and antiangiogenic activity of citrus bioflavonoid, the hesperidin (HES), on N-methly-N-nitrosourea (MNU) induced mammary tumors in Sprague-Dawley (SD) rats with reference to histopathology and immunohistochemical expressions of PCNA, Ki-67 and VEGF markers. Female SD rats of age 30-45 days were divided into five groups with six animals in each group. Group I was kept as environment/negative control group and Group II served as MNU (@50mg/kg BD) /tumor positive control group, whereas Group III aided as vehicle (1% CMC) control group. MNU exposed rats of Groups IV and V were administered orally with HES at doses 80 and 160 mg/kg body weights respectively. Total 19 out of 24 MNU exposed rats developed mammary tumors which were classified histopathologically as benign and malignant mammary tumors. HES treated rats showed low mitotic count in tumor samples compared to MNU control group. HES revealed dose dependent antihyperproliferative activity in tumor tissues through lowered immuno-expressions of PCNA and Ki-67 markers. Angiogenic activity in HES treated rat tumor samples was considerably decreased through suppression of VEGF expression.

Introduction

Cancer remains to be one of the most dreaded, major serious life threatening and widespread disease in nature, affecting humans, domestic animals, wild mammals, birds and fishes also, among which the mammary tumors are the most frequently occurring tumor in humans as well as in pet animals such as dogs and cats. It is estimated that, in 2018, breast cancer has shared 15% of all cancer deaths in women (Zahra and Shaheen, 2020) whereas approximately 1 in 3 dogs and 1 in 6 cats at some stage in their life, develop neoplasia and almost 50% of dogs over the age of 10 develop cancer (Kidd C. 2008; Davis and Ostrander, 2014) Moreover, dogs develop tumors approximately twice as frequently as

their human counterparts wherein mammary tumor contribute almost 52% of all tumors (MacEwen, 1990; Hahn *et al.,* 1994).

We are in a new era of medicine, where innovations have amplified our ability to attack the causes of diseases by many folds. Even after this much advancement in the medical and technological sciences, cancer is still a major cause of mortality in both developing and developed countries and we are still struggling for the treatment of cancers. Thus, there is always a constant need of developing alternative or synergistic anticancer drugs with minimal side-effects. Breast cancer models in animals have been used extensively to study the efficacy of various therapies and new treatment schedules, and for monitoring response to therapy. Rodents, specifically rats, are more frequently used in breast cancer research as rat mammary tumor shares similarities in histology and hormone responsiveness with human breast cancer (Mollard *et al.,* 2011; Abdelmegeed and Mohammed, 2018). Among different strains, the Sprague-Dawley rat strain is highly regarded for studies designed to investigate the mammary carcinogenesis as it bears histological features of mammary tumors closely resembling to that of human and canine mammary tumors (Schedin *et al.,* 2000; Gal *et al.,* 2020).

The two most commonly used chemical carcinogen for mammary tumorigenesis are 7,12-dimethylbenz(a)anthracene (DMBA) and N-methyl-N- nitrosourea (MNU). The MNU is highly specific for mammary gland that induces more estrogen dependent, aggressive, locally invasive tumors and in contrast to DMBA it does not require metabolic activation (Thompson and Adlakha, 1991).

In the last few decades, use of chemically synthesized drugs has not significantly improved the overall survival rate of patients with cancer (Choudhari *et al.,* 2020). As a result, trend toward finding new strategies and more efficient drugs with lesser side effects has increased. Recently, naturally occurring plant compounds/phytochemicals have been explored in an attempt to create novel chemopreventive and chemotherapeutic agents. Bioflavonoids are such phytochemicals derived from certain citrus fruits that have been demonstrated to possess antioxidative, antiproliferative and proapoptotic effects on different types of cancer (Choi and Kim, 2011).

A hesperidin (HES), bioflavonoid, isolated from citrus fruits reported to possess various biological activities such as anti-inflammatory, analgesic, anti-oxidant, antibacterial, antiviral, anti-neoplastic, anti-mutagenic and immunomodulatory activity etc. (Garg *et al.,* 2001; Siddiqi *et al.,* 2015). Previously, Anti-neoplastic effects of hesperidin have been deciphered against cancer of bone, colorectum, esophagus, liver, lung, kidney and breast cancers (Devi *et al.,* 2015; de Oliveira *et al.,* 2020). In spite of the fact that hesperidin possesses a number of significant biological activities, there is paucity of information especially in vivo activity of hesperidin against mammary tumors. Till now, based on our current knowledge, research describing chemopreventive activity of hesperidin against MNU induced mammary tumors in rats has not been described before. Therefore, in light of above inference the present study was designed to evaluate ameliorative effects of hesperidin on MNU induced mammary tumors in SD rats.

Materials and Methods

Animals

Female Sprague-Dawley (SD) rats (n=30) aged 30-45 days were procured from Zydus

Life Sciences, Ahmedabad, Gujarat and housed in clean and disinfected cages at the laboratory animal house in the Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Navsari Agriculture University, Navsari.

Rats were maintained in an environment controlled room at a temperature of 22±3˚C, relative humidity of 40 to 70 percent and photoperiod of 12 hours' light and 12 hours' dark. They were fed with standard rat pellet diet (M/S VRK nutrition solution, Pune) and water *ad libitum.* The study was carried out during the period of November-2019 to July-2020 in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Navsari, Gujarat.Ethical clearance for performing the experiment on rats was obtained from Institutional Animal Ethics Committee (IAEC) (No. 076-VCN-VPP-2019).

Chemicals and reagents

N-methyl-N-nitrosourea (MNU) was obtained from Reddy 'N' Reddy Pharmaceuticals, Hyderabad, Telangana. Hesperidin (HES) was purchased from Sigma-Aldrich company, USA.

Dose formulation and dosing

The drug MNU was handled with proper precautionary measures and weighed in light protected/ dark area. The MNU was dissolved immediately prior to its use in physiological saline, adjusted to pH 4 with acetic acid.

The MNU solution was injected intraperitoneally near ventral midline of the animal, half way between the third and fourth pair of the mammary glands. Hesperidin has very poor solubility, hence the suspension was made in 1% carboxymethyl cellulose (CMC).

Experimental design

Rats were randomly divided into 5 groups with 6 animals in each group. Group I was kept as environment control group. All animals of groups II, III, IV and V were injected with MNU @50 mg/kg body weight (BW) intraperitoneally (IP) on 1st, 30th and 60th day of experiment. Group II was kept as tumor/positive/MNU control. Group III was vehicle/VEH (1% CMC orally/day) control group for test substance hesperidin. Group IV received low dose (@80 mg/kg BW/day orally) of hesperidin for 16 weeks. Group V was given high dose (@160 mg/kg BW/day orally) of hesperidin for 16 weeks. At the end of experiment animals were euthanized by ether inhalation, tumor samples were excised, examined grossly and dimensions were measured using digital vernier caliper. Tumor samples were collected in 10% neutral buffered formalin for sequential histopathological and immunohistochemical studies.

Clinical observations

All animals were palpated regularly twice a week for any growth in mammary glands and visible tumor mass. The following parameters were taken into consideration:

Average latency period (in days)- period from MNU administration to the appearance of the first tumor in each animal/group

Tumor incidences- number of rats carrying the tumors expressed as percentage

Tumor volume- calculated using the formula for an ellipsoid tumor: $V= \frac{4}{3}\times\pi\times l\times b\times h$ and expressed in mm^3(Sorensen *et al.,* 2001).

Histopathology

Formalin fixed tissues were processed for routine histopathology using paraffin

embedding technique as per the standard protocol (Luna, 1968). Tissue sections of 4-5 µm thickness were taken on egg albumin coated slides for hematoxylin and eosin (H&E) staining, whereas duplicate section of thickness 3-4 µm were taken on poly-L-lysine coated slides for immunohistochemical staining. The H&E stained sections of rat mammary tumors were evaluated microscopically and classified according to the criterion given by Russo (Russo, 2015).

Mitotic Index

The MI was calculated by counting the number of mitotic figures in 30 random high-power magnification fields (HPFs) (400x) in H&E stained sections of tumours. The region of the tumor sample with highest overall mitotic activity was chosen for evaluation(Kumar *et al.,* 2010). Cells at any stage of mitosis were counted and the mitotic index in each group was expressed as Mean±Standard Error.

Immunohistochemistry (IHC)

Tissue sections taken on poly-L-lysine coated slides were deparaffinised and subjected to indirect/ peroxidase anti-peroxidase (PAP) immunohistochemistry method. For the purpose anti-PCNA (P8825, Sigma- Aldrich, USA),anti-Ki-67 (AB9260, Sigma- Aldrich, USA) and anti-VEGF (ABS82, Sigma-Aldrich, USA) antibodies were used. After deparaffinization, tissue sections were cleared in xylene and hydrated through series of graded alcohol and distilled water. Antigen retrieval was achievedby heat induced antigen retrieval (HIAR) protocol in 0.1 M citrate buffer, pH-6.0 (C9999, Sigma-Aldrich, USA).Endogenous peroxidase activity was quenched by incubating the sections with 3% hydrogen peroxide in distilled water for 10 min at room temperature (RT) followed by washing with phosphate buffered saline (PBS), pH-7.4. Then the sections were incubated with primary antibodies (anti-PCNA/anti-Ki-67) in humidified chamber at RT for 30 min. For anti-VEGF antibody, sections were incubated overnight in humidified chamber at 4ºC.The sections were washed with PBS, thereafter, incubated with HRP conjugated secondary antibody (K5007, REAL EnVision Detection System, DAKO, Denmark) for 40 min in humidified chamber at RT. After rinsing the sections in PBS, the peroxidase activity was visualized by treating the slides with freshly prepared3,3'-diaminobenzidine (DAB) substrate (provided with DAKO REAL EnVision Detection System). Then the slides were washed with PBS and finally counterstained with Gill III hematoxylin (GH) (1051740500, Sigma-Aldrich, USA) for 10-15 seconds. Negative controls were incubated with PBS instead of primary antibody.

The labelling indices for PCNA and Ki-67 were calculated by manually counting positive and negative nuclei (minimum 1000 cells) at higher magnification (x 40) in 8-10 microscopic field. For PCNA every immunostained (brown) nucleus was considered as positive (Pena *et al.,* 1998), whereas the analysis of Ki-67 stained cells involved detection of the cells with high Ki-67 content (dark brown) (Kinra and Malik, 2020). The cells exhibiting distinct cytoplasmic staining (brown) were considered to express VEGF. At least 1,000 cells were counted at higher magnification (x 40) in 8-10 microscopic fields to obtain the percentage of VEGF positive cells(Martano *et al.,* 2015).

Statistical analysis

All data was expressed as Mean±Standard Error (SE). Statistical analysis was performed using SPSS Advanced Statistic 20.0 software (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Duncan's

test was performed to determine intergroup differences in tumor volumes of each group. Chi-square test was implied to analyzed tumor incidences and frequencies. Values of $P<0.05$ were considered statistically significant.

Results and Discussion

Gross observations

The first palpable tumor appeared in group II on 69th day post carcinogen (MNU) administration with lowest average latency periods and highest tumor incidences. Overall 19 (79.00%) out of 24 MNU exposed rats developed tumors (Fig 1; Table 1).The intraperitoneally inoculation of MNU at dose 50 mg/kg body weight on 1st, 30th and 60th day of experiment confirmed 100% mammary tumor induction in female SD rats of MNU (group II) and VEH (group III) control groups which are consistent with the results reported by Murray *et al.,* (2009) and Faustino-Rocha *et al.,* (2016).

The results of current investigation suggest the dose dependent significant ($p < 0.05$) antitumor activity of HES (Table 2). Tumor volumes of MNU induced tumors ranged from 402.02 mm^3 to 15,684 mm^3.

The mean tumor volume (Table 1) of HES treatment groups IV and V were comparatively less than MNU control group II. Choi and Kim (2011) have proposed the possible mechanism involved in anticancer effects of hesperidin is through promotion of apoptosis by upregulating the p53 gene. In addition, it has been evidenced that HES arrests tumor growth by down-regulating the expression of Bcl-2 and upregulating the expression of caspase-3 (Donia *et al.,* 2018). The anticarcinogenic property of HES that could have inhibited tumor induction is considered to be attributed to its potent antioxidant nature related to its chemical configuration and its antioxidant activity increases with increased concentration (Ahmadi and Shadboorestan, 2016).

Histopathology

A total of 33 mammary tumors were collected from 19 animals and classified as benign (1/33; 3.03%) and malignant (32/33; 96.97%) epithelial neoplasms (Table 3; Fig 2). Group-wise mitotic counts were found to be 4.25±0.18/hpf, 4.22±0.14/hpf, 4.07±0.16/hpf and 3.8±0.11/hpf in groups II, III, IV and V respectively.

Immunohistochemistry

Cellular proliferation is fundamental part of carcinogenesis playing important role in initiation, promotion and progression steps and assessment of a tumor's proliferation potential is useful for determining tumor malignancy. Tumors were evaluated immunohistochemically for proliferative activity using anti-PCNA (Fig. 3& Cart 1) and anti-Ki-67 (Fig. 4 & Chart 1) antibodies. Tumors from HES treatment groups (IV & V) showed reduced mean PCNA and Ki-67 expressions in comparison to MNU control group. These results signify anti-hyperproliferative activity of HES in mammary tumors through reduction of PCNA as well as Ki-67 expressions with its more effective activity at higher dose i.e. at 160 mg/kg body weight. The potent *in vivo* antihyperproliferative activity of HES has also been previously reported by Siddiqi *et al.,* (2015), Kamaraj *et al.,* (2009) and Mahmoud *et al.,* (2017) in chemically induced tumors of kidney, lung and liver respectively.

Angiogenesis is essential for cancer development and growth. Without a blood supply, the tumor cannot grow beyond 1-2 mm^3 in diameter and become necrotic or apoptotic (Nishida *et al.,* 2006).

Table.1 Average tumor latencies, tumor incidences and average tumor volumes in MNU exposed animals

Group no.	Average Tumor Latencies (in days)	Tumor Incidences (%)	Tumor volumes (in mm^3)
II (MNU control)	75.33±2.59	100	5833.63±1315.27
III (VEH control)	80.17±2.86	100	3654.41±962.17
IV (HES @80mg/kg and MNU)	75.80±2.58	83.33	2736.77±749.63
V (HES @160mg/kg and MNU)	93.50±16.50	33.33	739.54±218.04

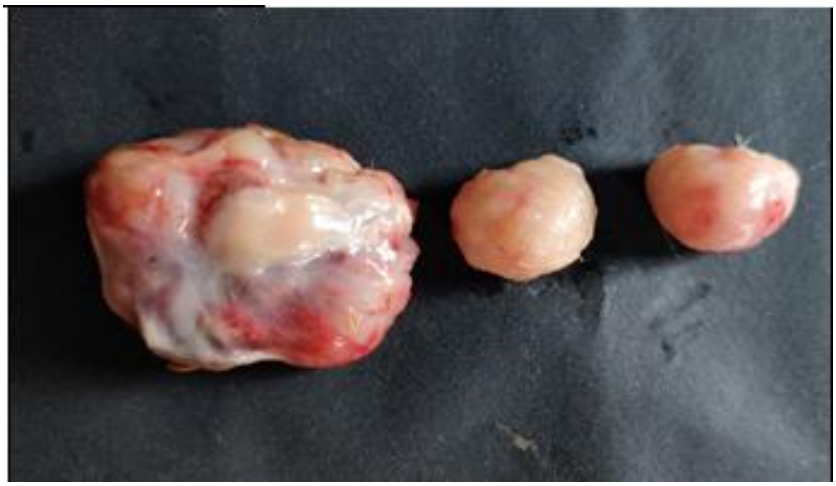
Table.2 Tumor induction in MNU exposed animals

Group no.	Animals with tumors	No. of tumors
II (MNU control)	6	12
III (VEH control)	6	9
IV (HES @80mg/kg and MNU)	5	9
V (HES @160 mg/kg and MNU)	2	3
Total	**19**	**33**
Chi. sq. (x^2)	8.316	10.36
Significance (*p*)	0.01	0.006

Table.3 Histological classification of mammary tumors induced by MNU

Type of Neoplasms	Number of tumors
Tubular adenoma	1
***In situ* papillary carcinoma**	1
***In situ* cribriform carcinoma**	1
Invasive papillary carcinoma	12
Invasive cribriform carcinoma	7
Invasive Comedocarcinoma	4
Invasive tubular carcinoma	6
Invasive adenoid cystic carcinomas	1
Total	**33**

Fig.1 a. Rat showing multiple tumor masses. **b**. Excised tumors from tumor bearing rat. **c**. Tumor dimension measurement using digital vernier caliper



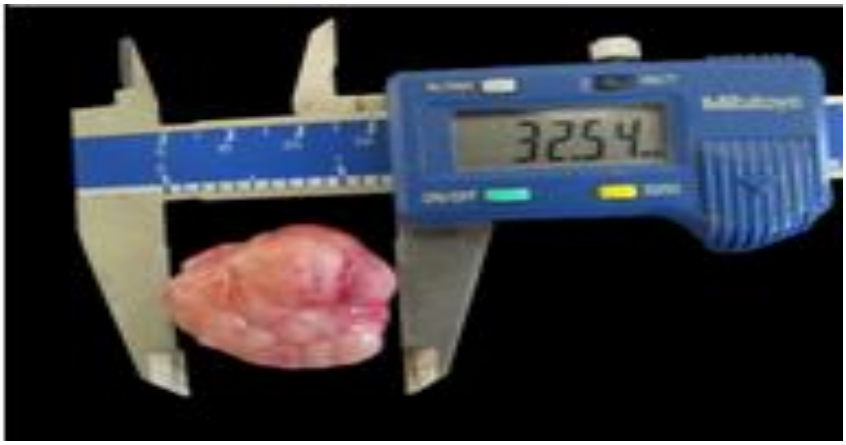


Fig.2 Representative photographs of mammary tumor tissues.**a.** Invasive cribriform carcinoma with numerous secondary luminas (H&E ×80). **b.** Invasive Comedocarcinoma with central necrotic area (H&E ×800). **c.** Invasive papillary carcinoma with scanty fibrovascular core (H&E ×800).

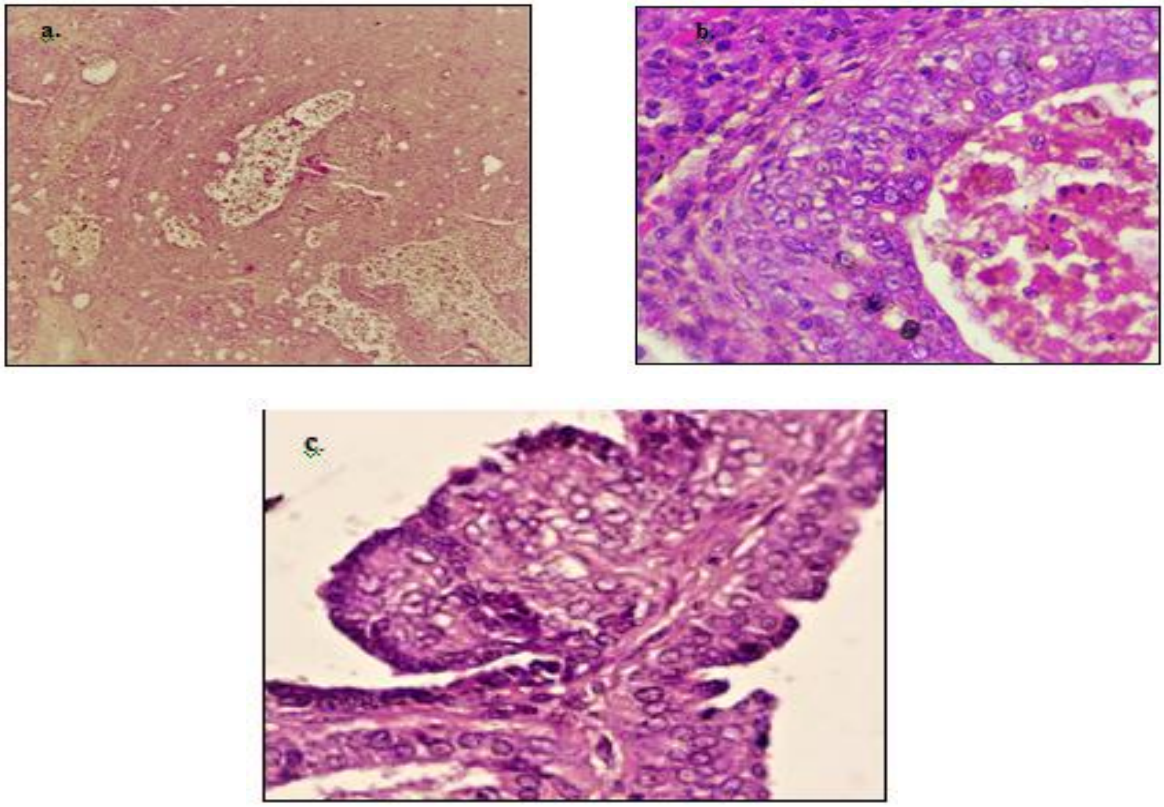


Fig.3 Representative photographs of mammary tumor tissues. **A.** MNU control rats showed strong positive Ki-67 nuclear immunolabelling (IP-DAB-Gill III haematoxylin ×800). **B.** MNU exposed and HES (@160 mg/kg BW) treated rats showed comparatively reduced and weak Ki-67 nuclear immunolabelling (IP-DAB-Gill III haematoxylin ×800).

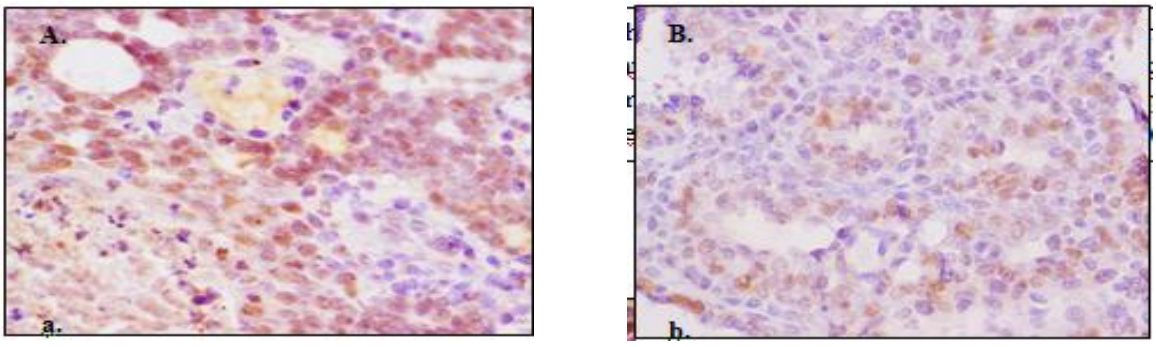


Fig.4 Representative photographs of mammary tumor tissues. **a.** MNU control rats showed strong positive PCNA nuclear immunolabelling (IP-DAB-Gill III haematoxylin ×800).**b.** MNU exposed and HES (@160 mg/kg BW) treated rats showed comparatively reduced and weak PCNA nuclear immunolabelling (IP-DAB-Gill III haematoxylin ×800).

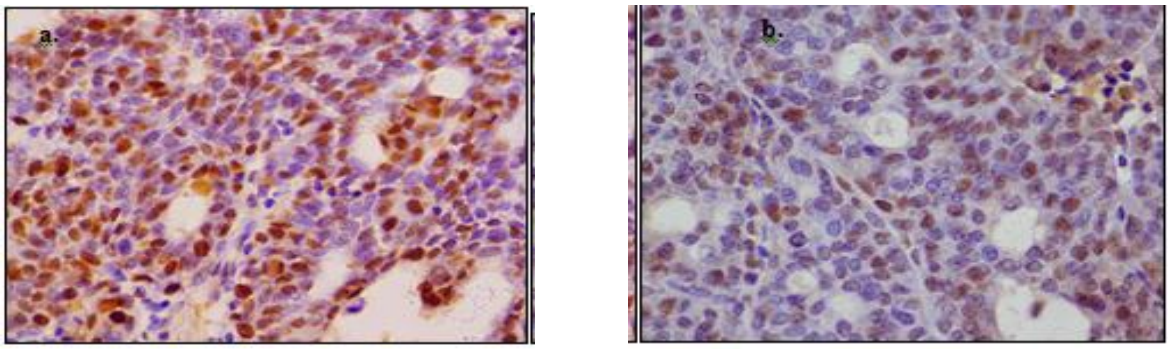


Fig.5 Representative photographs of mammary tumor tissues. **a.** MNU control rats showed strong positive VEGF cytoplasmic granular immunolabelling (IP-DAB-Gill III haematoxylin ×800). **b.** MNU exposed and HES (@160 mg/kg BW) treated rats showed comparatively weak VEGF cytoplasmic granular immunolabelling (IP-DAB-Gill III haematoxylin ×800).

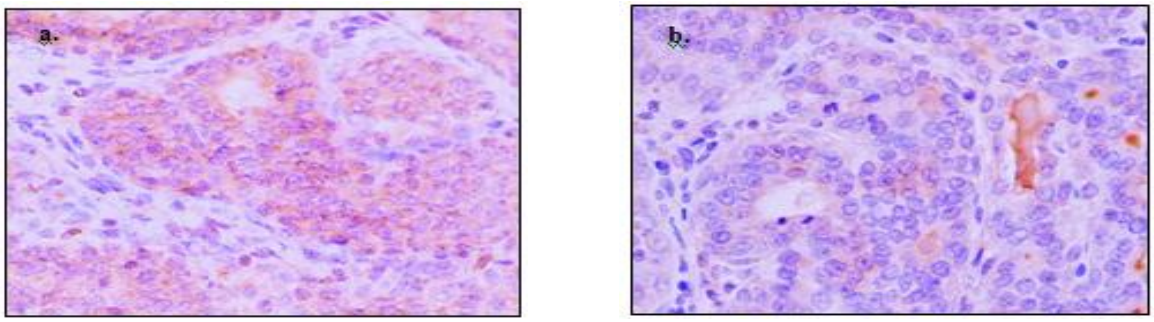
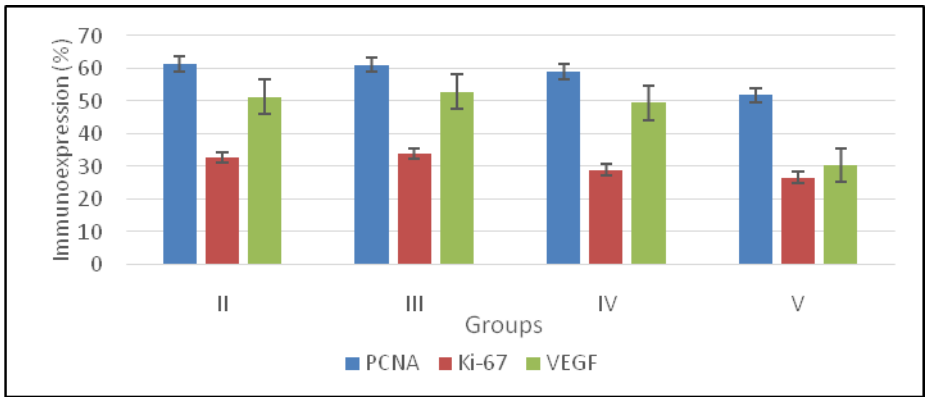


Chart.1 Immunohistochemical expression of PCNA, Ki-67 and VEGF markers in different groups



Vascular endothelial growth factor (VEGF), a homodimeric glycoprotein, is the key mediator of angiogenesis (the formation of new blood vessels) and vascular permeability (Thordarson *et al.,* 2001). The current investigation revealed remarkable dose dependent antiangiogenic effect of HES on MNU induced mammary tumors (Fig. 5 & Chart 1). The least expressions of VEGF in tumors of group V were indicating the effective VEGF suppressive activity of HES at higher dose i.e. at 160 mg/kg body weight. This was the first study enlightening the VEGF modulating effect of HES in mammary tumors. Our investigation supports former studies pertaining to antiangiogenic effects of HES though blocking the AKT/mTOR signaling pathways and suppression of PECAM expression (Kim, 2015).

The present study confirms the usefulness of hesperidin as an anticancer agent against mammary tumors. Hesperidin revealed its potent antihyperproliferative activity through down regulation of PCNA and Ki-67 expressions in induced tumors. Additionally, HES showed antiangiogenic activity through suppression of VEGF in tumor tissues. Thus, hesperidin could be potential chemopreventive agent against such conditions. Our results also suggest the dose dependent activity of HES with better effects at higher (160mg/kg BW) dose. However, further studies need to be performed to clearly understand chemopreventive efficacy of hesperidin and underlying mechanisms in prevention of cancer.

Acknowledgements

This project was partially supported by the Department of Veterinary Pharmacology and Toxicology, NAU, Navsari- 396450, Gujarat, India. The authors gratefully acknowledge to Zydus Life Sciences, Ahmedabad, Gujarat for providing female SD rats to carry out this study. We also thank to the Dean, College of Vet. Sci. and A.H., NAU, Navsari for providing funds and facilities for present research work.

References

Abdelmegeed, S. M., and Mohammed, S. (2018). Canine mammary tumors as a model for human disease. Oncology letters, 15(6), 8195-8205.

Ahmadi, A., and Shadboorestan, A. (2016). Oxidative stress and cancer; the role of hesperidin, a citrus natural bioflavonoid, as a cancer chemoprotective agent. Nutrition and cancer, 68(1), 29-39.

Choi, E. J. and Kim, G. H. (2011). Anti-/pro-apoptotic effects of hesperetin against 7, 12-dimetylbenz (a) anthracene-induced alteration in animals. Oncology Reports. 25(2): 545-550.

Choudhari, A. S., Mandave, P. C., Deshpande, M., Ranjekar, P., and Prakash, O. (2020). Phytochemicals in cancer treatment: From preclinical studies to clinical practice. Frontiers in pharmacology, 10, 1614.

Davis, B. W. and Ostrander, E. A. (2014). Domestic dogs and cancer research: a breed-based genomics approach. ILAR journal, 55(1), 59-68.

de Oliveira, J. M. P. F., Santos, C. and Fernandes, E. (2020). Therapeutic potential of hesperidin and its aglycone hesperetin: Cell cycle regulation and apoptosis induction in cancer models. Phytomedicine. 73: 1-14.

Devi, K. P., Rajavel, T., Nabavi, S. F., Setzer, W. N., Ahmadi, A., Mansouri, K. and Nabavi, S. M. (2015). Hesperidin: A promising anticancer agent from nature. Industrial Crops and Products. 76: 582-589.

Donia, T. I., Gerges, M. N., and Mohamed, T. M. (2018). Amelioration effect of

Egyptian sweet orange hesperidin on Ehrlich ascites carcinoma (EAC) bearing mice. Chemico-biological interactions, 285, 76-84.

Faustino-Rocha, A. I., Silva, A., Gabriel, J., da Costa, R. G., Moutinho, M., Oliveira, P. A., Gama, A., Ferreira, R. and Ginja, M. (2016). Long-term exercise training as a modulator of mammary cancer vascularization. Biomedicine and Pharmacotherapy. 81: 273-280.

Gal, A. F., Stan, L., Tăbăran, F., Rugină, D., Cătoi, A. F. and Andrei, S. (2020). Chemopreventive Effects of Propolis in the MNU-Induced Rat Mammary Tumor Model. Oxidative Medicine and Cellular Longevity. 2020: 1-13. DOI: 10.1155/2020/4014838.

Garg, A., Garg, S., Zaneveld, L. J. D. and Singla, A. K. (2001). Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytotherapy Research. 15(8): 655-669.

Hahn, K. A., Bravo, L. I. N. A., Adams, H., and Frazier, D. L. (1994). Naturally occurring tumors in dogs as comparative models for cancer therapy research. In Vivo, 8(1), 133-144.

Kamaraj, S., Ramakrishnan, G., Anandakumar, P., Jagan, S., and Devaki, T. (2009). Antioxidant and anticancer efficacy of hesperidin in benzo (a) pyrene induced lung carcinogenesis in mice. Investigational New Drugs, 27(3), 214-222.

Kidd, C. (2008). The many challenges of veterinary oncology. The Canadian Veterinary Journal, 49(11), 1132.

Kim, G. D. (2015). Hesperidin inhibits vascular formation by blocking the AKT/mTOR signaling pathways. Preventive Nutrition and Food Science, 20(4), 221.

Kinra, P., and Malik, A. (2020). Ki 67: Are we counting it right?. Indian Journal of Pathology and Microbiology, 63(1), 98.

Kumar, P., Kumar, R., Pawaiya, R. S., and Puttaswamy, M. B. (2010). Diagnostic significance of mitotic index and AgNOR count in canine mammary tumours. Brazilian Journal of Veterinary Pathology. 3(1): 41-45.

Luna, L. G. (Ed.). (1968). Manual of histologic methods of the armed forces institute of pathology. McGraw-Hill.

MacEwen, E. G. (1990). Spontaneous tumors in dogs and cats: models for the study of cancer biology and treatment. Cancer and Metastasis Reviews, 9(2), 125-136.

Mahmoud, A. M., Mohammed, H. M., Khadrawy, S. M., and Galaly, S. R. (2017). Hesperidin protects against chemically induced hepatocarcinogenesis via modulation of Nrf2/ARE/HO-1, PPARγ and TGF-β1/Smad3 signaling, and amelioration of oxidative stress and inflammation. Chemico-Biological Interactions, 277, 146-158.

Martano, M., Restucci, B., Ceccarelli, D. M., Lo Muzio, L., and Maiolino, P. (2015). Immunohistochemical expression of vascular endothelial growth factor in canine oral squamous cell carcinomas. Oncology letters, 11(1), 399-404.

Mollard, S., Mousseau, Y., Baaj, Y., Richard, L., Cook-Moreau, J., Monteil, J., Funalot, B. and Sturtz, F. G. (2011). How can grafted breast cancer models be optimized?. Cancer biology and therapy, 12(10), 855-864.

Murray, T. J., Ucci, A. A., Maffini, M. V., Sonnenschein, C. and Soto, A. M. (2009). Histological analysis of low dose NMU effects in the rat mammary gland. BioMed Central Cancer. 9(1): 1-9. DOI: https://doi.org/10.1186/1471-2407-9-267.

Nishida, N., Yano, H., Nishida, T., Kamura, T.

and Kojiro, M. (2006). Angiogenesis in cancer. Vascular Health and Risk Management. 2(3): 213–219.

Pena, L. L., Nieto, A. I., Pérez-Alenza, D., Cuesta, P., & Castano, M. (1998). Immunohistochemical detection of Ki-67 and PCNA in canine mammary tumors: relationship to clinical and pathologic variables. Journal of Veterinary Diagnostic Investigation, 10(3), 237-246.

Russo, J. (2015). Significance of rat mammary tumors for human risk assessment. Toxicologic Pathology. 43(2): 145-170.

Schedin, P., Mitrenga, me T. and Kaeck, M. (2000). Estrous cycle regulation of mammary epithelial cell proliferation, differentiation and death in the Sprague-Dawley rat: a model for investigating the role of estrous cycling in mammary carcinogenesis. Journal of Mammary Gland Biology and Neoplasia. 5(2): 211-225.

Siddiqi, A., Hasan, S. K., Nafees, S., Rashid, S., Saidullah, B., and Sultana, S. (2015). Chemopreventive efficacy of hesperidin against chemically induced nephrotoxicity and renal carcinogenesis via amelioration of oxidative stress and modulation of multiple molecular pathways. Experimental and molecular pathology, 99(3), 641-653.

Sorensen, A. G., Patel, S., Harmath, C., Bridges, S., Synnott, J., Sievers, A., Yoon, Y. H., Lee, E. J., Yang, M. C., Lewis, R. F. and Harris, G. J. (2001). Comparison of diameter and perimeter methods for tumor volume calculation. Journal of Clinical Oncology. 19(2): 551-557.

Thompson, H. J. and Adlakha, H. (1991). Dose-responsive induction of mammary gland carcinomas by the intraperitoneal injection of 1-methyl-1-nitrosourea. Cancer research, 51(13), 3411-3415.

Thordarson, G., Lee, A. V., McCarty, M., Van Horn, K., Chu, O., Chou, Y. C., Yang, J., Guzman, R.C., Nandi, S. and Talamantes, F. (2001). Growth and characterization of N-methyl-N-nitrosourea-induced mammary tumors in intact and ovariectomized rats. Carcinogenesis. 22(12): 2039-2047.

Zahra, K. and Shaheen, R. (2020). Prevention of Sartan-e-Sadi (Breast Cancer) in Unani System of Medicine-A.

How to cite this article:

Atul Prabhakar Urkude, Priti D. Vihol, Jignesh M. Patel, Jatin H. Patel2, Raseshkumar D. Varia, Vishal L. Saurathiya and Milind U. Dalvi. 2021. Chemopreventive Efficacy of *Hesperidin* against *N-methyl-N-nitrosourea* Induced Mammary Tumors in SD Rats. *Int.J.Curr.Microbiol.App.Sci.* 10(06): 344-355. doi: https://doi.org/10.20546/ijcmas.2021.1006.037