Ind. J. Pharm. Edu. Res., 2025; 59(4):1466-1473.
https://www.ijper.org

Original Article

Wogonoside Mitigates Inflammation and Cell Proliferation via Modulation of NF-κB Signalling Pathway in DMBA-Induced Mammary-Preclinical Model

Chenyang He, Yu Zhang, Yan Diao, Xingcong Ma, Guochao Mao, Xinyu Wei, Yang Zhao*

The Comprehensive Breast Care Center, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, CHINA.

ABSTRACT

Objectives: To hypothesis the effect of wogonoside against DMBA-induced Breast Cancer (BC) via TNF-α induced NF-κB signaling pathway a molecular appraoch. **Materials and Methods:** A 25 mg/kg Body Weight (BW), DMBA mixed dosage was used to produce BC with 1 mL olive oil by subcutaneous injection. We observed reduced body weight, elevated hepatic marker (AST, ALP, ACP and ALT), phase I (Cyt-b5 and Cyt-p450) and lipid peroxidative (TBARS and LOOH) markers and reduced antioxidant and xenobiotic enzymes in DMBA induced cancer-bearing rats. Moreover, the molecular study revealed that up-regulation of, inflammatory (NF-κB p65, TNF-α, IL-6 and IL-1β) markers in DMBA treated cancer-bearing animals. **Results:** Oral supplementation of wogonoside substantially reverted back to normal levels of these DMBA-induced rats have biochemical and molecular indicators. **Conclusion:** Overall, these findings supported wogonoside anti-inflammatory and anti-cell proliferation activity against DMBA-induced breast cancer through modification of the NF-κB signaling pathway.

Keywords: Wogonoside, DMBA, Inflammation, Cell proliferation, NF-κB signaling.

Correspondence:
Prof. Yang Zhao
The Comprehensive Breast Care Center, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an-710004, CHINA.
Email: szhaoy@xjtu.edu.cn

Received: 18-03-2025;
Revised: 24-05-2025;
Accepted: 03-07-2025.

INTRODUCTION

Breast Cancer (BC) leading cause of cancer-associated mortality and most repeatedly identified non-cutaneous malignancy in women all over the world.[1] Chemical carcinogens act as a key role in the formation of carcinogens, for those who intake excessive amounts of toxins in our foodstuff. The liver for metabolic activation of chemical carcinogens and some extrahepatic tissues, such as mammary glands and in the metabolic activation of hydrophobic chemicals such as DMBA.[2] Oxidative stress occurs by anfluctuate between the antioxidants and free radical formation, which initiate oxidative damage and tissue injuries.[3] This ROS is positively related to the deregulation of cellular macromolecules; this leads to structural and physiological changes in normal cell biology, which can lead to a variety of deteriorating damages, including cancer.[4,5]

Antioxidants act as the most important protecting mediator destroy ROS and suggested that efficacy in assessing the oxidative damage. The antioxidants act protection against oxidative stress with the aid in catalyzing the disproportionation responses of their substrate-free radicals produced *in vivo* via oxidative phosphorylation, cytochrome p450 metabolism and inflammatory progressions.[6] Oxidative Many key genes and proteins are regulated, influencing diverse signaling pathways such as cell growth and inflammation.[7] NF-κB activation the inhibitory protein IκB is phosphorylated, ubiquitinated and then proteolytically destroyed. When free NF-κB enters the nucleus and binds to its consensus sites, it causes it triggers gene production of cell proliferative proteins.[8] Activating Protein (AP-1), a transcriptional factor is an important regulator of different imduction processes. An AP-1 molecule is a dimeric complex made up of a heterodimer (c-Fos/c-Jun) or a homodimer (c-Jun/c-Jun) that enters the nucleus and attaches to its TRE consensus sequence areas and subsequently drives cell proliferative gene production.[9]

Phytochemicals modulate cellular differentiation and proliferation in a variety of signaling pathways that are inhibited in the carcinogenic process of breast cancer.[10,11] Wogonoside is a bioactive flavonoid glucuronide metabolite. wogonin, it has been an anticancer[12] and anti-inflammation-induced angiogenic activity.[13] Earlier research found that wogonoside protects against DSS-induced colitis by blocking NF-κB activation via the PI3K/Akt pathway.[14,15] Various studies have reported that some phytochemicals prevented NF-κB. In certain cancer situations, AP-1 signaling is inhibited by downregulating c-Jun and c-Fos,

DOI: 10.5530/ijper.20256082

two essential upstream factors of AP-1.[16-18] However, there are still no reports about the association between wogonoside and NF-κB signaling pathway in breast cancer. Therefore, in this hypothesis to the study, we explored the anti-inflammatory effect of wogonoside on DMBA-induced BC via the NF-κB pathway.

MATERIALS AND METHODS

Chemicals

Sigma Chemical Co., St. Louis, USA, provided the wogonoside and DMBA. Santa Cruz Biotechnology (USA) provided the primary antibodies.

Animal model and cancer induction

The 6-7 weeks-old adult female rats (140-160 g body weight) used in this present research work. This experimental study was conducted after obtaining approval from Hospital of Xi'an Jiao tong University, animal research ethics committee (Number: XJ20230809). It was carried out at Yeditepe University Faculty of Medicine Experimental Research Center. The rats maintained a regular laboratory environment. The BC was induced by a dose of 25 mg/kg bw., DMBA with 1 mL olive oil by subcutaneous injection.

Experimental design

Totally 24 ratsseparated into 4 groups n=6. Group 1 animals were considered as normal receiving olive oil. Group II and group III rats 25 mg/kg bw, DMBA was combined with 1 mL olive oil. In, group III rats were given 20 mg/kg bw, of wogonoside during the investigation time. Throughout the trial, Group IV rats were given a dose of wogonoside (20 mg/kg bw) exclusively. The body weight of the control and experimental animals were determined 0 week and final week during the investigation period of the experiment time, all animals were sacrificed. Mammary glands were quickly removed and cold saline was used to wash them. The blood was collected for biochemical analysis.

Biochemical investigation

Ice-cold salt was used to rinse the excised liver and Mammary Tissues (MT). In a Potter-Elvehjem homogenizer, a known quantity of tissue was homogenized in 0.1 M Tris-HCl buffer (pH 7.4) at 4ºC using a Teflon pestle at 600 rpm for 3 min. The homogenate was centrifuged for 10 min at 4ºC at 3000 g. Tissue homogenate, which was obtained from the supernatant, was utilized to test several biochemical parameters.

The LOOH level of plasma and Mammary Tissues (MT) by Jiang *et al.* (1992).[19] The plasma and MT in TBARS levels were determined by Ohkawa *et al.* (1979).[20] The GPx was assessed by a technique of Rotruck *et al.* (1973).[21] The CAT level by the manner of Sinha (1972).[22] The vitamin E by Desai (1984) method. The level of SOD analyzed by mode of Kakkar *et al.* (1984).[23] The GSH investigated by approach of Beutler and Kelley (1963).[24] The vitamin-C level was assessed by the way of Omaye *et al.* (1979).[25] The hepatic marker enzymes such as ALT and AST were estimated by the method of Bergmeyer *et al.* (1978).[26] The ACP and ALP were measured as elucidated in an earlier method.[6,26] The GR, Cyt-b5 and Cyt-p450 was analyzed in the manner of Carlberg and Mannervik (1985).[27] The GST was analyzed in the manner of Habig *et al.*, (1974)[28] and Omura and Sato (1964)[29] respectively.

Histopathological analysis

The experimental animals' breast tumor regions were promptly excised and their MT was then cleaned with a cool, normal saline solution. A section of the skin was embedded in paraffin and fixed in 10% formalin. Using a rotary microtome, sections with a thickness of 3-5μm were cut and stained with hematoxylin and eosin (H and E) dye. The areas that were discolored were examined under a light microscope and both the experimental and control groups' photomicrographs were captured.

Western blotting analysis

Protein concentrations were measured and 50gm protein samples from each group were subjected to SDS-PAGE. The separated proteins were then placed on a PVDF membrane and incubated for the entire night at 4ºC with 1:1000 dilutions of primary antibodies, including NF-κB p65, TNF-α, IL-6 and IL-1β. Following membrane incubation with 1:500 dilutions of protein bands, the particular secondary antibodies were then exposed to room temperature for 2 hr. The protein bands were then detected using an ECL kit method (Bio-Rad, USA).

Statistical analysis

The results are accompanied by the number of observations and are expressed as the Mean±Standard Deviation (SD). SPSS version 17.0 was used to do a one-way Analysis of Variance (ANOVA) for windows. A probability of less than 0.05 was deemed statistically significant for the results.

RESULTS

Effect of wogonoside on the body weight changes

Figure 1 indicated the body weight changes in normal and experimental animals. No substantial (p<0.05) body weight changes were observed in all rats by the initial study time. While reduced body weight modifications were identified at the end of experimental period in DMBA-alone when compared with the normal rats. Wogonoside-treated rats prevented body weight loss in DMBA-induced animals. Wogonoside alone and control rats demonstrated no body weight alterations were found.

Effect of wogonoside on lipid peroxidative markers and antioxidant status in plasma

Figure 2 shows the amount of lipid peroxidation and antioxidant markers in plasma of control and experimental rats. The

considerable ($p<0.05$) elevation of lipid peroxidative markers, decreased antioxidants was found in DMBA-alone when compared with normal rats. These quantities were significant ($p<0.05$) and retreated back to near normalcy in wogonoside-treated rats due to their antioxidant ability. Wogonoside alone and control rats exposed no changes were identified.

Effect of wogonoside on lipid peroxidative markers and antioxidant status in Mammary Tissues (MT)

The amounts of lipid peroxidative and antioxidant indicators in MT of normal and experimental rats are shown in Figure 3. When DMBA-induced rats were compared to normal rats, there was a significant ($p<0.05$) increase in lipid peroxidative indicators and a decrease in antioxidants. These quantities were significant ($p<0.05$) and retreated back to near normalcy in wogonoside-treated rats due to the antioxidant capability. In wogonoside alone and in control rats exposed no changes were found.

Effect of wogonoside on Hepatic Marker Enzymes (HME)

Figure 4 depicted the effect of wogonoside on HME in plasma levels such hepatic marker in control and experimental animals. When compared to normal rats, DMBA- rats had significantly ($p<0.05$) higher HME. Wogonoside-treated animals, on the other hand, had significantly lower HME ($p<0.05$) than cancer-bearing rats. There were no significant ($p<0.05$) differences between the control and experimental rats.

Effect of wogonoside on xenobiotic metabolizing enzymes

Figure 5 depicts the levels of Cyt p450, Cyt b5, GST and GR enzymes in normal and experimental MT. When DMBA-alone rats were compared to normal rats, there was a substantial ($p<0.05$) increase in phase-I enzymes and a decrease in phase-II enzymes. These activities were substantial ($p<0.05$) and retreated back to near normalcy in wogonoside-treated rats due to the

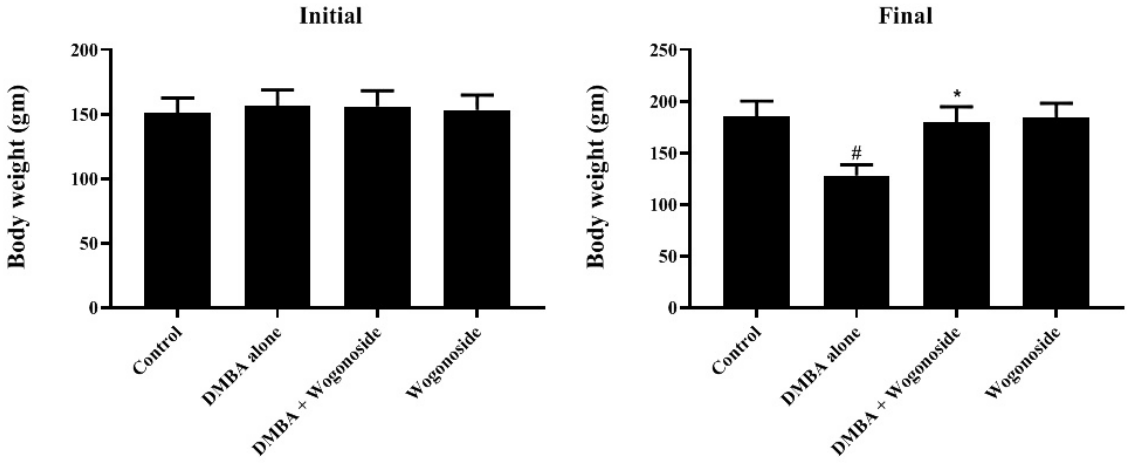

Figure 1: Variations in initial and end body weight of experimental and control animals in all groups. Within the group, statistical significance was compared as follows: DMBA-induced rats; DMBA+wogonoside treated rats. The values are reported as mean±SD for groups of 6 rats. At #,*$p<0.05$, the values are statistically significant.

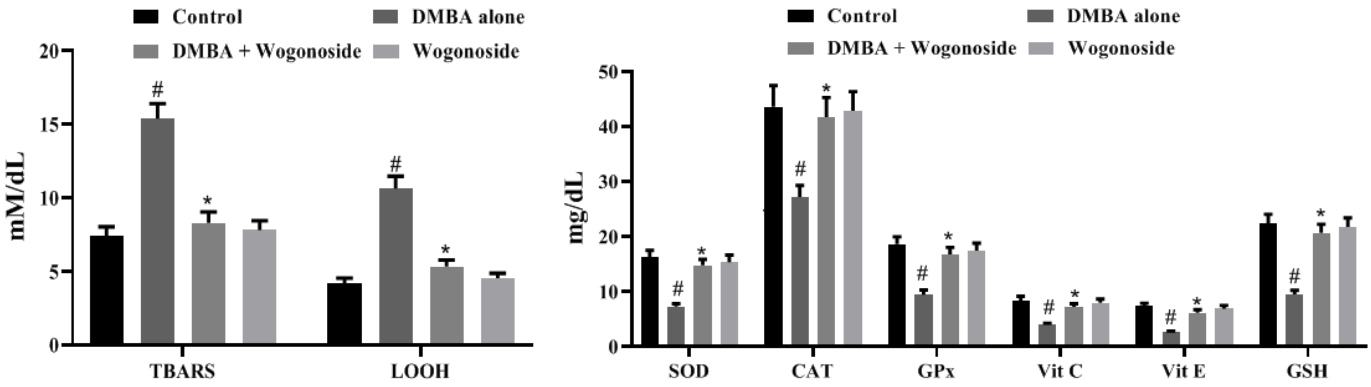

Figure 2: In all groups, the levels of lipid peroxidation and antioxidants were found in the plasma of control and experimental animals. Within the group, statistical significance was compared as follows: DMBA-induced rats; DMBA+wogonoside treated rats. The values are reported as mean±SD for groups of 6 rats. At #,*$p<0.05$, the values are statistically significant. SOD is the quantity of enzyme necessary to prevent 50% NBT reduction, CAT is the amount of H_2O_2 used per second and GPx is the amount of glutathione used per minute.

tumor inhibitory effect. Wogonoside alone and control rats exposed no changes were identified.

Effect of wogonoside on inflammatory marker in MT

Figure 6 displays western blotting was used to examine inflammatory markers in both normal and experimental rats. We noticed an increased expression of NF-κB p65, TNF-α, IL-6 and IL-1β in tumors taken from DMBA control rats, suggesting NF-κB p65 activation and subsequent translocation from cytosol to nucleus. On the contrary, wogonoside treatment attenuated these protein expressions compared to the DMBA control. These findings suggested wogonoside inhibited the triggering of NF-κB pathway induced by DMBA in mammary cancer.

Histopathological modifications of MT

Figure 7 demonstrated the histopathological changes of tumor and control tissues. DMBA-induced animals showed ductal hyperplasia is identified by significant proliferation in the mammary duct lumen. Whereas, wogonoside administration to DMBA-treated rats revealed a moderate development of cellular architecture were observed. Wogonoside-only treated rats had nearly normal ductal with normal epithelial cells.

DISCUSSION

Recently, there has been a lot of interest in the use of naturally occurring phytochemicals for cancer chemoprevention. A phytochemical called wogonoside, which can be found in horseradish, wasabi, cauliflower, cabbage, kale and brussels sprouts, may be useful in stopping the development of breast

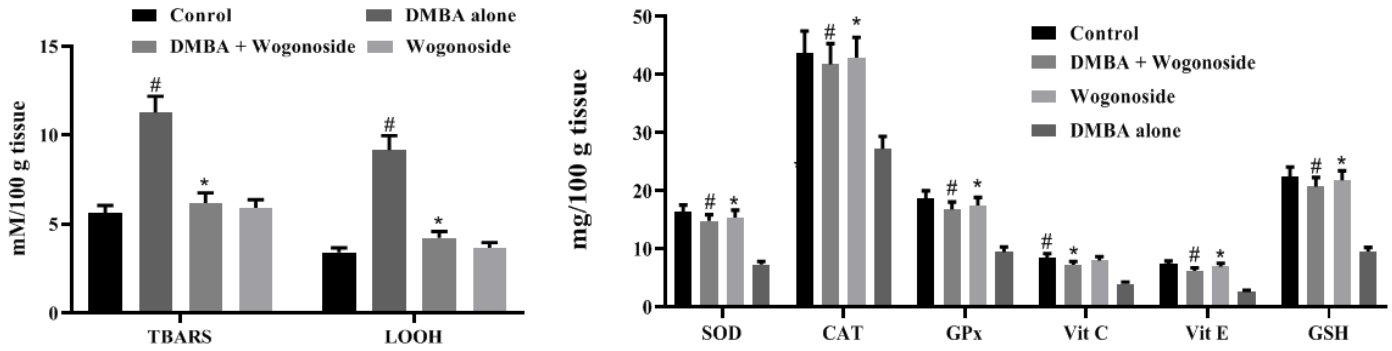

Figure 3: In all groups, the levels of lipid peroxidation and antioxidants were found in the mammary tissues of control and experimental animals. Within the group, statistical significance was compared as follows: DMBA-induced rats; DMBA+wogonoside treated rats. The values are reported as mean±SD for groups of 6 rats. At #,*$p<0.05$, the values are statistically significant. SOD is the quantity of enzyme necessary to prevent 50% NBT reduction, CAT is the amount of H_2O_2 used per second and GPx is the amount of glutathione used per minute.

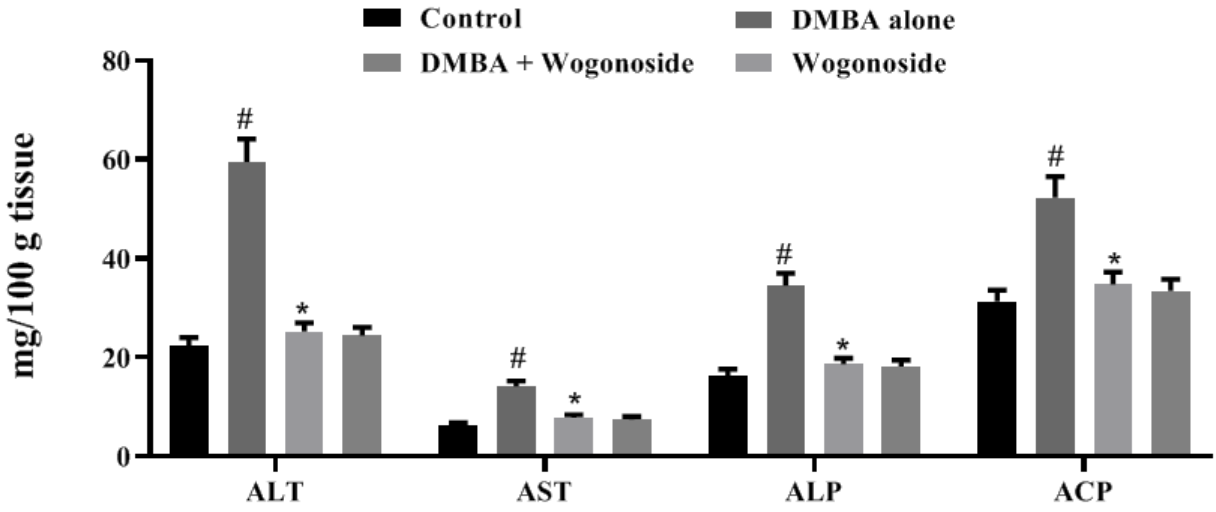

Figure 4: In all groups, the levels of liver marker enzymes were measured in the plasma of control and experimental animals. Within the group, statistical significance was compared as follows: DMBA-induced rats; DMBA+wogonoside treated rats. The values are reported as mean±SD for groups of 6 rats. At #,*$p<0.05$, the values are statistically significant.ALP and ACP liberate mol of phenol/min/mg protein, whereas ALT and AST liberate mol of pyruvate/min/mg protein.

cancer. The current study looked into how it can detoxify carcinogenic chemicals and scavenge free radicals.

Due to modifications in energy consumption during tumor formation, the total body weight of the DMBA-induced rats in this investigation was reduced. Moreover, an elevated level of LPO is crucial for the start of tumor development.[30] According to earlier research, the elevated TBARS level in tumor cells is linked to an excess of free radical generation and can be used as a gauge for the degree of tissue damage.[31] In the current investigation, we found that DMBA-induced animals had higher levels of LOOH and TBARS. And also we found increased liver marker enzymes in cancer-bearing animals due to the leakage from liver.[32,33] A previous study also reported the same findings in DMBA-induced cancer-bearing rats.[34] The wogonoside administration considerably modified these biochemical parameters and altered histopathological changes due to the antioxidant and free radical quenching effects.

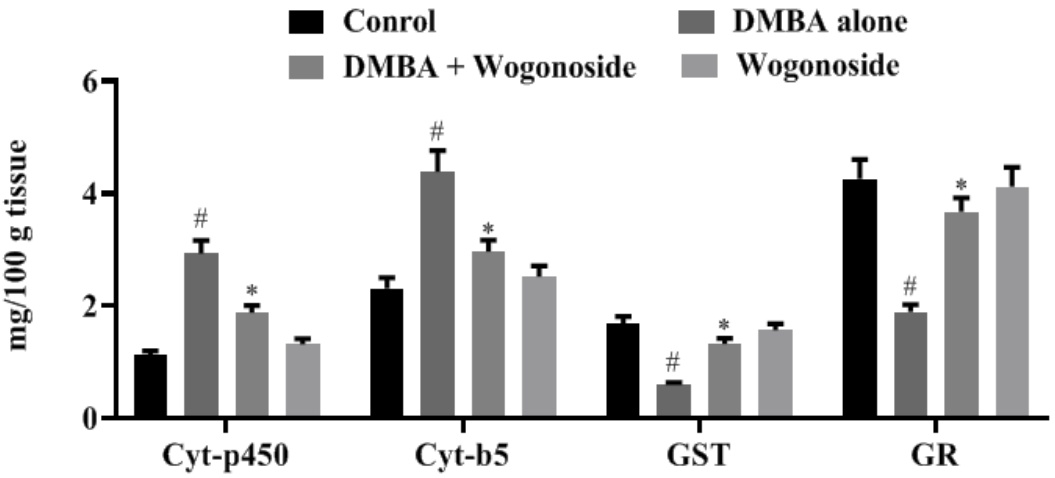

Figure 5: In both groups, the amounts of phase I and phase II biotransformation enzymes were found in the mammary tissues of control and experimental animals. Within the group, statistical significance was compared as follows: DMBA-induced rats; DMBA+wogonoside treated rats. The values are reported as mean±SD for groups of 6 rats. At $p<0.05$, the values are statistically significant. nmol/mg microsomal protein for Cyt-p450, nmol/mg microsomal protein for Cytb5, nmol/mg microsomal protein for GST, nmol of CDNB-GSH conjugate formed/mg microsomal protein/min for GR, nmol of NADPH oxidized/mg microsomal protein/min for GR.

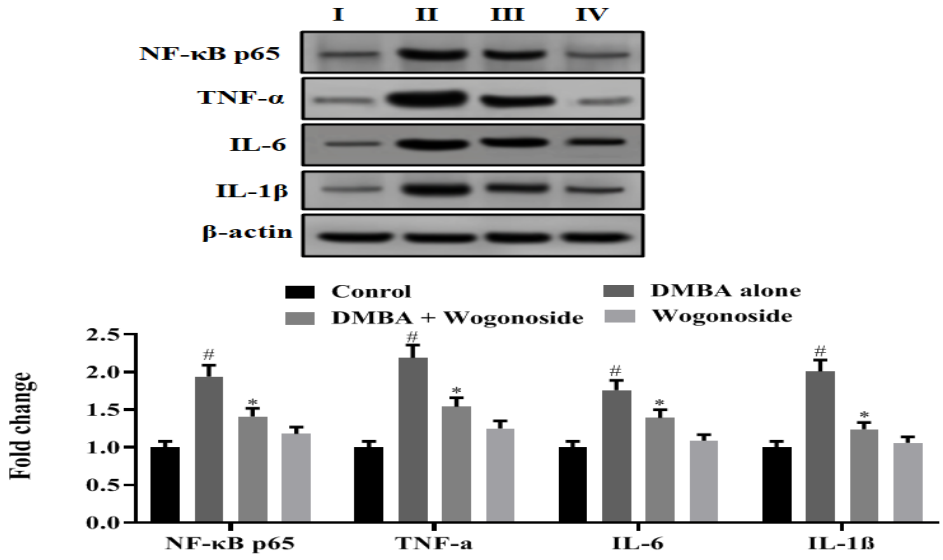

Figure 6: Representative photos show the effect of wogonoside on inflammatory markers in control and experimental rats, including NF-κB p65, TNF-α, IL-6 and IL-1β. Densitometry was used to quantify the band intensities, which were then normalized to the appropriate-actin loading control. Within the group, statistical significance was compared as follows: DMBA-induced rats; DMBA+wogonoside treated rats. The values are reported as mean±SD for groups of 6 rats. At $p<0.05$, the values are statistically significant. image7

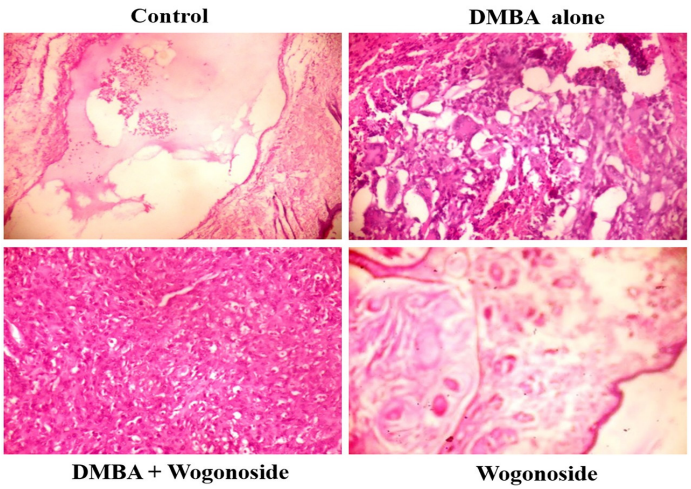

Figure 7: H and E stained histological alterations in rat mammary. The control and wogonoside-only treated mice had typical ductal with regular epithelial cells, with no evidence of hyperplasia or aberrant proliferation. In contrast, DMBA-only treated rats developed ductal hyperplasia, which was defined by significant proliferation in the mammary duct lumen. Wogonoside treatment resulted in significant and moderate development of cellular architecture in tumor tissue.

Augmented oxidative stress in the cumulative findings of several factors, including increased mitochondrial dysfunction, changed metal homeostasis and modified tissue antioxidant status.[35] DMBA causes significant reductions in antioxidant levels and promotes the production of pro-oxidants like reactive free radicals and electrophilic chemicals cause mammary gland malfunction and degeneration., which may be due to DMBA use of antioxidant enzymes in the removal of H_2O_2 and cancerous cells excessive production of ROS.[36,37] Therefore, in this hypothesis to the study the effect wogonoside in DMBA induced BC through TNF-α induced NF-κB pathway in rat model. This resultby an earlier report was the anticancer effect of wogonoside against MCF-7 breast cancer cellsin human blood plasma.[38,39] Biomarkers are widely used to track malignant diseases, particularly to evaluate how well pharmacotherapy is working as a treatment. When carcinogens interact with cells, the cells' structural integrity is compromised, which leads to the eventual cytoplasmic leaking of enzymes into the bloodstream.[40]

The enzymes involved in the conversion of amino acids to keto-acids are AST and ALT. Tissue damage is measured using pathophysiological diagnostic markers called AST and ALT. Increased liver cytosol leakage into the blood as a result of tissue damage may be the primary source of the spike in the functional activity of AST, ALT, ALP and ACP. One of the hallmarks of a malignant environment is tissue damage.[41] The current investigation found that the liver enzymes, including AST and ALT in the context of breast cancer; they suggest tumor growth. The study's elevated serum ALP values could be the result of DMBA's harmful effects on the liver.

ROS act as intracellular mediators, however, in release pose oxidative damage macromolecules and organelles, ultimately leads cell death.[42] Augmented ROS generation associated with the pathogenesis various cancer conditions, including breast cancer.[43] The present study indicates that DMBA initiation raises the levels of lipid peroxidative markers, phase I (Cyt-P450) enzymes, reduced body weight and decreased phase II (Cyt-b5, GR) enzymes and irregular histopathological changes. Phase II enzymes like GST and GR are examples of transferases that combine with hydrosoluble groups to form covalent adducts, which can then be added to DNA bases. The body can then eliminate these secondary products more quickly.[44] In the current investigation, we observed that rats treated with DMBA alone had higher levels of Cyt-P450 and Cyt-b5 and lower levels of GST and GR. Rats with mammary tumors had higher levels of phase-I enzymes and lower activity of phase-II enzymes.[45] In the present study rats receiving wogonoside treatment, however, saw a considerable change in these enzyme levels. Wogonoside triggers the induction of DMBA-induced breast cancer at the molecular level.

CYP1A1, a gene encoding the enzyme responsible for catalyzing the oxidative breakdown of DMBA, leading to the creation of DNA adducts. Benzyl and 2-phenylethyl isothiocyanates (BITC and PEITC) have been shown in numerous studies to promote phase-II enzymes that are involved for carcinogen detoxification and to inhibit the Cyt-P450 enzymes that are responsible for carcinogen activation.[46] The combined effects are what give in wogonoside their tumor-inhibiting properties. According to the study's findings, one in wogonoside, has the ability to suppress carcinogenesis in a comparable manner.

Animals with cancer had their liver tissue examined histopathologically and the results revealed dilated sinusoids and lost architecture. These alterations could result from the carcinogen's production of free radicals. Conversely, wogonoside-treated rats showed resolution of these alterations, which may be due to this compound's antioxidant and free radical-scavenging properties. A more dramatic impact was shown at 20 mg/kg bw. Animals receiving wogonoside treatment had hepatocytes with dilated sinusoids and moderate necrosis, which is their usual architecture. Consequently, it implies that the wogonoside may be able to shield people from carcinogens.

Inflammation plays a vital function in tumor angiogenesis and metastasis. Pro-inflammatory factors like TNF-α, IL-1β, IL-6 and NF-κB are the main molecular players that contribute to the inflammation of cancer axis. These particular inflammatory cytokines are controlling the tumor microenvironment that further accelerates tumorigenesis relating to different signaling pathways. Breast cancer is correlated with a rise in numerous cytokines and chemokines and their augments in tissue and circulation aggravate.[47] TNF-α is the primary inflammatory cytokine in control for tissue inflammation, IL-6 is a pleiotropic

cytokine responsible for cell growth and differentiation, NF-κB is a chief transcriptional mediator responsible for tissue inflammation, likewise IL-1β also a pro-inflammatory cytokine responsible for the pathogenesis of various diseases particularly cancer.[48] Previous studies reported that increased expression of these inflammatory markers in DMBA-induced BC is supported by this current study.[49] Inhibition of pro-inflammatory and enhanced anti-inflammatory cytokine is a protective effect against chemically induced breast carcinogenesis.[50] Present data of wogonoside-mediated prevention against IκBα phosphorylation and degradation and prevention with the translocation of activated NF-κB to the nucleus recommend that wogonoside-possibly impedes early events in DMBA-induced BC in animal model which regulates the immune response towards an infection. This finding in agreement with a previous report was the anti-inflammatory effect of wogonoside against dextran sulfate sodium-induced colon cancer by suppressing the inflammatory markers.[15] Another study reported that wogonoside suppresses inflammation process in MDA-MB-231 breast cancer cell lines.[51]

The overexpression of proliferative markers in DMBA alone rats stimulated by DMBA implies the initiation of a proliferative process in tumor cells. An earlier study found that higher expression of these proliferative markers in breast cancer, which is consistent with the current study.[52] Similarly wogonin treatment decreased the expression of these proliferative markers, point out that wogonoside possesses anti-proliferative properties, which is consistent with earlier research in colon cancer.[53]

CONCLUSION

Altogether over all data hypothesized that wogonoside improved body weight, decreased liver indicators, phase-I and lipid peroxidative markers and increased antioxidants and phase-II enzymes in DMBA-induced BC, as well as altered histology. Wogonoside decreasing the NF-κB signaling pathway, reduce inflammation and cell growth. This were leads wogonoside could be a mechanism for elucidating anti-inflammatory and anti-proliferative action on DMBA-induced mammary carcinogenesis. Furthermore, These findings suggested that wogonoside is a promising anti-cancer drug that should be studied further for the prevention and treatment of mammary carcinogensis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The research was supported by the "Basic-clinical" fusion innovation program of Xi'an Jiaotong University Health Science Center (No. YXJLRH2022052); Clinical research project of First Affiliated Hospital of Xi'an Jiaotong University (No. XJTU1AF-CRF-2022-026).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The local institutional animal ethics committee of The Second Affiliated Hospital of Xi'an Jiao tong University approved the experimental design (Registration number: XJ20230809).

ABBREVIATIONS

BC: Breast cancer; **DMBA:** 7,12-Dimethylbenz[a]anthracene; **MY:** Mammary tissues; **HME**: Hepatic marker enzyme; **NF-κB:** Nuclear factor kappa B; **IL-6:** Interleukin 6; **TNF-α:** Tumour Necrosis Factor alpha; **IL-1β:** Interleukin-1 beta.

SUMMARY

To summarize, wogonoside ability to You can minimize inflammation and cell proliferation by inhibiting the NF-κB signaling pathway. This could be a mechanism for understanding wogonoside anti-inflammatory via attenuation of NF-κB induced inflammatory and anti-proliferative effects on DMBA-induced breast carcinogenesis. Furthermore, wogonoside improved body weight, lowered liver indicators, phase-I and lipid peroxidative markers, elevated antioxidants and phase-II enzymes and altered histology in DMBA-induced BC. According to these findings, wogonoside is a promising anti-cancer medicine that should be explored further for the prevention and treatment of mammary carcinogensis.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424. doi: 10.3322/caac.21492, PMID 30207593.
2. Lin Y, Yao Y, Liu S, Wang L, Moorthy B, Xiong D, *et al.* Role of mammary epithelial and stromal P450 enzymes in the clearance and metabolic activation of 7,12-dimethylbenz(a)anthracene in mice. Toxicol Lett. 2012; 212(2): 97-105. doi: 10.1016/j.toxlet.2012.05.005, PMID 22595614.
3. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol. 2010; 38(1): 96-109. doi: 10.1177/0192623309356453, PMID 20019356.
4. Vera-Ramirez L, Sanchez-Rovira P, Ramirez-Tortosa MC, Ramirez-Tortosa CL, Granados-Principal S, Lorente JA, *et al.* Free radicals in breast carcinogenesis, breast cancer progression and cancer stem cells. Biological bases to develop oxidative-based therapies. Crit Rev Oncol Hematol. 2011; 80(3): 347-68. doi: 10.1016/j.critrevonc.2011.01.004, PMID 21288735.
5. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. J Bot. 2012; 2012: 1-26. doi: 10.1155/2012/217037.
6. Anbuselvam C, Vijayavel K, Balasubramanian MP. Protective effect of *Operculina turpethum* against 7,12-dimethylbenz(a)anthracene induced oxidative stress with reference to breast cancer in experimental rats. Chem Biol Interact. 2007; 168(3): 229-36. doi: 10.1016/j.cbi.2007.04.007, PMID 17531963.
7. Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans. 2007; 35(5): 1147-50. doi: 10.1042/BST0351147, PMID 17956298.
8. Gupta S, Bi R, Kim C, Chiplunkar S, Yel L, Gollapudi S. Role of NF-kappaB signaling pathway in increased tumor necrosis factor-alpha-induced apoptosis of lymphocytes in aged humans. Cell Death Differ. 2005; 12(2): 177-83. doi: 10.1038/sj.cdd.4401557, PMID 15647756.
9. Lu C, Shen Q, DuPré E, Kim H, Hilsenbeck S, Brown PH. cFos is critical for MCF-7 breast cancer cell growth. Oncogene. 2005; 24(43): 6516-24. doi: 10.1038/sj.onc.1208905, PMID 16027729.
10. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47(2): 389-94. doi: 10.1016/0003-2697(72)90132-7, PMID 4556490.

11. Bishayee A, Ahmed S, Brankov N, Perloff M. Triterpenoids as potential agents for the chemoprevention and therapy of breast cancer. Front Biosci (Landmark Ed). 2011; 16(3): 980-96. doi: 10.2741/3730, PMID 21196213.
12. Chen Y, Hui H, Yang H, Zhao K, Qin Y, Gu C, *et al.* Wogonoside induces cell cycle arrest and differentiation by affecting expression and subcellular localization of PLSCR1 in AML cells. Blood. 2013; 121(18): 3682-91. doi: 10.1182/blood-2012-11-466219, PMID 23487022.
13. Chen Y, Lu N, Ling Y, Gao Y, Wang L, Sun Y, *et al.* Wogonoside inhibits lipopolysaccharide-induced angiogenesis *in vitro* and *in vivo* via toll-like receptor 4 signal transduction. Toxicology. 2009; 259(1-2): 10-7. doi: 10.1016/j.tox.2009.01.010 , PMID 19428938.
14. Sun Y, Zhao Y, Yao J, Zhao L, Wu Z, Wang Y, *et al.* Wogonoside protects against dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-κB and NLRP3 inflammasome activation. Biochem Pharmacol. 2015; 94(2): 142-54. doi: 10.1016/j.bcp.2015.02.002, PMID 25677765.
15. Sun Y, Zhao Y, Wang X, Zhao L, Li W, Ding Y, *et al.* Wogonoside prevents colitis-associated colorectal carcinogenesis and colon cancer progression in inflammation-related microenvironment via inhibiting NF-κB activation through PI3K/Akt pathway. Oncotarget. 2016; 7(23): 34300-15. doi: 10.18632/oncotarget.8815, PMID 27102438.
16. Farombi EO, Shrotriya S, Surh YJ. Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappaB and AP-1. Life Sci. 2009; 84(5-6): 149-55. doi: 10.1016/j.lfs.2008.11.012, PMID 19081081.
17. Garg R, Ingle A, Maru G. Dietary turmeric modulates DMBA-induced p21ras, MAP kinases and AP-1/NF-kappaB pathway to alter cellular responses during hamster buccal pouch carcinogenesis. Toxicol Appl Pharmacol. 2008; 232(3): 428-39. doi: 10.1016/j.taap.2008.07.007, PMID 18687351.
18. Annamalai G, Suresh K. [6]-Shogaol attenuates inflammation, cell proliferation via modulate NF-κB and AP-1 oncogenic signaling in 7,12-dimethylbenz[a]anthracene induced oral carcinogenesis. Biomed Pharmacother. 2018; 98: 484-90. doi: 10.1016/j.biopha.2017.12.009, PMID 29287195.
19. Jiang ZY, Hunt JV, Wolff SP. Ferrous ion oxidation in the presence of Xylenolorange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem. 1992; 202(2): 384-9. doi: 10.1016/0003-2697(92)90122-n, PMID 1519766.
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2): 351-8. doi: 10.1016/0003-2697(79)90738-3 , PMID 36810.
21. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973; 179(4073): 588-90. doi: 10.1126/science.179.4073.588, PMID 4686466.
22. Sinha D, Biswas J, Sung B, Aggarwal BB, Bishayee A. Chemopreventive and chemotherapeutic potential of curcumin in breast cancer. Curr Drug Targets. 2012; 13(14): 1799-819. doi: 10.2174/138945012804545632, PMID 23140290.
23. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984; 21(2): 130-2. PMID 6490072.
24. Beutler E, Kelly BM. The effect of sodium nitrite on red cell GSH. Experientia. 1963; 19: 96-7. doi: 10.1007/BF02148042, PMID 13967892.
25. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol. 1979; 62: 3-11. doi: 10.1016/0076-6879(79)62181-x, PMID 440112.
26. Bergmeyer HU, Scheibe P, Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem. 1978; 24(1): 58-73. doi: 10.1093/clinchem/24.1.58, PMID 22409.
27. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol. 1985; 113: 484-90. doi: 10.1016/s0076-6879(85)13062-4, PMID 3003504.
28. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249(22): 7130-9. doi: 10.1016/S0021-9258(19)42083-8, PMID 4436300.
29. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, Purification and Properties. J Biol Chem. 1964; 239: 2379-85. doi: 10.1016/S0021-9258(20)82245-5, PMID 14209972.
30. Moselhy SS, Al mslmani MA. Chemopreventive effect of lycopene alone or with melatonin against the genesis of oxidative stress and mammary tumors induced by 7,12 dimethyl(a)benzanthracene in Sprague dawely female rats. Mol Cell Biochem. 2008; 319(1-2): 175-80. doi: 10.1007/s11010-008-9890-6, PMID 18682897.
31. Mishra P, Kale RK, Kar A. Chemoprevention of mammary tumorigenesis and chemomodulation of the antioxidative enzymes and peroxidative damage in prepubertal Sprague Dawley rats by biochanin A. Mol Cell Biochem. 2008; 312(1-2): 1-9. doi: 10.1007/s11010-008-9714-8, PMID 18273562.
32. Wang Z, Zhang X. Chemopreventive activity of honokiol against 7, 12-Dimethylbenz[a]anthracene-Induced mammary Cancer in Female Sprague Dawley Rats. Front Pharmacol. 2017; 8: 320. doi: 10.3389/fphar.2017.00320, PMID 28620301.
33. Padmavathi R, Senthilnathan P, Sakthisekaran D. Therapeutic effect of propolis and paclitaxel on hepatic phase I and II enzymes and marker enzymes in dimethylbenz(a) anthracene-induced breast cancer in female rats. Comp Biochem Physiol C Toxicol Pharmacol. 2006; 143(3): 349-54. doi: 10.1016/j.cbpc.2006.03.009, PMID 16720105.
34. Buendia I, Michalska P, Navarro E, Gameiro I, Egea J, León R. Nrf2-ARE pathway: an emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. Pharmacol Ther. 2016; 157: 84-104. doi: 10.1016/j.pharmthera.2015.11.003, PMID 26617217.
35. Arulkumaran S, Ramprasath VR, Shanthi P, Sachdanandam P. Restorative effect of Kalpaamruthaa, an indigenous preparation, on oxidative damage in mammary gland mitochondrial fraction in experimental mammary carcinoma. Mol Cell Biochem. 2006; 291(1-2): 77-82. doi: 10.1007/s11010-006-9199-2, PMID 16953336.
36. Lakshmi A, Subramanian SP. Tangeretin ameliorates oxidative stress in the renal tissues of rats with experimental breast cancer induced by 7,12-dimethylbenz[a]anthracene. Toxicol Lett. 2014; 229(2): 333-48. doi: 10.1016/j.toxlet.2014.06.845, PMID 24995432.
37. Huang Y, Zhao K, Hu Y, Zhou Y, Luo X, Li X, *et al.* Wogonoside inhibits angiogenesis in breast cancer via suppressing Wnt/β-catenin pathway. Mol Carcinog. 2016; 55(11): 1598-612. doi: 10.1002/mc.22412, PMID 26387984.
38. Grzegorczyk-Karolak I, Wysokińska H, Olas B. Studies on the antioxidant properties of extracts from the roots and shoots of two Scutellaria species in human blood plasma. Acta Biochim Pol. 2015; 62(2): 253-8. doi: 10.18388/abp.2014_944, PMID 26015995.
39. Hou WK, Xu YX, Yu T, Zhang L, Zhang WW, Fu CL, *et al.* Adipocytokines and breast cancer risk. Chin Med J (Engl). 2007; 120(18): 1592-6. doi: 10.1097/00029330-200709020-00009, PMID 17908478.
40. Sarhadi VK, Armengol G. Molecular biomarkers in cancer. Biomolecules. 2022; 12(8): 1021. doi: 10.3390/biom12081021, PMID 35892331.
41. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ. 2005; 172(3): 367-79. doi: 10.1503/cmaj.1040752, PMID 15684121.
42. de Almeida AJ, de Oliveira JC, da Silva Pontes LV, de Souza Júnior JF, Gonçalves TA, Dantas SH, *et al.* ROS: basic concepts, sources, cellular signaling and its implications in aging pathways. Oxid Med Cell Longev. 2022; 2022: 1225578. doi: 10.1155/2022/1225578, PMID 36312897.
43. Aggarwal V, Tuli HS, Varol A, Thakral F, Yerer MB, Sak K, *et al.* Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. Biomolecules. 2019; 9(11): 735. doi: 10.3390/biom9110735, PMID 31766246.
44. Gopalakrishnan T, Ganapathy S, Veeran V, Namasivayam N. Preventive effect of D-carvone during DMBA induced mouse skin tumorigenesis by modulating xenobiotic metabolism and induction of apoptotic events. Biomed Pharmacother. 2019; 111: 178-87. doi: 10.1016/j.biopha.2018.12.071, PMID 30583225.
45. Wang Z, Zhang X. Chemopreventive activity of honokiol against 7, 12-dimethylbenz[a]anthracene-induced mammary cancer in Female Sprague Dawley Rats. Front Pharmacol. 2017; 8: 320. doi: 10.3389/fphar.2017.00320, PMID 28620301.
46. Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer. 2009; 9: 187. doi: 10.1186/1471-2407-9-187, PMID 19531241.
47. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, *et al.* Inflammation and tumor progression: signaling pathways and targeted intervention. Signal Transduct Target Ther. 2021; 6(1): 263. doi: 10.1038/s41392-021-00658-5, PMID 34248142.
48. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity and disease. Cold Spring Harb Perspect Biol. 2014; 6(10): a016295. doi: 10.1101/cshperspect.a016295, PMID 25190079.
49. Akhouri V, Kumari M, Kumar A. Therapeutic effect of *Aegle marmelos* fruit extract against DMBA induced breast cancer in rats. Sci Rep. 2020; 10(1): 18016. doi: 10.1038/s41598-020-72935-2, PMID 33093498.
50. Esquivel-Velázquez M, Ostoa-Saloma P, Palacios-Arreola MI, Nava-Castro KE, Castro JI, Morales-Montor J. The role of cytokines in breast cancer development and progression. J Interferon Cytokine Res. 2015; 35(1): 1-16. doi: 10.1089/jir.2014.0026 , PMID 25068787.
51. Yao Y, Zhao K, Yu Z, Ren H, Zhao L, Li Z, *et al.* Wogonoside inhibits invasion and migration through suppressing TRAF2/4 expression in breast cancer. J Exp Clin Cancer Res. 2017; 36(1): 103. doi: 10.1186/s13046-017-0574-5, PMID 28774312.
52. Ma Z, Kim YM, Howard EW, Feng X, Kosanke SD, Yang S, *et al.* DMBA promotes ErbB2-mediated carcinogenesis via ErbB2 and estrogen receptor pathway activation and genomic instability. Oncol Rep. 2018; 40(3): 1632-40. doi: 10.3892/or.2018.6545 , PMID 30015966.
53. Wang M, Liu X, Chen T, Cheng X, Xiao H, Meng X, *et al.* Inhibition and potential treatment of colorectal cancer by natural compounds via various signaling pathways. Front Oncol. 2022; 12: 956793. doi: 10.3389/fonc.2022.956793, PMID 36158694.

Cite this article: He C, Zhang Y, Diao Y, Ma X, Mao G, Wei X, *et al*. Wogonoside Mitigates Inflammation and Cell Proliferation via Modulation of NF-Kb Signalling Pathway in DMBA-Induced Mammary-Preclinical Model. Indian J of Pharmaceutical Education and Research. 2025;59(4):1466-73.