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# IN-VIVO EVALUATION OF ANTICANCER ACTIVITY OF *VIGNA MUNGO* AND *VIGNA RADIATA*

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## ABSTRACT

*Vigna mungo* and *Vigna radiata* belongs to the very important group of plant food stuffs and is known as legumes. They are rich source of bioactive compounds apart from their nutritional compounds. In the present study seeds of both the plants were selected for the study. Seeds were subjected to the extraction by using ethanol water as a solvent system. Extracts obtained are then fractionated by using column chromatography. Total four fractions were collected. These fractions were subjected to the preliminary phytochemical analysis. Isolated fractions were evaluated for anticancer activity by using modified Murine tumor model. From column chromatography total four fractions were isolated, two from *Vigna mungo* (VMF1 &VMF2) and two from *Vigna radiata* (VRF3 & VRF4). VMF1 and VMF2 was indicating presence of the flavonoids and phenolic compounds in significant concentrations. VRF3 and VRF4 of *Vigna radiata* were indicating the flavonoid and phenolic compounds. In the Murine tumor model, all the results showing that VMF1, VMF2, VRF3 and VRF4 produced mild to moderate effect on tumor inhibition. *Vigna mungo* and *Vigna radiata* fractions were showing anticancer activity. This activity may be due to presence of antioxidant compounds which acts as radical scavengers

**Key words: *Vigna mungo, Vigna radiata*, Murine tumor model, anticancer activity**

## INTRODUCTION

In modern system of medicine, plants are playing very crucial role. Current medicine system still rest on the plant in a significant extent. Many important categories of medicines are obtained from the plants. Plants are considered as a largest segment of biodiversity. Due to plants gene expressions and variation in environment, each plant becomes unique. It is unique for its medicinal value due to its chemical composition. Plants are source of medicine as it contains active constituents. One species or plant can be used for different disorders **[1]**. Plants and animals possess bioactive compounds. These are classified according to different criteria like pharmacological or toxicological effects or chemical nature. Based on their chemical nature these are classified as glycosides, tannins, flavonoids, proanthocyanidins, terpenoids, resins, lignans, alkaloids, coumarins etc **[2]**. Food is obtained from the plants. Foods can be potential source of medicine or ex active principles with pharmacological activity. Foods consist of nutritional constituents and non-nutritional compounds. These components or secondary metabolites are biomolecules which possess the capacity to produce health benefits and maintains health of well-being. These are also known as bioactive compounds and available in small concentrations. Example includes phenolic compounds, flavonoids, organophosphorus compounds, phyto-estrogens, lycopene's, sterols, soluble dietary fibres etc **[3]**. In many parts of the world legumes and pulses are playing crucial role in food stuffs. Legumes are also providing carbohydrates, several water soluble vitamins, and minerals to human nutrition other than proteins **[4]**. Examples of legumes are Mung bean, adzuki bean, rice bean, black gram, etc **[5]**. Legumes are rich in bioactive compounds like phytoestrogens, flavonoids and other. Reported literature suggested that legume phytoestrogens and saponins have anticancer and hypocholesterolemic action. Saponins found to be effective in colon cancers. Application of food in treatment of diseases will improve in the future. Currently many scientists are in search of novel compounds having pharmacological potential. These compounds could be helpful for development new therapeutic agents or pharmaceutical excipients or ingredients of dietary supplements derived from natural products **[6]**.

*Vigna mungo* is one of the legume crop extensively cultivated in India. It is called as black gram in English, Masha in Sanskrit. Black gram is very important part of Indian

food. It is used in the form of cooked dhal, idli, hopper, papad and waries. Black gram seeds consist of moisture, proteins, fats, fibers, carbohydrates and minerals **[7]**. *Vigna radiata* is commonly called as Mung bean in Hindi and Bengali. It is called as Green gram in English, Moongi in Punjabi language. It is consumed as food in many countries from more than 3500 years. Mung bean is commonly consumed as food in Asian countries like India, China, Bangladesh and many western countries. Black gram and green gram are rich in bioactive components. Reported bioactive components are flavonoids, isoflavonoids, phytoestrogens, phenolic acids, enzymes, saponins, trypsin inhibitors, phytic acid, lectins, neutral detergent fibre, proteinase inhibitors, tocopherols, fatty acids, proteins and minerals. These bioactive components have been reported to produce different activities like anticancer, antioxidant, antimicrobial activity, anti-inflammatory, hypolipidemic, hypoglycemic, estrogenic, antiestrogenic, antiviral and antifungal activity **[8]**.

Genus vigna consists of different species. Anticancer effects of *Vigna unguiculata, Vigna angularis, Vigna subterranea etc.* on different cell lines were reported. Detailed review of literature suggested that polyphenols, flavonoids, trypsin inhibitors etc. are responsible for anticancer activities. Review of bioactive compounds of black gram and green gram suggested the presence of phytoconstituents like flavonoids, phenolic compounds, trypsin inhibitors etc. which is having anticancer properties. Therefore two legumes grown and consumed in India on large scale are green gram (*Vigna radiata*) and black gram (*Vigna mungo*) were selected for this study. Cancer is amongst the principal reasons (second leading) of death worldwide. Globally, about 1 death out of 6 deaths is due to cancer. In Indian women breast cancer is the most common cancer. One woman dies out of every 2 women newly diagnosed with breast cancer. Cervical cancer is the second largest cancer in India responsible for 22.86% of all cancer. In Indian men oral cancer is the most common. Tobacco leads to mortality of 3500 persons every day. Breast cancer is the most common in USA followed by lung and bronchus cancer. The fresh cancer cases per year are predicted to rise to 23.6 million by 2030. As per the WHO survey most common cancers are leading to the higher deaths are includes lung cancer, breast cancer and colorectal cancer. Recent cancer treatments have known for its adverse effects hence, there is need to identify compounds with less side effects and potent anticancer activity. Natural products

could be suitable choice for anticancer treatment. It is well known that natural products derived from plant and animals worked as source of compounds with good pharmacological properties including anticancer drugs. Aim of the present investigation is to isolate phytoconstituents from the legume plants like green and black gram and evaluate for the anticancer potential.

## METHODOLOGY

### Collection and Authentication of Plant Material:

Seeds of *Vigna mungo* and *Vigna radiata* were collected from local farmers located in Markal area near Pune, Maharashtra. Herbarium of the both the plants were prepared and sent to the Botanical Survey of India, Pune for authentication.

### Extraction:

Seeds of both the plants were collected and washed properly with the water. Foreign organic matter was separated. Seeds were then coarsely powdered. Coarse material was first defatted and then subjected for extraction. Continuous hot extraction method was used by using ethanol water solvent system (80:20) Hydro-alcoholic extracts were collected and concentrated by using Rotary Vacuum evaporator. Dried extract was kept in desiccator for storage.

### Isolation of fractions

Extract obtained from both the plants were subjected to column chromatography. Glass column was taken and stationary phase was prepared by pouring slurry of Silica Gel 60-120 in n-hexane solvent. Air bubbles were removed by tapping on column. Before pouring slurry cotton wool and 1cm thick Silica was added. After silica loading sample was added in column. Sample was prepared by mixing it with silica gel and then trituration. Initially n hexane was used then n hexane and chloroform in different ratios (70:30, 10:90) were added. Column was eluted with chloroform (100%). Further solvent polarity was changed using Chloroform and methanol in different ratios (80:20, 50:50, 30:70, 100). Different fractions were collected and labeled for the further analysis. During separation colored compounds were separated by visual identification. Colorless compounds were identified by UV and thin layer chromatography. Fractions with similar properties were mixed together and evaporated in rotary vacuum evaporator to isolate fraction.

### Phytochemical analysis of isolated fractions

Chemical constituents present in isolated fractions were identified by preliminary

phytochemical tests. Test for Glycosides, saponins, alkaloids, phenolic, flavonoids etc. were performed according to the procedure mentioned in the standard books.

**Acute Toxicity Study**

These were conducted as per the internationally accepted protocol drawn under the OECD guidelines in Swiss albino mice. Groups of animals were made. Each group had 6 animals. They were treated with all isolated fractions which include VMF1, VMF2, VRF3, and VRF4. Separate control group was prepared.

**Murine Tumor Model:**

Female Wistar Albino rats 7 to 8 weeks old and weighing around 150-200g was divided in to seven groups, each group containing six animals. In positive control group breast cancer will not be induced. Negative Control group with breast cancer was not received any treatment. Test group with breast cancer was received test samples. Tumor was induced by Injection of $1x10^6$ viable cells of MCF 7 cell lines at fourth position of inaugural mammary fat pads. After 7 days growth of tumor was observed continuously for 15 days. Upon induction, animals were treated orally for 21 days. Suspensions of standard paclitaxel and test samples were prepared with 0.5 % CMC. In this study dose of 200 and 400 mg/kg was selected. Following are the evaluation parameters which were studied during the animal study

**Evaluation parameters:**

a. General Behavioral studies

b. Feed and water consumption: The quantity of food consumed by rats in each cage was recorded on the day of commencement of treatment and twice a week thereafter.

c. Body weight: The body weights of each animal were recorded during pre-treatment, on the day of commencement of the treatment and daily thereafter to monitor the development of the tumor.

d. Assessment of the activity: The inhibitory rate (%) against growth of tumor was calculated.

e. Mean Survival Time: Mortality throughout the study all animals was supervised twice a day to look for dead or moribund to allow necropsy and gross pathological examination was carried out.

f. Hematological Parameters: On 0 day and 22nd day the animals were anesthetized with anesthetic ether, 1ml blood was collected by retro orbital puncture for evaluation of following parameters,

1. Hb count
2. Complete blood count

g. The animals were euthanized with overdose of Ketamine more than 30mg/kg

and mammary fat tissues were isolated for further evaluation.

h. Histopathological studies: Hematoxylin & Eosin (H & E) stained slides of liver, kidneys, lungs, heart, spleen and brain tissues were taken. These slides were examined under microscope by Pathologist to note Histopathological lesions, if any. Severity of the observed lesions were recorded as 0= No abnormality detected, 1= Minimal (<1%), 2= Mild (1-25%), 3= Moderate (26-50%), 4= Moderately Severe/Marked (51-75%), 5= Severe (76-100%). Distribution of the lesions was recorded as focal, multifocal and diffuse.

**Statistical Analysis**

All the experiments were performed in triplicates. Data were represented as mean ± SEM values. Statistical analysis of the data were performed by one-way ANOVA (Graph Pad Prism 8.1.1, Graph Pad Software, Inc., California) followed by Tukey's test.

**RESULTS AND DISCUSSION:**

**Identification and authentication of plant material**

Plant specimens were identified as *Vigna radiata* L. R. Wilczek with voucher specimen number DJS 04 and *Vigna Mungo* L. Hepper with voucher specimen number DJS 05. Both plants belong to the Fabaceae family.

**Extraction and isolation of fractions**

Total four fractions were collected from the *Vigna mungo* extract. Fraction no F2 and F4 shown single spot on TLC and were present in significant quantity and hence those two fractions were selected for the further study and labeled as VMF1 and VMF2 **(Table 1)**. From *Vigna radiata* fractions, fraction no F2 and F3 were selected for the further studies **(Table 2)**.

**Preliminary phytochemical analysis**

Preliminary phytochemical tests suggest that VMF1 and VMF2 was indicating presence of the flavonoids and phenolic compounds in significant concentrations. VRF3 and VRF4 of *Vigna radiata* were indicating the flavonoid and phenolic compounds.

**Acute toxicity study**

Acute toxicity study assessment showed that VMF1, VMF2, VRF3 and VRF4 did not produce toxic effect in the mice. All fractions were found to be safe and no mortality was observed up to 2000mg/kg. Therefore dose of 100, 200 and 400 mg/kg was selected.

**Effect of isolated fractions on body weight of animals**

Effect on body weight was determined at different phases of experiment **(Figure 1)**. Body weight was determined before injection of cell lines in the animals. It was considered as a normal. After injection of cancer cell lines again weight was determined and then

after treatment of the extracts. It was observed that weight was significantly reduced after cell line injection. After treatment of isolated fractions and standard body weight was brought back to the normal. *Vigna mungo* fractions were administered orally to the animals. VMF1, VMF2 was showing increase in body weight while VRF3 and VRF4 also showed positive effect. Determination of effect on organ weight during study is good screening tool during the studies. Change in organ weight is good indicator of health of organism. Cancer shows significant decrease in muscle and body mass **[9]**. Isolated fractions showing improvement in the body mass.

**Effect of isolated fractions on feed consumption of animals**

Effect of treatment was studied by determination of the feed consumption on animals **(Figure 2)**. Negative control was showing continuous decrease in feed consumption. Other groups after treatment with extracts were indicating improvement in consumption of feed. All fractions showing improvement in feed consumption. VMF1 among all fractions showed highest improvement in the feed consumption.

**Effect of isolated fractions on blood parameters**

**Effect of isolated fractions on water consumption of animals**

All groups when treated with cancer cell lines showed significant reduction in the water consumption. All the groups after fraction treatment showed improvement in the water consumption **(Figure 3)**.

After injection of cell lines WBC count was increased than the normal values **(Figure 4)**. It was also observed that RBC count, Hb count and other constants were decreased when treated with the cell lines. There was significant increase in lymphocyte concentration after cell line injection. It was observed that after treatment of fractions WBC count and lymphocyte count was decreased and brought back to the normal values. While Hb counts, RBC count values were increased after treatments.

**Effect of isolated fractions on weight of vital organs**

Effects of cancer cell lines on weight of vital organ were studied. Reduction in the weight of vital organs was observed after treatment of cell lines. Isolated fractions were showing improvement in the reduced weight in cancer induced animals **(Figure 5)**.

**Effect of isolated fractions and standard drug on tumor cells**

In **Figure a and b** multifocal mild neutrophilic/lymphocytic infiltration at

dermis and subcutis is observed **(Figure 6)**. Multifocal mild neovasularization in dermis and subcutis is noted which indicated Mild inflammation with neovasularization. In figures c, d and e large tracts of tumor cells with little stroma with fibrous tissue at subcutis is observed. Diffuse nuclear pleomorphism (variations in nuclear size and staining), without evidence of gland formation is noted. Increased nuclear cytoplasm ratio with eosinophilic cytoplasm was seen. Mitotic figures are observed (1-2/3 hpf) Multifocal moderate lymphocytic infiltration at tumor area (2+) and multifocal moderate neovasularization (3+) observed.

It showed low grade Solid Mammary Adenocarcinoma. When compared with rats of negative control group, Standard drug, VMF1, VMF2, VRF3 and VRF4 treated mice revealed reduced size, distribution and severity of tumor area. Further, increased necrosis at tumor site of treated rat suggests mitigatory effect. All groups shows moderate to mild cytotoxic activity.

**(Figure 7)** Microscopic examination of all the tissues of rats positive control group did not revealed any lesion of pathological significance. Rats of negative control group showed multifocal mild cytoplasmic vacuolations at hepatocytes of liver and tubules of kidneys suggestive of injection of test cell line produce degenerative lesions in liver and kidneys of mice. All the observed tissues of rats treated with Standard drug, VMF1, VMF2, VRF3 and VRF4 did not revealed any lesion of pathological significance and metastasis of tumor. Diffusely severe tract of neoplastic cells spread across complete subcutaneous area with diffuse moderate stroma along with fibrous tissue is observed in negative control group mice. Moderate cellular pleomorphism with round to oval nucleus and pink cytoplasm, moderate anaplasia and increased mitotic **Figures (7-10)** is also noted. Focal mild necrosis and diffuse moderate inflammation at tumor site with neovasularization is seen in positive control mice.

VMF1, VMF2, VRF3 and VRF4 were showing mild to moderate effect on tumor inhibition. *Vigna mungo* and *Vigna radiata* fractions are showing anticancer activity. Daily food habits and the drugs or medicines are vital in making sick body well. Diet of individual is proved to be helpful in prevention of different cancers **[10]**. Diet is most important factor in reducing cancer risk. Evidences suggest diet with natural phytoconstituents decreases risk for cancers **[11]**. It was reported that for adjuvant cancer treatment compounds with antioxidant

potential can be used as an adjuvant. This is related to their ability to produce apoptosis. Synthetic anticancer drugs produce side effects which are highly toxic and damage the normal cells. Flavonoids are showing lack of significant side effect. They are having inherent biological activity. This makes them ideal candidates for new agents. Flavonoids produce cytotoxic effects by various mechanisms. They can penetrate in cultured cells and modulate metabolic activities of the cell. They can act by inhibiting damage caused by oxidation, and lead to deactivation of carcinogenic compounds. They may cause cell cycle arrest and produce apoptosis. Polyphenol compounds may interact with enzymes and produces cytotoxic effects. Apoptosis is important part in tissue homeostasis. In cancer there is decrease ability of human cells to undergo apoptosis. External stimuli may stop apoptosis. Induction of apoptosis in cancer cells is a promising approach to develop new anticancer agent. Flavonoids have ability to induce apoptosis in cancer cells. Cell cycle arrest at G1/S or G2/M phase by alteration of regulatory proteins of the cells is caused by flavonoids. Many studies have revealed that antioxidant activities of polyphenols may contribute to the anticancer activities. Oxidative stress is higher in cancer cells than normal cells. Agents which scavenge ROS may kill cancer cells. Depending upon the structure and class flavonoids display different sensitivity and selectivity to the cancer cells. Effect of flavonoids on cancer cells is depends on the etiology of cancers. Sak reported various flavonoids for treatment of breast cancers. These includes quercetin, fisetin, galangin, kaempferol, rutin, naringenin, hesperidin, apigenin, luteolin, chrysin, tangeretin, wogonin etc **[12]**. Cytotoxic effect of fractions of *Vigna*

*mungo* and *Vigna radiata* is possibly due to presence of flavonoids and phenolics in isolated fractions **[13]**. Present study confirms that the fractions isolated from both the plants have phytoconstituents like flavonoids that have anticancer activity. Literature also suggesting that the phenolic and flavonoid are compounds responsible for anticancer activity. **Table 3** shows antitumor effect of control, negative control, standard, isolated fraction groups by studying extent of neoplastic cell, pleomorphism, anaplasia, mitotic figures, extent of necrosis, extent of inflammation, stromal reaction, and neovasularization.

**Table 1: Fractionation of *Vigna mungo* EE extract in Column chromatography**

| Sr. No | Mobile Phase | Ratio | Fraction | No of spots | Description | Rf value |
|---|---|---|---|---|---|---|
| 1 | n-hexane | 100:0 | F1 | 3 | Green color | 0.72, 0.21, 0.35 |
| 2 | n hexane: chloroform | 70:30 | 0 | --- | --- | --- |
| 3 | n hexane: chloroform | 10:90 | 0 | --- | --- | --- |
| 4 | Chloroform | 100:0 | 0 | --- | --- | --- |
| 5 | Chloroform: MeOH | 80:20 | F2 | 1 | Light yellowish/ greenish | 0.344 |
| 6 | Chloroform: MeOH | 50:50 | F3 | 2 | Colorless | 0.56, 0.81 |
| 7 | Chloroform: MeOH | 30:70 | F4 | 1 | Yellowish/cream colored | 0.86 |
| 8 | MeOH | 100:0 | 0 | 0 | --- | --- |

**Table 2: Fractionation of *Vigna radiata* EE extract in Column chromatography**

| Sr. No | Mobile Phase | Ratio | Fraction | No of spots | Description | Rf value |
|---|---|---|---|---|---|---|
| 1 | n-hexane | 100:0 | F1 | 2 | Greenish | 0.52, 0.34 |
| 2 | n hexane: chloroform | 80:20 | 0 | --- | --- | --- |
| 3 | n hexane: chloroform | 50:50 | 0 | --- | --- | --- |
| 4 | n hexane: chloroform | 30:70 | 0 | --- | --- | --- |
| 5 | Chloroform | 100:0 | 0 | --- | --- | --- |
| 6 | Chloroform: MeOH | 90:10 | 0 | --- | --- | --- |
| 7 | Chloroform: MeOH | 60:40 | F2 | 1 | Whitish yellow | 0.45 |
| 8 | Chloroform: MeOH | 20:80 | F3 | 1 | Yellowish cream colored | 0.61 |
| 9 | MeOH | 100:0 | 0 | 0 | --- | --- |

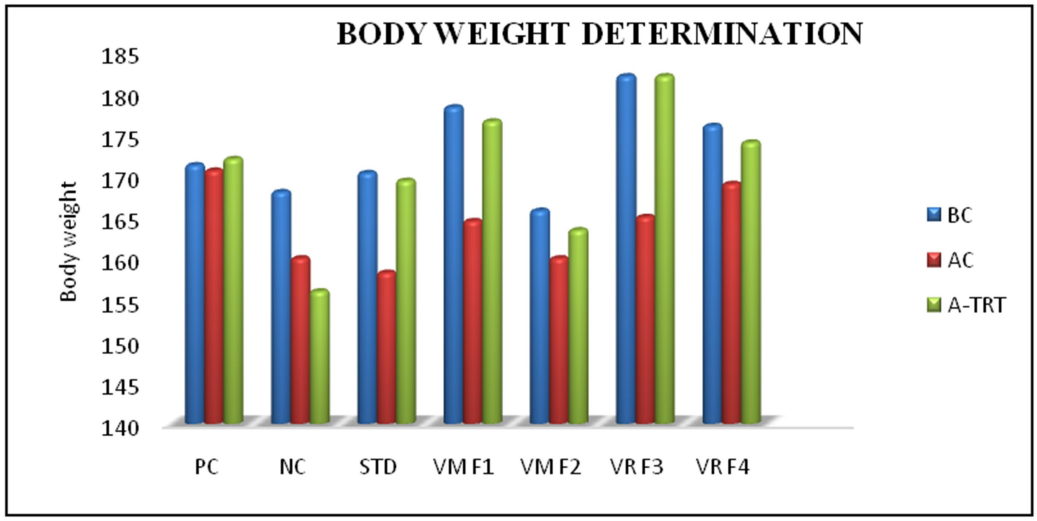



**Figure 1: Effect of isolated fractions on body weight of animals**

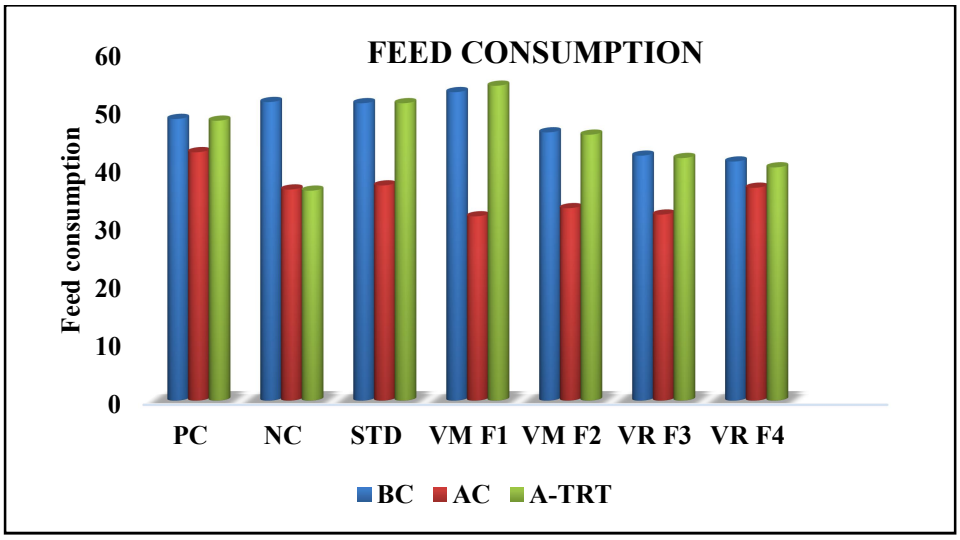



Figure 2: Effect of isolated fractions on feed consumption of animals

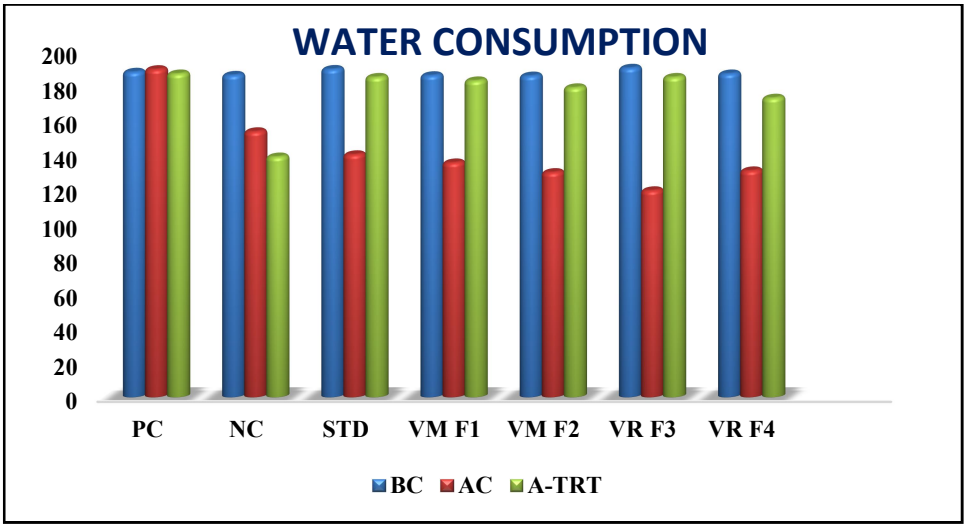



Figure 3: Effect of isolated fractions on water consumption of animals

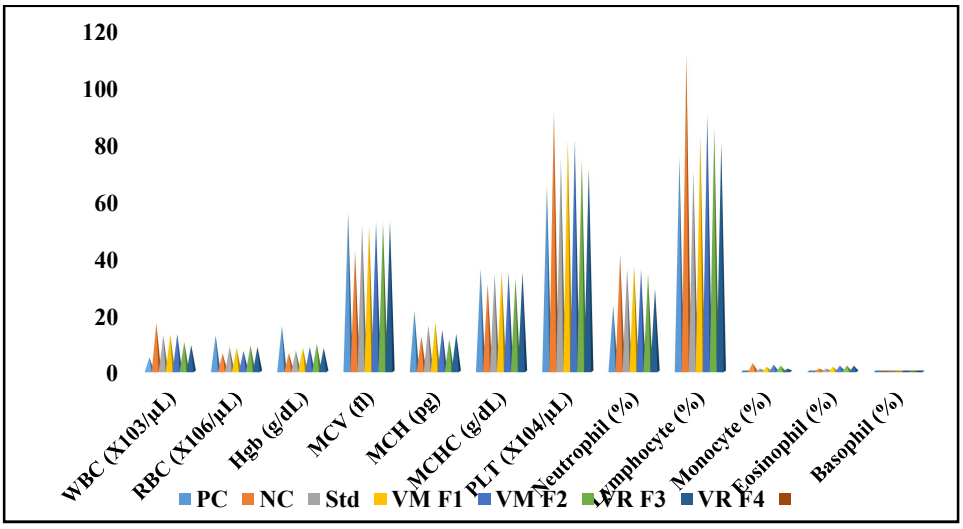



Figure 4: Effect of isolated fractions on blood parameters

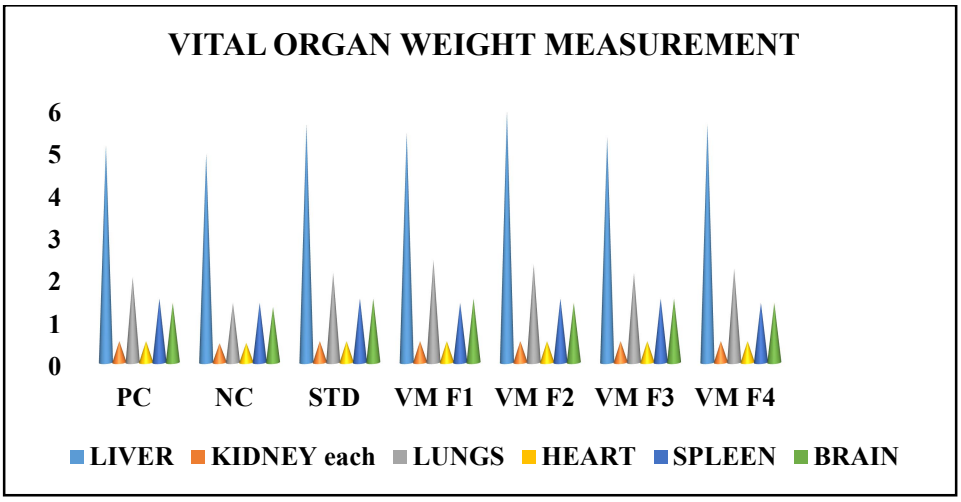
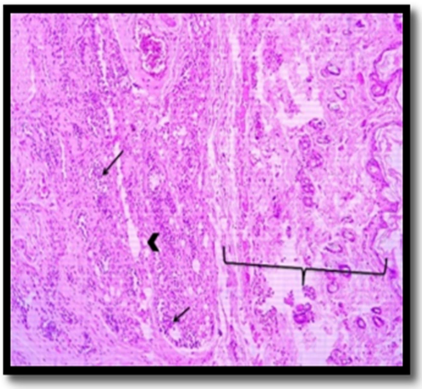
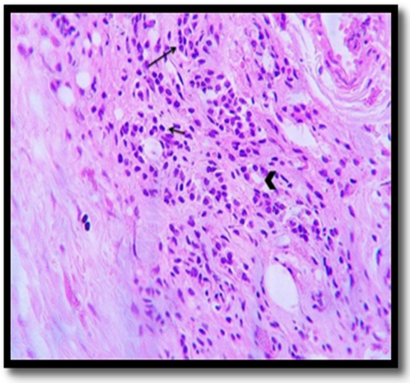


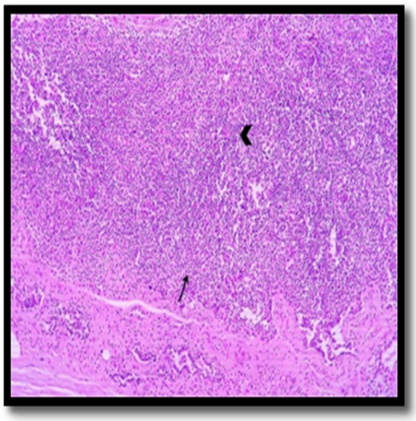

**Figure 5: Effect of isolated fractions on weight of vital organs**



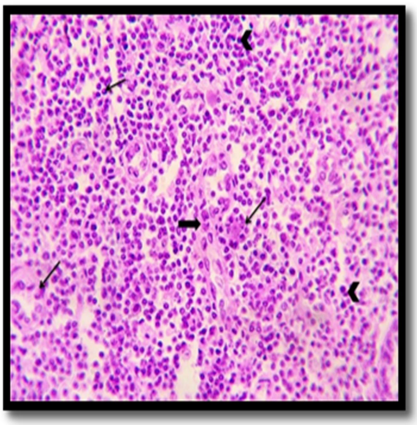
**a.** **Neutrophilic/lymphocytic infiltration (arrow) and neovasularization (arrow head).**



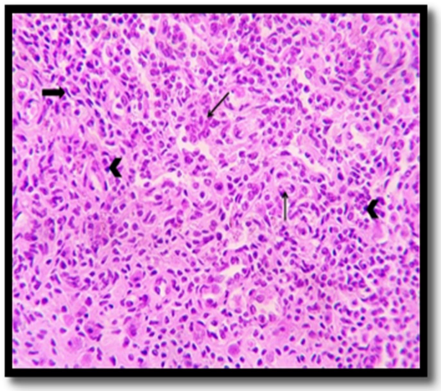
**b.** **Neutrophilic/lymphocytic infiltration (arrow)and neovascularization (arrow head).**



**c.** **Showing complete section of solid tumor at subcutis (arrow) and note prominent lymphocyte infiltration at tumor area (arrow head)**

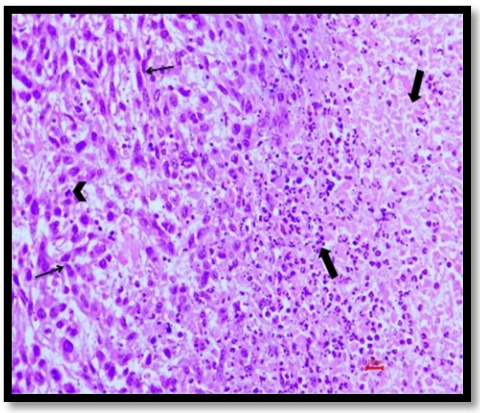


**d.** **Showing tumor cells (arrow), neovasularization(Large arrow) and lymphocyte infiltration (arrow head)**

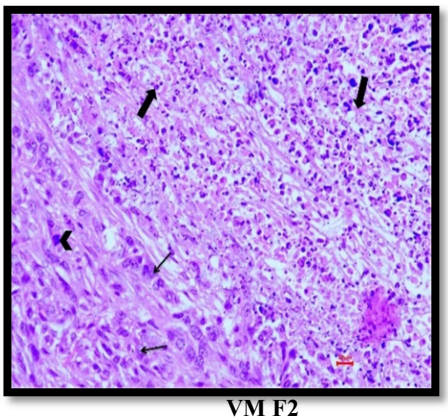


**e. Showing tumor cells (arrow), fibrosis (Large arrow) and lymphocyte infiltration (Arrow head)**

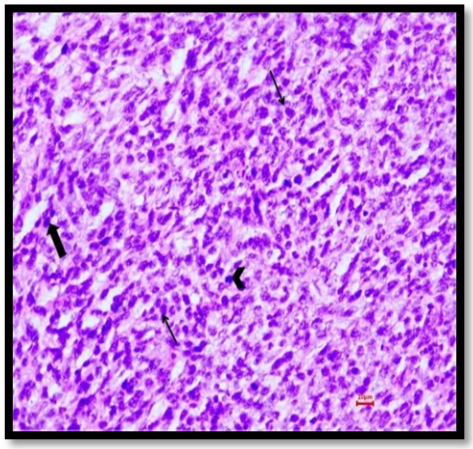
**Figure 6: Initial histopathological observations for development of the tumor**



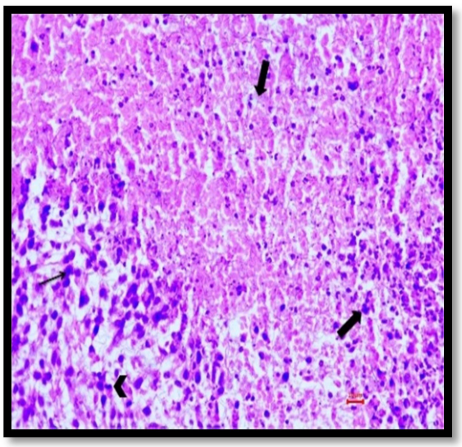
**VM F1**
**Showing neoplastic cells (small arrow) with stromal reaction, mitotic figures (arrow head), inflammation and necrosis (large arrow)**



**VM F2**
**Showing neoplastic cells (small arrow) with stromal reaction, mitotic figures (arrow head), inflammation (large arrow)**



**VR F3**
**Showing neoplastic cells (small arrow) with stromal reaction, mitotic figures (arrow head), inflammation and necrosis (large arrow)**



**VR F4**
**Showing neoplastic cells (small arrow) with stromal reaction, mitotic figures (arrow head), inflammation and necrosis (large arrow)**

**Figure 7: Effect of isolated fractions and standard drug on tumor cells**

**Table 3: Summary of microscopic observations of isolated fractions**

| Group | Extent of neoplastic cell | Pleomorphism (Cell / Nucleus Morphology) | Anaplasia | Mitotic figures | Extent of necrosis | Extent of inflammation | Stromal reaction | Neovasularization |
|---|---|---|---|---|---|---|---|---|
| **Positive Control** | **No abnormality detected** | | | | | | | |
| **Negative Control** | **Tracts of tumor cells spread across subcutaneous area, Diffuse severe** | **Moderate with round to oval nucleus & pink cytoplasm** | **Moderate** | **7-10/hpf** | **Focal mild** | **Diffuse moderate** | **Stroma with fibrous tissue, Diffuse, moderate** | **Multifocal moderate** |
| **Standard** | **Tracts of tumor cells spread across subcutaneous area, Multifocal mild** | **Mild with round to oval nucleus & pink cytoplasm** | **Mild** | **2-3/ 2 hpf** | **Diffuse severe** | **Focal mild** | **Stroma with fibrous tissue, Multifocal, mild** | **Multifocal mild** |
| **VM F1** | **Tracts of tumor cells spread across subcutaneous area, Multifocal mild** | **Mild with round to oval nucleus & pink cytoplasm** | **Moderate** | **3-5/hpf** | **Diffuse severe** | **Multifocal mild** | **Stroma with fibrous tissue, Multifocal, mild** | **Multifocal mild** |
| **VM F2** | **Tracts of tumor cells spread across subcutaneous area, Multifocal severe** | **Mild with round to oval nucleus & pink cytoplasm** | **Moderate** | **4-6/hpf** | **Multifocal mild** | **Multifocal moderate** | **Stroma with fibrous tissue, Multifocal, mild** | **Multifocal moderate** |
| **VR F3** | **Tracts of tumor cells spread across subcutaneous area, Multifocal mild** | **Mild with round to oval nucleus & pink cytoplasm** | **Moderate** | **3-5/hpf** | **Multifocal moderate** | **Focal mild** | **Stroma with fibrous tissue, Multifocal, moderate** | **Multifocal mild** |
| **VR F4** | **Tracts of tumor cells spread across subcutaneous area, Multifocal mild** | **Mild with round to oval nucleus & pink cytoplasm** | **Moderate** | **4-7/hpf** | **Diffuse moderate** | **Focal mild** | **Stroma with fibrous tissue, Diffuse, mild** | **Multifocal mild** |

## CONCLUSION

Natural bioactive compounds were isolated from the some plants of genus vigna. In the Murine tumor model, all the results showing that VMF1, VMF2, VRF3 and VRF4 produced mild to moderate effect on tumor inhibition. *Vigna mungo* and *Vigna radiata* fractions are showing anticancer activity. This activity may be due to presence of an antioxidant compound which acts as a radical scavenger. It is also due to apoptosis induction of cancer cells via mitochondrial pathway. These isolated compounds showed significant anticancer activity in the *in vivo* studies.

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