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Unveiling the Power of Traditional Soodha Vallathi Urundai against Gastric Cancer Cells

Revathi M M.D(s)[1*], Subash Chandran G. M.D (s)[2], Uma K.S. M.D(s)[3]

[1]Research scholar, Department of Pothu Maruthuvam,TN. Dr. MGR Medical University, Chennai-60003

[*2]Associate Professor, Department of Pothu Maruthuvam, Government Siddha Medical College, Palayamkottai-627002.

[3]Associate Professor, Department of Siddha, The TN Dr. MGR Medical University, Chennai-600032

*** Corresponding autho:**

Dr. G. Subash Chandran

Associate Professor, Dept. of Maruthuvam, Government Siddha Medical College, Palayamkottai,

Email ID: siddhadrgs21@gmail.com

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ABSTRACT

Introduction: Cancer-related deaths from gastric cancer (GC) are significant worldwide, and there is an urgent need for new, efficient therapies. Siddha medicine, known for its extensive historical use, presents potential anticancer compounds. Soodha Vallathi Urundai (SVU) is a time- honored remedy believed to have healing benefits; however, its effect on gastric cancer remains largely unexplored. This study aimed to assess the anticancer efficacy of SVU in a gastric cancer model (MKN-45P), focusing on tumor development, angiogenesis, blood parameters, and metabolic indicators.

Methodology: Three groups of Balb/c mice (n=6) received injections of MKN-45P tumor cells and then administered SVU (200 mg/kg). Tumor advancement and blood were assessed, including biochemical tests, immunohistochemistry for CD31, LDH, and TNF-α, and white blood cell counts, were performed.

Results: SVU treatment markedly inhibited tumor growth (tumor size in the SVU-treated group: 2.45 ± 0.63 cm versus 3.02 ± 0.73 cm in the MKN-45P group, $p < 0.05$). The phrase of CD31 in tumors was reduced in SVU-treated sector (0.32 ± 0.12) relative to the MKN-45P group (0.72 ± 0.21, $p < 0.01$). Haematological examination showed elevated WBC levels (14.1 ± 0.754 $\times 10^3/\mu L$ in the SVU-treated group compared to 11.2 ± 1.58 $\times 10^3/\mu L$ in the MKN-45P group, $p < 0.05$). LDH levels were considerably lower in SVU group (0.136 ± 0.024 IU/L compared to 0.226 ± 0.0115 IU/L in the MKN-45P group, $p < 0.01$).

Conclusions: SVU showed notable anticancer effects, decreasing tumor size, angiogenesis, and metabolic indicators, while enhancing immune response with minimal toxicity. These findings indicate that SVU may be a viable treatment option for gastric cancer, necessitating additional research.

Keywords: *Balb/c mice, MKN-45Ptumor cells, Biochemical test, Immunohistochemistry*

1. INTRODUCTION

In the world, gastric cancer (GC) is the third most common cause of cancer-related death and the fifth most common cancer overall, especially prevalent in East Asia and South India . With advancements in multimodal treatment approaches, including targeted treatments, radiation, chemotherapy, and surgery, the prognosis of advanced gastric cancer (AGC) remains poor due to high recurrence rates and chemoresistance[1, 2]. The limitations of conventional therapies in complementary and alternative medicine (CAM), particularly traditional systems like Ayurveda and Siddha, for potential anticancer agents are notable. Siddha medicine, one of the most ancient forms of traditional medicine practiced in Tamil Nadu, India, encompasses a wide variety of herbal, mineral, and metal-based formulations with therapeutic potential. A study involving 101 meta-analyses of randomized controlled trials found that combining herbal medications with chemotherapy dramatically increased tumor response rates, survival rates, and quality of life in patients with advanced gastric cancer while also reducing adverse drug effects.[3,4]

Soodha Vallathi Urundai is a classical polyherbal and mineral Siddha formulation traditionally indicated for chronic inflammatory conditions and believed to possess detoxifying and rejuvenating properties, its potential anticancer effects through mechanisms like apoptosis and anti-proliferation, underlining the need for more clinical investigation[5,6]. Though

widely used in clinical practice, the pharmacological mechanisms and anticancer potential of this formulation have not been systematically studied or scientifically validated.

Preliminary ethnopharmacological reports suggest that certain individual components of Soodha Vallathi Urundai possess bioactive compounds with antioxidant, anti-inflammatory, and anticancer activities[7,8]. However, the combinatorial effect of the formulation on malignant cells, particularly gastric cancer cells, remains unexplored.

This study aims to assess the anticancer potential of Soodha Vallathi Urundai in MKN-45P cell lines, focusing on key parameters such as cell viability, apoptosis induction, and modulation of signaling pathways relevant to cancer progression. By leveraging traditional knowledge with modern biomedical research, this work seeks to contribute to integrative oncology approaches for the treatment of gastric cancer.

2. MATERIALS AND METHODS

Preparation of Soodha vallathi urundai (SVU)

To prepare the formulation, 100.8 grams of Senkottai was first taken and subjected to purification as per classical Siddha procedures. 8.4 grams each of Kurosani Omam, Thaneervittan Kilangu, and Nilapanai Kilangu were sun-dried separately and finely ground for six hours to obtain a uniform powder. Once powdered, 100 grams of the purified Senkottai was gradually added to the herbal mixture and ground thoroughly to ensure proper blending. Following this, 8.4 grams each of Soodham (Valai Rasam) and Pooram were incorporated into the blend and further ground into a homogenous paste. The resulting mixture was shaped into 22 pills of equal weight and size, ready for use in subsequent biological assays.

Chemicals and Reagents

Dulbecco's Modified Eagle Medium (DMEM), Penicillin-Streptomycin (PenStrep), Trypsin-EDTA, Ham's F12 nutrient mixture, fetal bovine serum (FBS) and phosphate-buffered saline (PBS) were obtained from Thermo Fisher Scientific (Invitrogen, USA). MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), dimethyl sulfoxide (DMSO), and other high-purity chemicals were sourced from Sigma-Aldrich.

Cell Lines and Culture Conditions

The ATCC supplied the cell lines for this study. Dulbecco's Modified Eagle Medium (DMEM)/Ham's F12 was complemented with 10% heat-inactivated fetal bovine serum, penicillin, and streptomycin. Cells were cultured at 37°C with 5% CO_2 until confluent, then subcultured using a dissociation solution and incubated in 96-well plates.

Cell Cycle Analysis

The cell cycle influence was assessed using Propidium Iodide/RNase buffer staining. Cells (2.5×10^5) were plated in 6-well plates overnight, treated with SVU (0 and 10µg/ml) for 24h, and exposed to RT (0 and 2Gy) for 48h. Post-trypsinization, cells were fixed in 70% ethanol, washed, resuspended in PBS with RNase and propidium iodide, incubated at 37°C, and analyzed by flow cytometry.

Invivo study

Mice

Six-week-old female BALB/c nude mice are obtained from whizbang bio research laboratory, Chennai, and maintained under specific pathogenfree conditions in the K.M.College of Pharmacy,

Madurai(IAEC/REVATHYM/TNMGRMU/Ph.D/M.D(s)/KMCP/137/2021-22). Mice are kept for 1 week in our animal

facility prior to tumor inoculation. All procedures are performed according to the Guidelines for Animal Experimentation of CPCSEA, New Delhi.

Cell lines

MKN-45 human gastric cancer cell lines are utilized, with a variant called MKN-45P that induces ascites and peritoneal spread in vivo, obtained from the Department of Pharmacology, JSS College of Pharmacy and Tamilnadu.

These cell lines are sub cultured in RPMI 1640 medium (Gibco BRL Life Technologies) with 10% fetal bovine serum and an antibiotic-antimycotic solution (amphotericin B, streptomycin, and penicillin) under standard conditions (5% CO2, 37°C). For inoculation, cells in exponential growth are harvested with trypsin-EDTA, producing a single-cell suspension with 90% viability, confirmed via trypan blue exclusion.

Experimental grouping and dosage regimen

The mice were divided into the following groups, with $n = 6$ in each group:

- **Group I** served as the normal control and received a vehicle.

- **Group II** served as the model group and received 1 × 10^6 MKN-45P cells subcutaneously.
- **Group III** received 1 × 10^6 MKN-45P cells subcutaneously along with the Siddha drug. Group III was administered the test drug orally (P.O.) for 14 days. At the end of the experiment, all mice were euthanized, and blood samples were collected to estimate biochemical parameters.

Preparation of murine peritoneal metastasis model

Mice received intraperitoneal injections of MKN-45P cells (5 × 10^6), as reported earlier. [9]. A group of mice (n = 10) received oral administration of the Siddha formulation *Soodha Vallathi Urundai* (200 mg/kg), beginning 5 days after tumor ce For the control group (n = 10), the same volume of PBS was given. As per Hu et al.,[11], there is no notable difference in experimental results when comparing normal IgG and PBS used for control groups.ll injection. This treatment was repeated daily for a total of 10 doses per mouse[10]. For the control group (n = 10), an equal volume of PBS was administered. According to Hu *et al*., [11], there is no significant difference in experimental outcomes between the use of normal IgG and PBS for control groups.

All mice were euthanized on day 18 post-tumor inoculation. Maximum abdominal circumference and ascites volume were measured. To recover peritoneal fluid, 1 mL of PBS was injected. Total number and weight of mesenteric tumors were also evaluated[12,13].

Body weight

Body weights were documented every two weeks during the conditioning phase, before and after exposure to the test substance.

Organ Weight

After completing the experimental protocol and euthanizing the animals, a thorough gross necropsy was conducted on each mouse. The liver, spleen, kidneys, lungs, heart, brain, and testes were excised for weight analysis. Each organ was rinsed with cold saline, dried with sterile paper, and weighed using a calibrated electronic balance with 0.01 g sensitivity. Consistency in dissection was prioritized to reduce errors and tissue damage. Individual organ weights were recorded, and relative organ weights were calculated to account for body mass variations among the animals for statistical analysis.

Haematological Parameters

At the end of the experimental period, all animals were euthanized, and blood samples were collected through cardiac puncture under sterile conditions. The samples were divided into two portions: one for hematological analysis in EDTA-coated tubes and the other for serum biochemical parameters in plain tubes. Hematological parameters such as RBC count, Hb concentration, PCV, MCV, MCH, MCHC, WBC count, platelet count, and ESR were analyzed using an automated hematology analyzer; ESR was measured by the Westergren method. Differential leukocyte counts were conducted manually on stained blood smears, while serum uric acid and CRP levels were quantified using diagnostic kits, all following GLP standards.

Evaluation of LDH and TNF-α in MKN-45P-Induced Gastric Cancer

To evaluate tumor progression and systemic inflammation in a gastric cancer model induced by MKN-45P, serum levels of lactate dehydrogenase (LDH) and tumor necrosis factor-alpha (TNF-α) were assessed in experimental mice. Blood samples were collected post-euthanasia, and serum was separated via centrifugation and stored at −80°C for analysis. LDH activity was measured using a colorimetric assay kit that quantifies the enzymatic conversion of lactate to pyruvate coupled with NAD+ reduction, with absorbance read at 340 nm and expressed in U/L. LDH serves as an indicator of cell membrane integrity and tumor burden due to its elevation in hypoxic environments and correlation with tumor aggression, as noted by Lv et al. (2020)[14]. TNF-α levels were measured using a sandwich ELISA specific for mouse TNF-α, following standard protocols, with absorbance at 450 nm to determine concentrations from a standard curve. TNF-α acts as a marker for inflammation and immune response, reflecting roles in the tumor microenvironment and related signaling pathways, supported by Balkwill (2009)[13]. Measurements were conducted in duplicate for reliability.

Histological Examinations

The stomach was swiftly removed, cut out, and washed in a chilled saline solution. A section of stomach was preserved in a 10% neutral formalin solution, dehydrated using alcohol, and subsequently embedded in paraffin. Sections of 4–5 µm thickness were prepared using a rotary microtome. The sections were colored with haematoxylin–eosin (H&E) dye to examine histopathological alterations

Image analysis

Images are captured for four distinct quadrants of each tumor section at 3,100 magnification. In assessing the vascular structures in peritoneal tumors, accurately counting the vessels is challenging due to their varying shapes. Consequently, we will analyze the CD31-stained vessel region by converting images to grayscale and establishing a uniform threshold for all

slides using Scion software, which is based on the NIH Image program for Windows. The area of the vessels surrounding tumor cells is represented as pixels per high-power field[16].

Statistical analysis

Data are presented as means ± standard deviation. ANOVA and Newman-Keuls tests analyze significance ($P < 0.05$), using GraphPad Prism 3.0 software.

3. RESULT AND DISCUSSION

The results obtained from this study suggest that the test drug has significant cytotoxic activity against MNK-45P cells, highlighting its potential as a therapeutic agent for the treatment of gastric carcinoma. The results demonstrated a dose-dependent decrease in cell viability with increasing concentrations of SVU, reaching complete inhibition (0% viability) at concentrations ≥ 50 µg/mL(Figure1). The calculated IC_{50} value was 15.68 µg/mL for both cell lines, indicating potent anticancer activity. Recent studies have highlighted the efficacy of various natural compounds in inhibiting gastric cancer cell proliferation, showing that polysaccharides from natural products effectively suppress tumor growth and induce apoptosis in lung cancer cells via Toll-like receptor 4-mediated reactive oxygen species generation, endoplasmic reticulum stress , and apoptosis in gastric carcinoma cells[17,18].

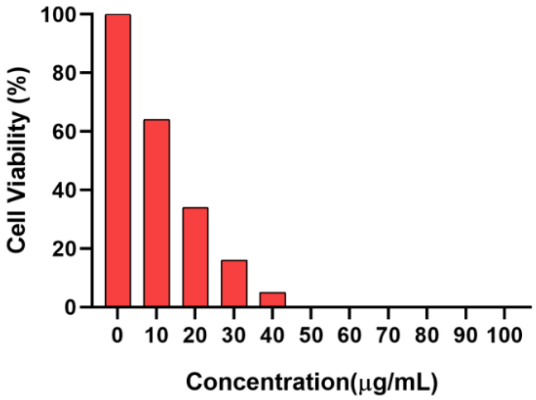
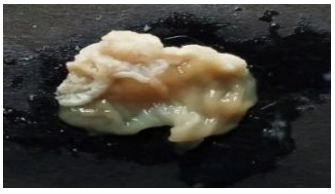


Figure1. Effect of SVU Sample Concentration on MNK-45P Cell Viability (MTT Assay)

Values are expressed as mean ± SEM. Statistical significance (p) calculated *p>0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

The SUV cytotoxicity observed may involve the induction of apoptosis, a mechanism commonly targeted by anticancer agents. Recent research has identified several natural compounds that induce apoptosis in gastric cancer cells, including fucoidan , which inhibited cell proliferation and induced apoptosis in human ovarian cancer cells by modulating the PI3K/Akt signalling pathway.



Group -1 control

Group -2 MKN-45P cell line

Group -3 MKN-45Pcell line +SUV (200mg/kg)

Figure 2. Morphological Evaluation of Soodha Vallathi Urundai on Stomach

Additional studies are required to clarify the fundamental molecular mechanisms and to assess the in vivo effectiveness of the experimental drug in preclinical models (Figure 2). Moreover, combination therapy with standard chemotherapeutic agents could be explored to enhance the therapeutic efficacy and overcome resistance mechanisms.

Body Weight Changes

The mice's body weight was tracked consistently during the experimental duration to assess both general health and potential toxicity of the Soodha Vallathi Urundai (SVU) treatment (Table 1). The initial average body weight of the control group was 34.8 ± 2.07 g, while the group injected with only MKN-45P cells had an average weight of 31.7 ± 3.62 g. Interestingly, the group treated with both MKN-45P cells and SVU (200 mg/kg) started with a lower average body weight of 27.3 ± 1.15 g. At the end of the study, the MKN-45P group showed a significant decrease in body weight (20.5 ± 7.32 g), suggesting cachexia or cancer-associated weight loss. This pattern is consistent with previous reports that describe tumor-induced metabolic dysfunction and systemic inflammation leading to reduced body mass in cancer models[19,20]. The control group also experienced weight loss (19.7 ± 6.36 g), possibly due to non-specific effects such as stress, tumor burden, or the vehicle used for SVU administration.[21]

Table 1. Effect of Soodha Vallathi Urundai n Body Weight Analysis of Normal and Experimental Animals.

Group	Initial Body Weight (g)	Final Body Weight (g)
Control	34.8 ± 2.07	19.7 ± 6.36
Only MKN-45P Cells	31.7 ± 3.62	20.5 ± 7.32
MKN-45P Cells + SVU 200 mg/kg	27.3 ± 1.15	36.3 ± 2.59

Values are represented as mean ± SEM. Statistical significance (p) *p>0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Body weight is the key phenomenon in cancer studies; a marked decrease or increase in body weight suggests the impact of the test drug in experimental groups. In our study, we observed a significant decrease in body weight in Group II (only MNK-45P) compared to the other experimental groups, which serve as a negative control. However, we noted a significant increase in the treated Group III compared to their initial body weight.

Table 2. Effect of Soodha Vallathi Urundai on Organ Weight of Normal and Experimental Animals

Group	Stomach Weight (mg/g)
Control	22.5 ± 7.2
Only MKN-45P Cells	28.2 ± 9.47
MKN-45P Cells + SVU 200 mg/kg	42 ± 3.42

Values are expressed as mean ± SEM .Statistical significance (p) calculated *p>0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

As shown in Table 2, the stomach weight was notably influenced by both tumor induction and Soodha Vallathi Urundai (SVU) treatment. In the control group, the stomach weight was 22.5 ± 7.2 mg/g, which served as a baseline reference for healthy organ mass. Mice injected with MKN-45P cells alone exhibited an increase in stomach weight (28.2 ± 9.47 mg/g), which may reflect local tumor growth, inflammatory infiltration, or edema, commonly associated with gastric tumor development[22]. Strikingly, the group treated with SVU (200 mg/kg) in the presence of MKN-45P cells showed a further increase in stomach weight (42 ± 3.42 mg/g). While initially suggesting pathological hypertrophy, the low standard deviation in comparison to the tumor-only group indicates a regulated tissue response rather than tumor expansion. SVU may promote protective hypertrophy or mucosal thickening as part of a regenerative mechanism. Histopathological analysis is needed to determine if increased weight results from tumor suppression, hyperplasia, or adaptive responses. These findings show SVU's dual role in potentially altering tumor dynamics and impacting organ physiology beneficially, warranting further mechanistic studies. Organ weight reflects tumor volume enrichment, with Group I showing nominal values, Group II increasing due to tumor volume, and Group III increasing due to drug effects on blood and mucous secretion.

Hematological profiles provide essential insights into both disease progression and the systemic effects of therapeutic interventions. In this study, MKN-45P tumor induction and subsequent treatment with the Siddha formulation Soodha

Vallathi Urundai (SVU) led to modest but notable variations in several hematological indices (Figure 3). There was no significant change in RBC count among the groups, suggesting that neither the tumor burden nor SVU treatment induced overt erythropoietic stress or anemia. However, WBC count was significantly elevated ($14.1 \pm 0.754 \times 10^3/\mu L$; $p < 0.05$) in the SVU-treated group compared to control, indicating a possible immune-stimulatory or inflammatory response to the treatment or tumor interaction. This observation aligns with previous studies reporting that herbal immunomodulators, such as Echinacea purpurea and *Withania somnifera*, can stimulate leukocytosis and enhance host defense mechanisms[18]. In the tumor-bearing group, hemoglobin levels and packed cell volume (PCV) were significantly elevated compared to controls, indicating a potential compensatory erythropoietic response to tumor-induced hypoxia[23]. SVU treatment led to a slight decline in these values, suggesting a normalization effect possibly due to reduced tumor burden or systemic stress. Differential WBC counts remained stable, revealing that SVU did not adversely impact immune cell ratios. Mean corpuscular hemoglobin (MCH) was also within normal ranges across groups, indicating intact erythrocyte function. Thus, SVU treatment appears to lack hematological toxicity and may enhance leukocyte activity, supporting its role as a safe immunomodulatory adjunct in cancer therapy. Additionally, increased angiogenesis is vital for tumor growth, with Group II (MNK-45P) exhibiting elevated total hemoglobin levels, while Group III (MNK-45P + SVU 200 mg/kg) showed increased WBC counts, indicating stronger immune activation with SVU treatment.

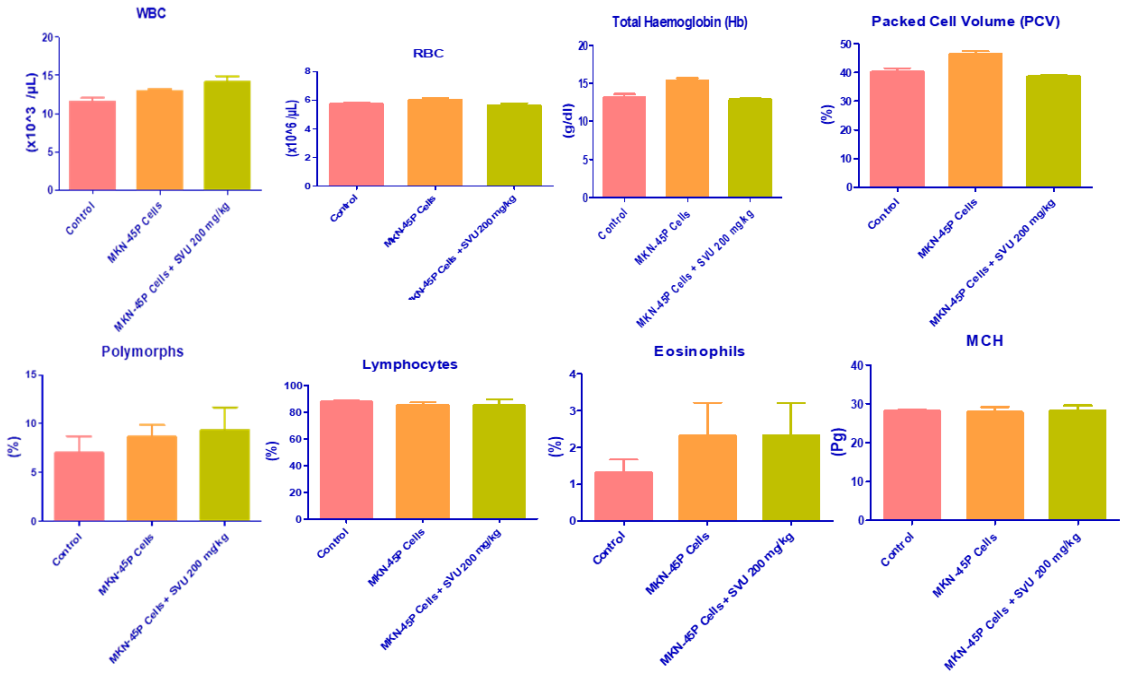


Figure 3. Effect of Soodha Vallathi Urundai on Haematological Parameters of Normal and Experimental Animal

Values are expressed as mean ± SEM. Statistical significance (p) calculated by *p>0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table 3. Effect of Soodha Vallathi Urundai on Biochemical Parameters (Lactic Acid Dehydrogenase) of Normal and Experimental Animals

Group	LDH Activity (IU/L)
Control	0.109 ± 0.00513
Only MKN-45P Cells	**0.226 ± 0.0115** **
MKN-45P + SVU 200 mg/kg	0.136 ± 0.024 (ns)

Values are expressed as mean ± SEM. Statistical significance (p) calculated ,*p>0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Lactic acid dehydrogenase (LDH) is an intracellular key glycolytic enzyme often used as a biomarker of tissue damage or tumor metabolic activity, especially in cancer models that exhibit enhanced anaerobic glycolysis converting pyruvate to lactate. —a phenomenon known as the Warburg effect (Warburg, 1956)[24]. As shown in Table 3, LDH levels were significantly elevated in the MKN-45P tumor-bearing group (0.226 ± 0.0115 IU/L; $p < 0.01$) compared to controls, indicating increased metabolic stress and tumor burden. This aligns with the known association between tumor progression and enhanced LDH release due to cell turnover, necrosis, and altered energy metabolism Elevated LDH levels correlate with increased tumor size, metastasis, and poor prognosis in gastric cancer[25]. Interestingly, the group treated with SVU (200 mg/kg) exhibited a marked reduction in LDH levels (0.136 ± 0.024 IU/L), approaching near-normal levels and showing no statistical significance when compared to the control group. This finding suggests the tumor-suppressive role of SVU, potentially through mechanisms involving reduced glycolytic flux or improved mitochondrial function in tumor cells. The normalization of LDH further supports the anti-cancer potential of SVU, indicating its ability to modulate key biochemical markers of tumor progression without inducing cytotoxicity to healthy tissues.LDH measurement helps assess the efficacy of therapeutic interventions and the progression of the disease

Tumor Necrosis Factor-alpha (TNF-α) is a pro-inflammatory cytokine that plays a dual role in cancer progression. While initially involved in antitumor immunity, persistent elevation of TNF-α is often associated with tumor growth, metastasis, and systemic inflammation, especially in gastrointestinal cancers[26].

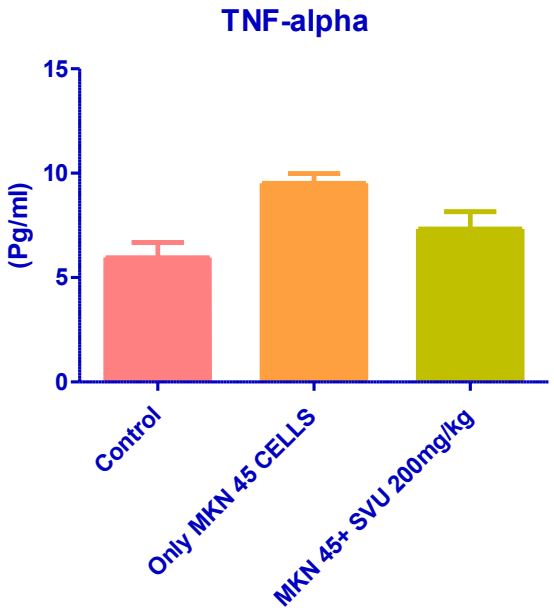


Figure 4. Effect of Soodha Vallathi Urundai on Biochemical Parameters (TNF-A) of Normal and Experimental Animals

Values are expressed as mean ± SEM. Statistical significance (p) calculated by *p>0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

In this study (Figure 4), TNF-α levels were significantly elevated in the MKN-45P tumor group (9.52 ± 0.46 pg/mL; $p < 0.05$), reflecting a pro-inflammatory tumor microenvironment. Elevated TNF-α has been correlated with tumor-induced immune dysregulation, angiogenesis, and promotion of metastatic behavior[27]. However, SVU-treated animals showed a reduction in TNF-α levels (7.33 ± 0.826 pg/mL) compared to the MKN-45P group, though the difference did not reach statistical significance. This trend towards normalization may point to SVU's immunomodulatory or anti-inflammatory effects, potentially mitigating tumor-associated systemic inflammation. The partial reduction in TNF-α further supports earlier observations (from LDH and WBC data) that SVU may play a role in regulating the tumor microenvironment, possibly by suppressing inflammatory mediators or restoring immune homeostasis [28].

4. IMMUNOHISTOCHEMICAL ANALYSIS

To evaluate the impact of the Siddha formulation Soodha Vallathi Urundai on angiogenesis, disseminated tumors (over 3 mm) are randomly selected from each group (n = 6) and subjected to immunohistochemical staining. Formalin-fixed, paraffin-embedded sections undergo staining using a labeled streptavidin-biotin method. Three-micrometer sections are deparaffinized with xylene and rehydrated with ethanol. Endogenous peroxidase activity is blocked by incubation in 0.3%

H2O2, followed by washing in PBS. After blocking nonspecific binding, sections are treated with a mouse monoclonal antibody against CD31. Detection is facilitated by using the Histofine SAB-PO kit. Visualization of reaction products occurs after incubating with a 3,3'-diaminobenzidine solution and counterstaining with Mayer's hematoxylin. Negative controls are included[29, 30].

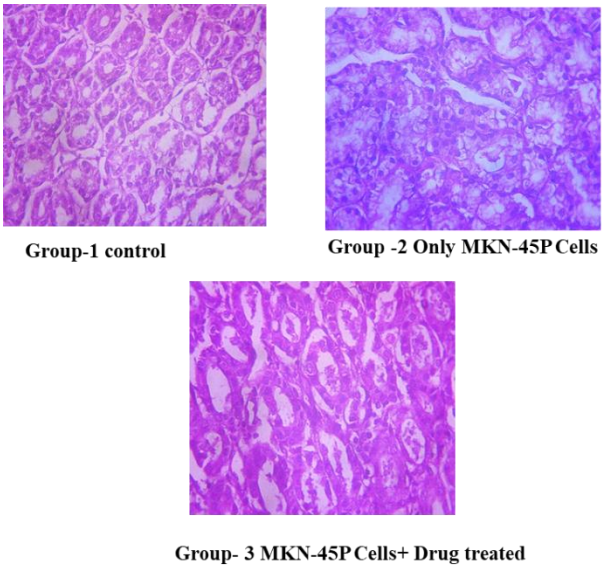


Figure 5. Histopathological Evaluation of Soodha Vallathi Urundai on Stomach Specimens of Normal and Experimental Animals: (Mnk-45p Cell Line-Induced Gastric Carcinoma in Balb/c Mice Model)

Histopathological analysis revealed significant differences among the control and other treatment groups(Figure 5). Group I (control) showed the gastroesophageal junction with non-neoplastic gastric and squamous epithelium, with no trace of neoplasm in other layers of cells. Group II (only MNK-45P) showed the gastroesophageal junction with a gastro-polygonal lesion lined by low-grade neoplastic columnar epithelium and gastric glands containing Paneth and goblet cells. Group III (MNK-45P cell line + SVU 200 mg/kg) showed the gastroesophageal junction with non-neoplastic gastric columnar lining epithelium and underlying edematous submucosa, which also showed scattered inflammatory infiltrates.

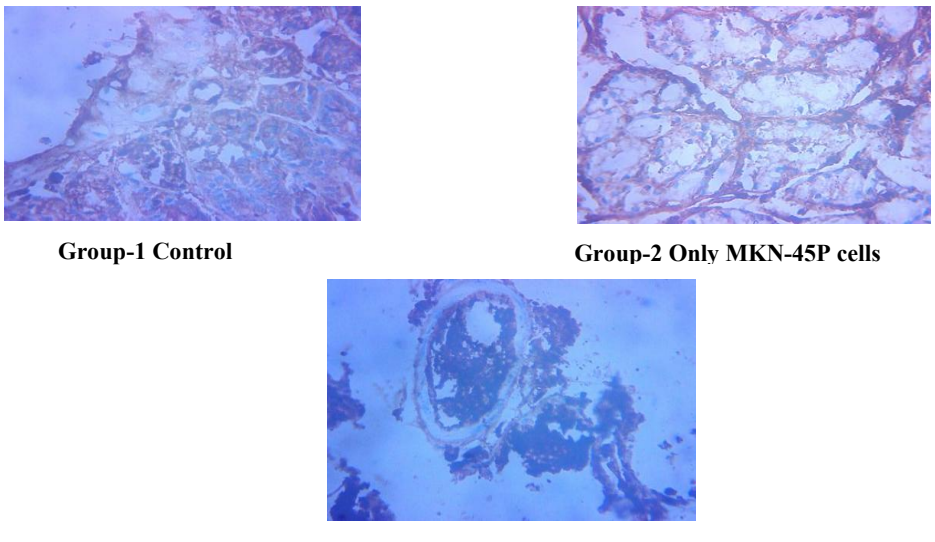


Figure 6. Immunohistochemical Evaluation of Gastric Tissue Markers in Control and Experimental Mice Treated with Soodha Vallathi Urundai (SVU) in a MNK-45P Cell Line–Induced Gastric Carcinoma Model

The CD31 marker for endothelial cells was utilized to assess tumor angiogenesis and invasion capability in the gastric cancer model induced by the MNK-45P cell line (Figure 6). CD31 positivity indicates neovascularization and vascular remodeling

linked to tumors, both of which are essential for cancer progression and spread.

Group I (Control): CD31 staining indicated membranous positivity restricted to normal blood vessels (+), aligning with baseline physiological angiogenesis. No signs of tumor cell involvement were detected, affirming a healthy gastric structure.

Group II (MNK-45P only): CD31 expression was significantly elevated (++), demonstrating strong membranous positivity in both blood vessels and adjacent tumor cells. This signifies active tumor-driven angiogenesis and vascular invasion, essential characteristics of advanced gastric carcinoma.

Group III (MNK-45P + SVU 200 mg/kg): CD31 staining was limited to blood vessels, similar to the control group, yet showed heightened positivity (++), which may suggest a reparative neovascular response absent of tumor cell infiltration. The lack of CD31-positive tumor cells indicates that SVU treatment hindered tumor-induced angiogenesis and invasion, while maintaining normal vascular remodeling

CD31, also known as PECAM-1 (Platelet Endothelial Cell Adhesion Molecule-1), is a widely used immunohistochemical marker for evaluating angiogenesis, vascular invasion, and microvessel density (MVD) in gastric cancer, particularly in peritoneal metastasis. It is present on endothelial cells, making it a reliable indicator for quantifying MVD, which is strongly associated with tumor progression, metastasis, and poor prognosis in gastric cancer[31]. CD31 immunostaining is instrumental in highlighting tumor-associated vasculature, helping identify vascular invasion and neovascularization in metastatic peritoneal implants[32], Journal of Cancer Research and Therapeutics). Additionally, CD31 can help distinguish vasculogenic mimicry (VM), where tumor cells mimic vascular structures, contributing to metastatic potential and resistance to therapies[17]. In a gastric carcinoma model induced by MNK-45P in Balb/c mice, CD31 was employed to assess the effects of a test medication (SVU 200 mg/kg): Group I (control) showed membrane positivity in blood vessels only (+) with no tumor cells; Group II (MNK-45P only) displayed membrane positivity around both tumor cells and blood vessels (++); Group III (MNK-45P + SVU) exhibited membrane positivity confined to blood vessels but with increased vascular presence (++). These findings support the utility of CD31 as a critical marker for tracking tumor angiogenesis, progression, and metastasis in gastric cancer, especially with peritoneal involvement.

5. CONCLUSION

This research highlights the Siddha preparation Soodha Vallathi Urundai (SVU) as a promising anti-cancer agent, particularly against gastric cancer. SVU influenced tumor development and angiogenesis, as indicated by altered CD31 levels, suggesting a potential impact on blood vessel formation. Hematological assessments revealed no significant toxicity from SVU, with increased white blood cell counts and reduced lactate dehydrogenase (LDH) levels, hinting at altered tumor metabolism. Despite signs of tumor-induced cachexia, SVU exhibited protective effects. The findings suggest SVU's therapeutic potential, warranting further investigation into its mechanisms and effectiveness in complex preclinical models for cancer treatment.

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