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Suppression of DMBA-induced cervical epithelial carcinoma by *Glycine max* (L.) Merr. extracts: Implications for phytochemical- based interventions

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Abstract
Cervical cancer remains one of the leading causes of cancer-related mortality among women worldwide. Although conventional chemotherapeutic agents are effective, their associated toxicity has intensified the search for safer phytochemical-based interventions. *Glycine max* (L.) Merr. (soybean) is a rich source of isoflavones, phenolics, flavonoids, and other bioactive compounds with documented antioxidant and anticancer properties. Purpose: This study investigated the chemoprotective effects of different solvent extracts of *G. max* seeds against 7,12-dimethylbenz[a]anthracene (DMBA)-induced cervical epithelial carcinogenesis in rats through morphological and histopathological evaluation. Female rats (150 250 g) were allocated into vehicle, negative control (DMBA), positive control (paclitaxel), and five experimental groups treated with different *G. max* (L.) Merr. seed extracts following DMBA induction. Gross morphological alterations of the uterus, cervix, ovaries, and oviducts were examined, followed by histopathological assessment of cervical, uterine, and ovarian tissues using hematoxylin and eosin staining. Results showed that DMBA administration induced pronounced pathological changes characterized by uterine congestion, hemorrhage, ovarian enlargement with nodularity, cervical thickening, and cervical epithelial dysplasia marked by basal hyperplasia, loss of epithelial polarity, pleomorphism, and increased mitotic activity. Paclitaxel markedly restored reproductive organ morphology and reduced dysplastic alterations. Among the soybean extracts, the ethanolic extract demonstrated the greatest improvement in gross morphology, while experimental group 5 exhibited complete restoration of normal cervical histoarchitecture without evidence of dysplasia or invasive carcinoma. Other extracts provided partial protection with varying degrees of improvement in vascular congestion, ovarian morphology, and epithelial integrity. *G. max* (L.) Merr. seed extracts effectively suppressed DMBA-induced cervical epithelial lesions and preserved reproductive tissue architecture, indicating significant chemopreventive potential. The superior efficacy of the ethanolic extract suggests that solvent-dependent phytochemical composition plays a critical role in biological activity. These findings support soybean-derived phytochemicals as promising candidates for the development of multitarget, plant-based interventions for the prevention and management of cervical epithelial carcinogenesis. Further molecular and phytochemical investigations are warranted to elucidate the underlying mechanisms and facilitate translational application.

Keywords: *Glycine max* (L.) Merr., Cervical cancer, DMBA, Morphology, Histology

Introduction

One of the most prevalent cancers that affect women globally, cervical cancer continues to be a significant public health concern, especially in low- and middle-income nations where access to screening and immunization programs is restricted. Cervical intraepithelial neoplasia, a precancerous lesion, is the first stage of the disease, which starts in the epithelial cells lining the cervix and progresses to invasive carcinoma. Approximately 99% of instances cervical cancer globally are thought to be caused by persistent infection with high-risk strains of the human papillomavirus (HPV), particularly HPV-16 and HPV-18 (World Health Organization [WHO], 2026). Disease susceptibility and development are further increased by additional risk factors including smoking, long-term oral contraceptive usage, early sexual activity, multiple sexual partners, immunosuppression, and co-infection with human immunodeficiency virus(de Martel et al., 2017).

Cervical cancer is the fourth most common cause of cancer-related mortality among women globally, with about 660,000 new cases and about 350,000 deaths per year, according to recent estimates (Gao et al., 2025). Due to weak healthcare infrastructure, low screening rates, and restricted access to HPV vaccination programs, the burden of disease is disproportionately higher in developing nations. Cervical cancer still causes large socioeconomic and healthcare costs worldwide, despite major advancements in preventive measures including HPV vaccination and cervical screening (WHO, 2026).

HPV oncogenes E6 and E7 are integrated into the host genome throughout the pathophysiology of cervical cancer, which results in the inactivation of important tumor suppressor proteins such p53 and retinoblastoma protein (pRb). Uncontrolled cellular proliferation, apoptosis suppression, genomic instability, and ultimately malignant transformation of cervical epithelial cells are the outcomes of this disruption. Cervical carcinogenesis and tumor progression have also been linked to molecular pathways such as nuclear factor kappa B (NF-κB), phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase (MAPK), and vascular endothelial growth factor (VEGF) signaling (Gao et al., 2025). Although patient survival

has increased as a result of modern therapeutic procedures such as radiation, chemotherapy, surgery, and targeted medicines, treatment-associated toxicity, chemoresistance, recurrence, and metastasis continues to be significant clinical issues.
Glycine max (L.) Merr. extracts and their bioactive components have been shown in numerous studies to suppress the growth of cervical cancer cells and trigger programmed cell death via a variety of molecular mechanisms. One of the main isoflavones found in soybeans, genistein, has been shown to inhibit the growth of cervical cancer cells by causing cell cycle arrest, preventing angiogenesis, and encouraging mitochondrial-mediated apoptosis. Additionally, genistein reduces cellular proliferation, migration, and metastatic potential in cervical carcinoma cells by modulating multiple signaling pathways implicated in cancer progression, such as focal adhesion kinase (FAK), MAPK, PI3K/Akt, and NF-κB pathways (Zhang et al., 2023).
In a similar vein, isoflavone combinations made from soy that comprise daidzein, genistein, and glycitein have shown negligible toxicity to normal cells but considerable cytotoxic effects against HeLa cervical cancer cells. By activating caspase-3 and cleaving poly (ADP-ribose) polymerase (PARP), these substances cause apoptosis while downregulating anti-apoptotic proteins like Bcl-2 and Bcl-xL (Xiao et al., 2011). More recently, it was demonstrated that isoflavone-rich soybean extracts limit the growth of cervical cancer cells by suppressing cyclin-dependent kinases that drive cell cycle progression, increasing p21 expression, and causing G1 and G2/M phase arrest (Sukhamwang et al., 2024).
Glycine max's phytoestrogenic isoflavones, which structurally resemble endogenous estrogens and can bind estrogen receptors to modulate hormone-dependent signaling pathways involved in tumor growth, are chiefly responsible for the plant's anticancer capabilities. Furthermore, soybean phytochemicals have potent antioxidant properties that might lessen DNA damage and oxidative stress, two important factors in the development of cancer. *Glycine max* extract may be a viable option for the creation of safer and more potent treatment approaches against cervical cancer due to the synergistic effects of these bioactive substances.
Therefore, research into *G. max* (L.) Merr. extract as a possible anticancer agent is crucial given the rising incidence of cervical cancer and the drawbacks of traditional treatments. The potential of soybean-derived chemicals as supplementary or alternative therapeutic approaches for cervical cancer management is highlighted by the availability of several bioactive constituents that can target different molecular pathways implicated in cervical carcinogenesis.

MATERIAL AND METHODS

Collection of plant

Seeds were obtained from Nawabshah, Sind, Pakistan. Plant was identified by expert taxonomist at GC Women University, Lahore, Punjab, Pakistan and voucher specimen (Botanical number: GC. Herb. Bot.3782) was saved in the department of IMBB, The university of Lahore, Lahore Pakistan along with its voucher number for further use in future.

Preparation of Plant Extract/ Plant extraction

To prepare the extract, the seeds were grounded in a powder form with the help of grinding machine taking full hygienic and aseptic measures. In first step of fractional distillation, 35 gram of powdered soybean seeds were added in 350 mL of ethyl acetate, left overnight on shaker and then after 24 hours centrifuged on 4000 rpm for 15 minutes, the supernatant was separated and was kept for further analysis, and the residue was added in 250 mL of N-hexane, left overnight on shaker. After 24 hours this mixture was centrifuged at 4000 rpm for 15 min, the supernatant was collected in separate container and residue was added in 250 ml of chloroform and again placed in shaker for 24 hours, followed by centrifugation of sample at 4000rpm for 15 minutes. Supernatant was separated and residue was added in ethanol 250 mL and whole procedure was repeated (Sasidharan et al., 2011). Extracts were lyophilized at −80 °C for 3 days (FreeZone 6 Plus, Labconco, Kansas City, MO, USA) and stored at −80 °C. Dried extracts were milled to a fine powder (MM301 Retsch, Haan, Germany; 30 Hz for 60 s), which was stored at −80 °C until extraction (Maisl et al., 2023).

Animal Selection

Adult female Sprague–Dawley rats weighing between 150 and 200 g were procured from the Animal House Facility of the Institute of Molecular Biology and Biotechnology and were housed in standard stainless-steel cages under controlled environmental conditions, including a temperature of 25 ± 1°C and relative humidity of 60–70%, while maintaining standard laboratory husbandry practices. The animals were provided with free access to a standard laboratory diet and water throughout the experimental period. Only healthy adult female Sprague–Dawley rats within the specified weight range of 150–200 g was included in the study, whereas sick, diseased, or physically compromised female rats, as well as all male rats irrespective of their health status, were excluded from the experimental protocol.

Ethical approval

Ethical approval (Ref-IMBB/BBBC/24/343B) was taken prior to the experimental studies

Grouping of animals

1. Vehicle (only DMSO)
2. Negative control group (Only DMBA)
3. Positive control group (DMBA+ paclitaxel 6mg/mL)
4. Experimental group 1 (DMBA +ethanolic extract of seeds)
5. Experimental group 2 (DMBA +ethyl acetate extract of seeds)
6. Experimental group 3 (DMBA + N-hexane extract of seeds)
7. Experimental group 4 (DMBA +chloroform extract of seeds)

8. Experimental group 5 (DMBA + water)

Induction of cervical cancer

The experiment was initiated by administering 0.2 mL of 7,12-Dimethylbenz[a]anthracene DMBA (1mg/mL of normal saline) intravaginally twice weekly to all rats for the induction of cervical cancer. The animals were subsequently monitored regularly for the appearance and progression of clinical signs and symptoms associated with cervical cancer development. The only exception was the vehicle group, which did not receive DMBA treatment and was maintained as a normal healthy control group to provide baseline physiological and histological parameters for comparison with the diseased and treatment groups (Anisimov et al., 2000; Goerttler et al., 1980)

Treatment with extracts

Animals in the vehicle group received no treatment and served as the normal healthy control. The negative control group was administered with DMBA only for cervical cancer induction and received no therapeutic intervention. The positive control group received DMBA followed by treatment with paclitaxel (6mg/mL) administered intravaginally in a volume of 0.2 mL. Experimental groups 1 to 5 were induced with DMBA and subsequently treated with different seed extracts, including ethanolic extract (Experimental Group 1), ethyl acetate extract (Experimental Group 2), n-hexane extract (Experimental Group 3), chloroform extract (Experimental Group 4), and aqueous extract (Experimental Group 5). Treatment in all experimental groups was initiated following successful DMBA induction, and each animal received 0.2 mL of the respective formulation via the intravaginal route. A total of two doses were administered per week for the duration of the treatment period under standardized experimental conditions. This treatment regimen was designed to evaluate and compare the therapeutic efficacy of different solvent extracts of the seeds against DMBA-induced cervical cancer.

Morphology of animals

All animals were euthanized humanely under anesthesia(chloroform) and subjected to a complete gross morphology. Following dissection, major internal organs including the ovaries, utres and cervix were excised and examined for changes in size, shape, color, texture, congestionand tumor formation. Particular attention was given to the cervix and uterus for evidence of cervical lesions, enlargement, nodules, or abnormal tissue growth associated with DMBA-induced cancer. Representative photographs of gross lesions were recorded whenever necessary. The observed morphological findings were compared among the experimental groups to assess disease progression and the therapeutic efficacy of the administered treatments (Parkinson et al., 2011; Kittel et al., 2004; U.S. Food and Drug Administration [FDA], 2000).

Histopathology of tissues

Animals were dissect for collection of tissues in which rats were placed in properly covered glass chambers. Chloroform-soaked cotton (dose of chloroform was according to surgical anaesthesia in rodent) was placed in that chamber, after 5-10 minutes rats were surgically anesthetized for organ collection. Ovaries, uterus and cervix were fixed in 10% neutral formalin. The specimens were dehydrated in descending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 4-5µm thickness will be cut and stained with hematoxylin and eosin and examined under microscope (Slaoui et al., 2017).

RESULTS

Morphological Changes in animals

Gross morphological evaluation of the female reproductive system demonstrated marked differences among the experimental groups. The vehicle rats exhibited a normal bicornuate uterus with symmetrical uterine, thickening, or luminal contents, and no evidence of cysts, nodules, or neoplastic lesions. Bilateral ovaries were pale pink, oval, and smooth to slightly nodular, while the oviducts appeared as highly coiled, translucent, thread-like structures. The cervix was firm, thick-walled, and displayed a smooth pale-white external surface. In contrast, the rats in negative control group showed severe pathological alterations characterized by diffuse uterine congestion, multifocal hemorrhage, hyperemia, tissue thickening, enlargement of the uterine horns, ovarian enlargement with irregular nodularity, congested oviducts, and a thickened hyperemic cervix suggestive of inflammatory and neoplastic progression. Treatment with paclitaxel (6 mg/mL) markedly improved gross morphology, resulting in only mild residual congestion, reduced hemorrhagic lesions, restoration of near-normal ovarian morphology, preservation of uterine architecture, and normalization of cervical appearance. Similarly, animals treated with the ethanolic extract demonstrated partial restoration of normal uterine morphology with reduced hemorrhage, minimal ovarian irregularities, preserved oviductal structure, and a relatively normal cervix. The ethyl acetate extract moderately improved congestion and swelling, with maintenance of the bicornuate uterine configuration, slight ovarian nodularity, and minimal cervical hyperemia. Administration of the n-hexane extract resulted in persistent but less severe uterine congestion and focal hemorrhage, accompanied by mild ovarian nodularity, slight oviductal vascular prominence, and mild cervical thickening without obvious neoplastic lesions. The chloroform extract exhibited moderate therapeutic effects with noticeable reductions in vascular congestion and hemorrhage, preservation of uterine symmetry, near-normal ovarian morphology, and minimal cervical abnormalities. Likewise, treatment with the aqueous seed extract produced partial protection against DMBA-induced damage, as evidenced by moderate uterine congestion with occasional focal hemorrhage, preservation of ovarian and oviductal morphology, and mild cervical thickening without visible neoplastic changes. Overall, DMBA administration induced significant gross pathological alterations in the female reproductive organs, whereas paclitaxel and the various seed extracts demonstrated varying degrees of protective and restorative effects, with the positive control and ethanolic extract groups showing the most pronounced improvements (Figure 1).

Histology of rats

Histopathological examination revealed normal cervical, uterine, and ovarian architecture in the rats of vehicle, with only a corpus luteum observed in the ovary and no evidence of dysplasia or malignancy. In contrast, animals in negative control group exhibited mild to moderate cervical dysplasia characterized by basal layer hyperplasia, loss of epithelial polarity, mild pleomorphism, prominent nucleoli, and occasional mitotic figures, although no invasive carcinoma was detected. The positive control group demonstrated moderate to severe cervical dysplasia with pronounced basal hyperplasia and loss of keratinocyte polarity, while the ovaries retained normal follicular development. Experimental groups showed varying degrees of histological improvement following treatment. Experimental group 1 exhibited mild to moderate dysplasia similar to the negative control, whereas experimental groups 2 and 4 showed only mild dysplastic changes with preservation of normal uterine and ovarian morphology; group 2 additionally displayed eosinophilic stromal infiltration and multiple corpora lutea. Experimental group 3 showed persistent moderate to severe dysplasia, indicating limited therapeutic efficacy. Notably, experimental group 5 demonstrated complete restoration of normal cervical histology with no evidence of dysplasia or invasive carcinoma, while maintaining normal uterine tube and ovarian structures, suggesting the strongest protective and therapeutic effect among the treatment groups (Figure 2)

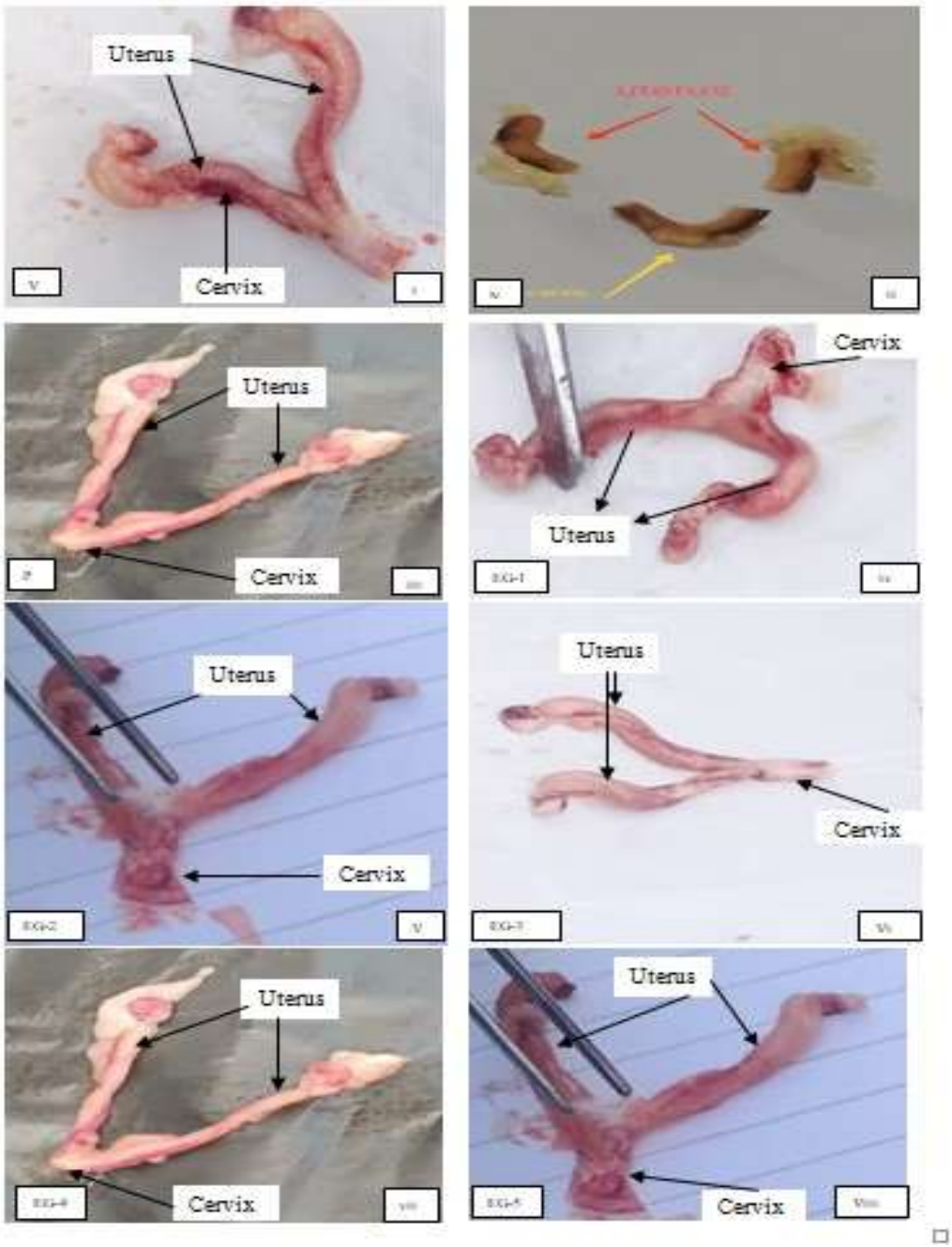
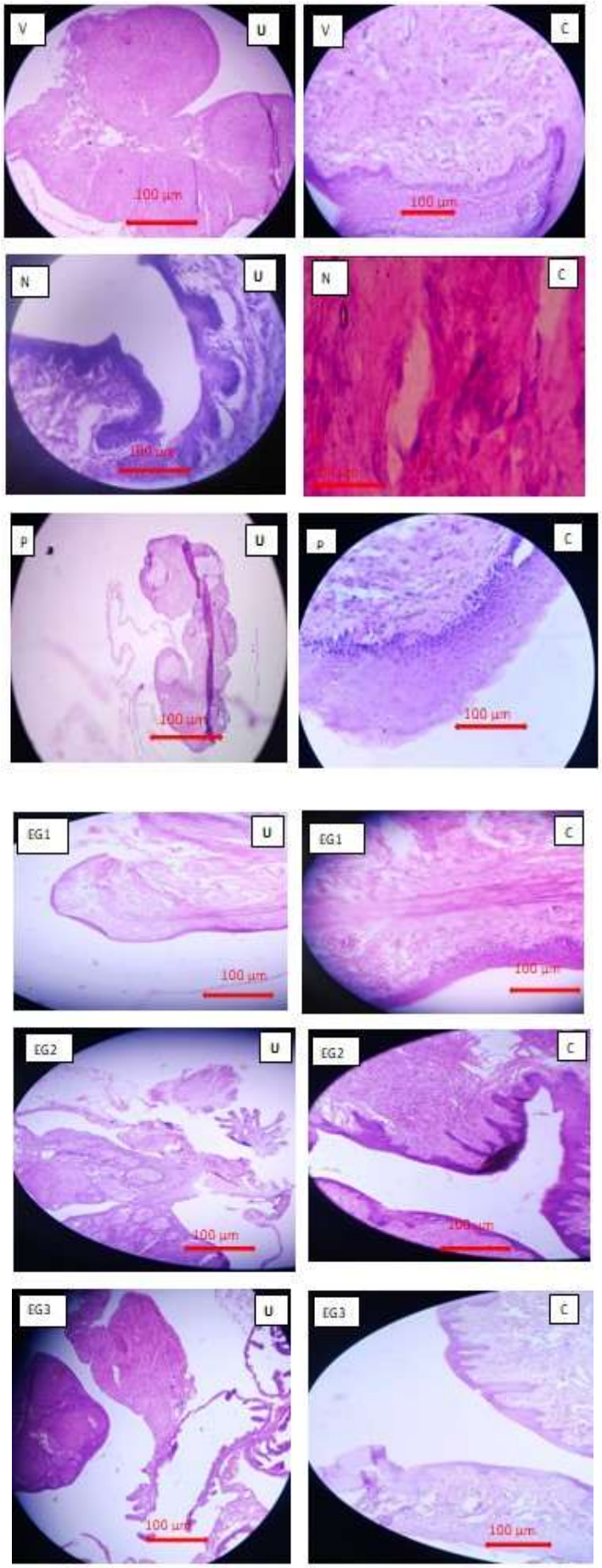


Figure 1 Morphological changes in uterus and cervix of animals
Vehicle group (v), Negative control group (N), positive control group (P), Experimental group 1 (EG-1), Experimental group 1 (EG-1) , Experimental group 2 (EG-2) , Experimental group 3 (EG-3) , Experimental group 4 (EG-4) , Experimental group 5 (EG-5)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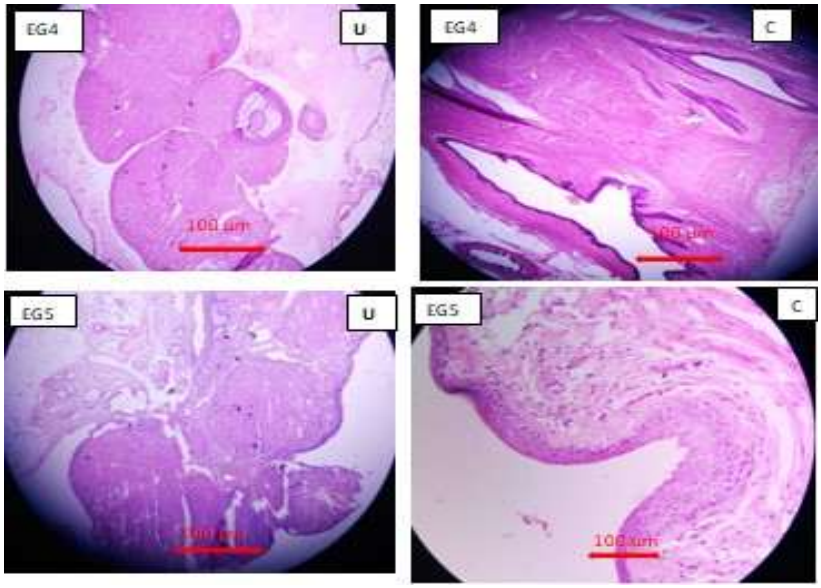

Figure 2 Histological changes in animals
Vehicle group (v), Negative control group (N), positive control group (P), Experimental group 1 (EG-1), Experimental group 1 (EG-1) , Experimental group 2 (EG-2) , Experimental group 3 (EG-3) , Experimental group 4 (EG-4) , Experimental group 5 (EG-5)
Uterus (U). Cervix (C)

DISCUSSION

The present study demonstrates that *G. max* (L.) Merr. seed extracts effectively attenuate DMBA-induced cervical epithelial carcinogenesis, as evidenced by marked improvements in both gross morphology and histopathological architecture of the female reproductive tract. DMBA administration produced characteristic pathological alterations including uterine congestion, hemorrhage, ovarian nodularity, cervical thickening, and cervical epithelial dysplasia, whereas treatment with soybean extracts substantially mitigated these abnormalities. Among the tested preparations, the ethanolic extract exhibited the greatest macroscopic restoration, while experimental group 5 completely normalized cervical histoarchitecture, suggesting that the chemoprotective efficacy of *G. max* (L.) Merr. is strongly influenced by extraction solvent and phytochemical composition. These findings reinforce the growing evidence that soybean-derived phytochemicals possess multitarget anticancer activities capable of interrupting early stages of epithelial carcinogenesis (Jan et al., 2022; Shoaib et al., 2022; Mahajan et al., 2021). The pathological changes induced by DMBA in the present study are consistent with its established role as a potent polycyclic aromatic hydrocarbon that requires metabolic activation by cytochrome P450 enzymes to generate electrophilic metabolites capable of forming DNA adducts and initiating carcinogenesis (Murray et al., 2022; Murray et al., 2020; Rendic & Guengerich, 2021). Persistent oxidative stress, chronic inflammation, mitochondrial dysfunction, and genomic instability generated by DMBA collectively promote epithelial hyperplasia, dysplasia, and neoplastic transformation (Qurat-ul-Ain & Choudhary, 2020). The severe congestion, diffuse hemorrhage, cervical hyperemia, and epithelial disorganization observed in the negative control animals therefore represent typical manifestations of chemically induced reproductive tract carcinogenesis and validate the experimental model employed in this investigation. Similar pathological features have been consistently reported in experimental models of chemical carcinogenesis, confirming that DMBA remains a robust model for evaluating natural chemopreventive agents (Murray et al., 2020).

Gross morphological evaluation demonstrated that soybean extracts markedly reduced vascular congestion, uterine enlargement, hemorrhagic lesions, and ovarian abnormalities. Such improvements likely reflect suppression of inflammatory vascular remodeling and restoration of tissue homeostasis. Chronic inflammation is now recognized as a hallmark of carcinogenesis because inflammatory mediators stimulate angiogenesis, enhance cellular proliferation, inhibit apoptosis, and facilitate progressive genomic damage (Hanahan, 2022). Consequently, reduction of these macroscopic lesions suggests that *G. max* (L.) Merr. interrupts early inflammatory events that precede irreversible malignant transformation. Similar anti-inflammatory effects have been described for soybean-derived phytochemicals in several experimental cancer models, where attenuation of inflammatory cytokines and oxidative injury preceded histological recovery (Li et al., 2020; Morvaridzadeh et al., 2020; Shahrajabian et al., 2022).

Histopathological assessment provides stronger evidence for the chemopreventive potential *G. max* (L.) Merr. DMBA-treated animals exhibited basal layer hyperplasia, epithelial disorganization, nuclear pleomorphism, loss of cellular polarity, prominent nucleoli, and increased mitotic activity, all of which are characteristic features of cervical intraepithelial neoplasia (Xue et al., 2019; Hanahan, 2022). Importantly, invasive carcinoma was absent, indicating that the present model successfully reproduced premalignant cervical lesions and therefore permitted evaluation of interventions targeting the initiation and promotion phases of carcinogenesis. Restoration of normal epithelial stratification in the treated groups indicates that soybean extracts inhibited progression of dysplastic lesions rather than merely reducing inflammation. Complete normalization of cervical histology in

experimental group 5 is particularly noteworthy because reversal of epithelial dysplasia remains one of the principal objectives of chemopreventive strategies against cervical cancer (Hanahan, 2022; Ravi et al., 2022).

The superior efficacy of the ethanolic extract is biologically plausible considering the phytochemical composition of soybean. Ethanol efficiently extracts isoflavones, flavonoids, phenolic acids, saponins, phytosterols, and tocopherols, many of which possess complementary antioxidant and anticancer activities (Isanga & Zhang, 2008; Yingngam, 2016). Among these constituents, genistein has received particular attention because it modulates numerous signaling pathways implicated in cervical carcinogenesis. A recent comprehensive review concluded that genistein suppresses cervical cancer cell proliferation, induces apoptosis, inhibits migration and invasion, and enhances sensitivity to both chemotherapy and radiotherapy through regulation of ERK, PI3K/Akt/mTOR, MAPK, and caspase-dependent signaling pathways. These mechanistic observations provide a strong molecular framework for interpreting the histological restoration observed in the present study (Murray et al., 2023).

The therapeutic effects observed in current investigation are unlikely to result from a single bioactive compound. Instead, they probably reflect synergistic interactions among multiple phytochemicals naturally present in soybean seeds. Recent reviews emphasize that soy isoflavones act through pleiotropic mechanisms involving attenuation of oxidative stress, inhibition of NF-κB-mediated inflammatory signaling, suppression of angiogenesis through VEGF regulation, modulation of estrogen receptor signaling, induction of mitochondrial apoptosis, and inhibition of epithelial proliferation (Kaufman-Szymczyk et al., 2022; Rahman et al., 2024). Such multitarget pharmacology represents a major advantage over conventional single-target therapeutics because carcinogenesis involves simultaneous dysregulation of numerous interconnected molecular pathways.

The differential therapeutic responses among solvent fractions further support the importance of extraction methodology. Experimental group 3, corresponding to the n-hexane extract, exhibited persistent moderate-to-severe dysplasia, whereas the ethanolic and other intermediate-polarity extracts demonstrated greater protection. Solvent polarity directly influences recovery of biologically active phenolic compounds and isoflavones, with ethanol generally providing higher yields of genistein, daidzein, glycitein, and related polyphenols than non-polar solvents (Luthria et al., 2007; Barnes, 2010). Consequently, the superior biological activity of the ethanolic extract likely reflects greater concentrations of these compounds rather than increased extract quantity alone. This observation has practical implications for future development of standardized soybean-based phytopharmaceuticals because extraction procedures critically determine phytochemical composition and therapeutic consistency (Wu et al., 2005; Luthria et al., 2007).

Paclitaxel markedly improved tissue morphology and served as an appropriate pharmacological reference. Interestingly, the highest-performing soybean extract produced histological restoration comparable to the positive control, highlighting the potential of phytochemical-based interventions as complementary therapeutic strategies. This observation is consistent with accumulating evidence that genistein enhances responsiveness of cervical cancer cells to conventional chemotherapy while simultaneously reducing proliferative signaling and promoting apoptosis (Del Carmen et al., 2024; Sahin et al., 2019). Experimental studies have also demonstrated that soybean isoflavones may sensitize cervical cancer cells to radiation and cytotoxic agents, suggesting potential utility as adjuvant therapies rather than replacements for established treatment protocols (Hillman & Singh-Gupta, 2011; Kim et al., 2005).

Preservation of normal ovarian follicles, corpora lutea, uterine architecture, and oviductal morphology in several treatment groups further suggests that soybean extracts exerted selective chemoprotective effects without producing obvious reproductive tissue toxicity during the experimental period. This observation may have translational importance because fertility preservation remains a major concern during cervical cancer management, particularly among younger women (Munro et al., 2003; Kang et al., 2023). Although dedicated reproductive toxicity studies remain essential, the maintenance of normal reproductive histology observed here supports the favorable safety profile generally associated with soybean-derived phytochemicals (Cederroth et al., 2012).

The mechanisms responsible for the observed histological recovery are likely multifactorial. Oxidative stress represents one of the earliest events following DMBA exposure, resulting in lipid peroxidation, oxidative DNA damage, mitochondrial dysfunction, and activation of redox-sensitive transcription factors including NF-κB and AP-1 (Matsumoto et al., 2017; Klaunig et al., 2011). Soybean polyphenols possess potent free radical scavenging activity and enhance endogenous antioxidant defense systems, thereby limiting oxidative injury and mutation accumulation (Messina, 2016; Rizzo et al., 2023).. Simultaneously, isoflavones suppress inflammatory cytokines, inhibit cyclooxygenase-2 expression, modulate PI3K/Akt and STAT3 signaling, promote Bax-mediated apoptosis, downregulate Bcl-2 expression, activate caspase-3 and caspase-9, and interfere with angiogenic pathways. Collectively, these complementary mechanisms may explain the remarkable restoration of cervical epithelial organization observed following treatment (Del Carmen et al., 2024; Sahin et al., 2019).

Despite these encouraging findings, several limitations should be acknowledged. The present investigation relied primarily on morphological and histopathological evaluation without quantitative assessment of oxidative stress biomarkers, inflammatory mediators, proliferative indices, apoptosis-related proteins, or angiogenic markers. Consequently, the proposed molecular mechanisms remain inferential. Furthermore, phytochemical characterization of the individual solvent extracts was not performed, preventing direct correlation between biological activity and concentrations of genistein, daidzein, glycitein, or total phenolics (Kim et al., 2020). Future studies should integrate chromatographic fingerprinting (HPLC-MS/MS or LC-

QTOF-MS), quantitative phytochemical analysis, immunohistochemistry for Ki-67, p53, Bcl-2, Bax, cleaved caspase-3, VEGF, COX-2 and NF-κB, together with transcriptomic and metabolomic approaches to establish mechanistic links between soybean phytochemicals and suppression of cervical carcinogenesis (Ding et al., 2016; Kim et al., 2020). Evaluation of HPV-associated molecular pathways, although beyond the scope of the present chemically induced model, would also improve the translational relevance of future investigations (Alshammari et al., 2024; Ullah et al., 2019).

Overall, the present findings provide compelling experimental evidence that *G. max* (L.) Merr. seed extracts suppress DMBA-induced cervical epithelial dysplasia and restore normal reproductive tissue architecture (Messina, 2016; Ullah et al., 2019). The pronounced efficacy of the ethanolic extract and the complete histological normalization observed in experimental group 5 strongly suggest that soybean-derived phytochemicals exert synergistic chemopreventive effects through modulation of oxidative stress, inflammation, apoptosis, and proliferative signaling Banerjee et al., 2008; Alshammari et al., 2024). Given the increasing interest in plant-derived multitarget therapeutics, these results support *G. max* as a promising candidate for the development of phytochemical-based interventions aimed at preventing or delaying cervical epithelial carcinogenesis. Future studies integrating phytochemical standardization, molecular mechanistic analyses, pharmacokinetic evaluation, and translational animal models will be essential to facilitate clinical development of soybean-derived chemopreventive agents (Rietjens et al., 2017; Russo et al., 2021).

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