ANTICANCER ACTIVITY AND CYTOTOXICITY OF MORINGA OLEIFERA AND CALOTROPIS PROCERA: AN IN VITRO STUDY

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Abstract : - Many medicinal plants are used as chemo preventive and antitumor agents in various experimental models of malignancies. Moringa oleifera and Calotropis procera is a plant that contains various phytochemical agents, which have been used for medical purposes including analgesic, antihelmintic, anti-hyperglycemic, hepatoprotective, and antimicrobial, anti-fertility, among others. Moringa oleifera seeds and Calotropis procera leaf extracts have also been proposed as potential anticancer agents. Cancer is one of the leading causes of death all over the worldwide. Few reports reveal the anticancer activity of Moringa oleifera and Calotropis procera leaf extract in cancer cells. We investigated the effect of both drug extract in colon and liver cancer cell lines. We hypothesized that both drug extracts will inhibit the growth of cancer cells. The Moringa oleifera seeds and Calotropis procera leaf extract was tested in colon and liver cell lines for a period of 24, 48, and 72 hrs of incubation and compared with the positive control which was Vinblastine. The proliferation rate of Caco2 (human colon carcinoma) and Hepg2 (human liver carcinoma) cell after treatment with different concentrations of extracts were determined using the colorimetric 3-(4,5-dimethylthiazol-2-yl)- 2,5- diphenyl tetrazolium bromide (MTT) assay. The concentration inhibiting 50% of cell growth (IC50) was calculated

Keywords- Calotropis procera, Moringa oleifera, Colon cancer, Liver cancer, inhibitory concentration,

Introduction - Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. In the United States, as the leading cause of death, it accounts for 25% of all the deaths in humans presently. It is considered as an adversary of modernization and advanced pattern of socio-cultural life dominated by Western medicine. Multidisciplinary scientific investigations are making best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine. Recently, a greater emphasis has been given towards the researches on complementary and alternative medicine that deals with cancer management. Several studies have been conducted on herbs under a multitude of ethno botanical grounds. *Ayurvedic aspects of Cancer (Arbuda)*- The disease *Arbuda*[i] was prevalent during the *Vedic* period. In *Atharvaveda*[ii], there is reference of *Arbuda* and its management. *Arbuda* is one of the surgical diseases and was explained in detailed by *Acharya Sushruta* the pioneer of ancient *Ayurvedic* texts that have remarkable similarities with modern interpretation of cancer[iii]. While searching the literature one finds that *Arbuda* is the nearest clinical correlated with the externally visible cancerous growth. Many diseases can be correlated with *Arbuda* but Cancer is a parallel disease for it. The signs and symptoms of *Arbuda* can be very well explained in terms of Cancer. *Sushruta Samhita*[iv] and *Charaka Samhita*[v], two well-known classics, have described cancer as inflammatory or non inflammatory swelling and mention them as either *Granthi* or *Arbuda*. *Acharya Madhava* while describing *Arbuda* opines that the vitiated *Doshas* afflict the *Mamsa* and *Rakta*[vi] both to produce a swelling. *Sushruta Samhita*, *Charaka Samhita* as well as *Astanga Hridaya*[vii], which was written much later, give very detailed description about this subject. The *Doshas* get vitiated in any part of the body and afflicting the *Mamsa dhatu* later produce swelling. This lesion is circular, fixed, slightly painful, big in size, broad based, slowly growing and it does not suppurate; same is called *Arbuda*. These clinical symptoms which are parallel for malignant growth are correlated with cancer. There are no specific references available in classics regarding direct correlation of colon cancer and liver cancer. But *Ayurveda* has different approach toward disease as *Acharya Charaka* explains it is not necessary to specify each disease with their specific name it may differ as per location, shape and size. The diagnosis of any *Anukta* disease can be done on the basis of *Dosha*, *Dushya*, & *Srotas*. The helpful methodology of *Ayurveda* has been partitioned into four classifications as *Prakritisthapani Chikitsa* (health maintains), *Roganashani Chikitsa* (disease fix), *Rasayana Chikitsa* (reclamation of typical capacity) and *Naishthiki Chikitsa* (profound methodology)[viii]. Finding the reason for a sickness is the fundamental objective of *Ayurvedic* treatment. There are no specific references available in classics regarding direct management of colon cancer and liver cancer. But by avoiding cause (*Nidana Parivarjana*) and by following *Samshodana Chikitsa (* purification process*) Rasyana Chikitsa (*rejuvenating process*)* one can increase the life span.

Need of Anticancer Study- Medicinal herbs are important for cancer treatment due to their multiple chemical compounds for discovering new active materials against cancer[ix].

ShigruBeeja - is herbal drug which has *Ushna*, *Tikshanaguna*, *Madhur*, *Tiktarasa* and *Katuvipaka* along with its alkylating properties (*Kshara*).Various *Acharya* mentioned *Shigru* in context of diseases like *Arbuda*, *Granthi*,*Apchi*, *Galganda*, *Gulma*,*Udarroga* which is co-related with cancerous growth.

Arkapatra -is also having *Laghu, Ruksha, Tikshnaguna* and *KatuVipaka* along with its alkylating properties (*Kshara*).*Arka* is also given in the context of diseases like *Arbuda, Granthi,Apchi, Galganda, Gulma,Udarroga* which is co-related with cancerous growth. Hence, plant derived compounds can serve as a source for anticancer therapy. Therefore *Shigrubeeja* & *Arkapatra*has been selected for In-Vitro anticancer activity.

Aims &Objectives-

1. To study the anticancer activity of *Shigrubeeja (Moringa oleifera seed)*

2. To study the anticancer activity of *Arkapatra (Calotropis procera leave)*

3. To compare the anticancer activity of *ShigruBeeja* and *Arkapatra*.

4. To evaluate, elaborate & discussion of cancer as per modern and *Ayurveda.*

Material method-

Plant material–*Calotropis procera leaf* (*Arkapatra*) and *Moringa oleifera seed (shigrubeeja)* was collected seasonally, authenticated by a plant taxonomist in Department of *Dravyaguna* NIA Jaipur Rajasthan to prepare alcoholic extract/aqueous extracts that used for detection the cytotoxicity properties.

1. Preparation of *Shigrubeeja* extract-Testing and approval of above drugs have done under the supervision of DOLCAS BOTANOSYS PVT.LTD. Bikaner.

Step 1- *Moringa oleifera* seeds (*Shigrubeeja*) have been evaporated in the dry shade.

Step 2- Size reduction of above given drugs (*Shigrubeeja)* in Mixer-Grinder.

Step 3- Extraction of drugs (*Shigrubeeja)* have been extracted in two different method for 3 hours and repeated the cycle for three times at the specific temperature of 70-80°C the volume of solvent must be 3-4 times higher as above-mentioned drugs.

Step 4-Removal of liquid extract done by filtration method and filtered drugs were obtained.

Step 5- To obtain thick syrup like formation the concentration & distillation procedure have been done under vacuum at the specific temperature of 55-60°C.and it has been dried under vacuum at 60°C.

Step 6- After the above procedure removal of dry flakes/Lumps have been done and fine powdering formation were collected.

2. Preparation of *Arkapatra* extract-

Step 1-*Calotropisprocera* leaves (*Arkapatra)* have been evaporated in the dry shade.

Step2- Size reduction of above given drugs (*Arkapatra)* in Mixer-Grinder.

Step 3- Extraction of drugs (*Arkapatra*) have been extracted in two different method for 3 hours and repeated the cycle for three times at the specific temperature of 70-80°C the volume of solvent must be 3-4 times higher as above mentioned drugs.

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Step 6 - After the above procedure removal of dry flakes/Lumps have been done and fine powdering formation were collected.

Study group- The study was divided into 4 groups of each test drugs-
The in-vitro anticancer activity of *Shigrubeeja* and *Arkapatra* was examined under different medium which is listed below.

In-vitro anticancer activity of *Shigrubeeja* on CaCo2 cell line

- **Study group-1** -The aqueous extract of *Shigrubeeja*was tested for anticancer activity w.s.r to colon cancer.
- **Study group -2**- The methanol extract of *Shigrubeeja* was tested for anticancer activity w.s.r to colon cancer.
- **Control group-** Vinblastine was used as positive control of the study.

In-vitro anticancer activity of *Shigrubeeja* on Hepg2 cell line

- **Study group-1** - The aqueous extract of *Shigrubeeja*was tested for anticancer activity w.s.r to liver cancer
- **Study group -2**- The methanol extract of *Shigrubeeja* was tested for anticancer activity w.s.r to liver cancer
- **Control group-** Vinblastine was used as positive control of the study.

In-vitro anticancer activity of *Arkapatra* on Caco2 cell line

- **Study group**-1 - The aqueous extract of *Arkapatra* was tested for anticancer activity w.s.r to colon cancer.
- **Study group** -2- The methanol extract of *Arkapatra* was tested for anticancer activity w.s.r to colon cancer.
- **Control group**- Vinblastine was used as positive control of the study

In-vitro anticancer activity of *Arkapatra* on *Hepg2* cell line

- **Study group-1** - The aqueous extract of *Arkapatra*was tested for anticancer activity w.s.r to liver cancer
- **Study group -2**- The methanol extract of *Arkapatra* was tested for anticancer activity w.s.rto liver cancer
- **Control group**- Vinblastine was used as positive control of the study.

Human cell lines & Postive control- The following cancer cell line was used to test anticancer activity.

(a) Colon cancer cell line- (Caco2 cell line)

(b) Liver cancer cell line- (Hepg2 cell line)

(c) Positive control- Vinblastine

In Vitro Anticancer activity and cytotoxic assay –

Cell culture maintenance-

CaCo2(human colon carcinoma) and HepG2 (human Liver carcinoma) cell lines were procure/FromATCC (USA). Cells were maintained in MEM supplemented with 10%FBS (fetal bovine serum), 1% antibiotic and anti-mycotic at 37ºC with 5% CO_2.

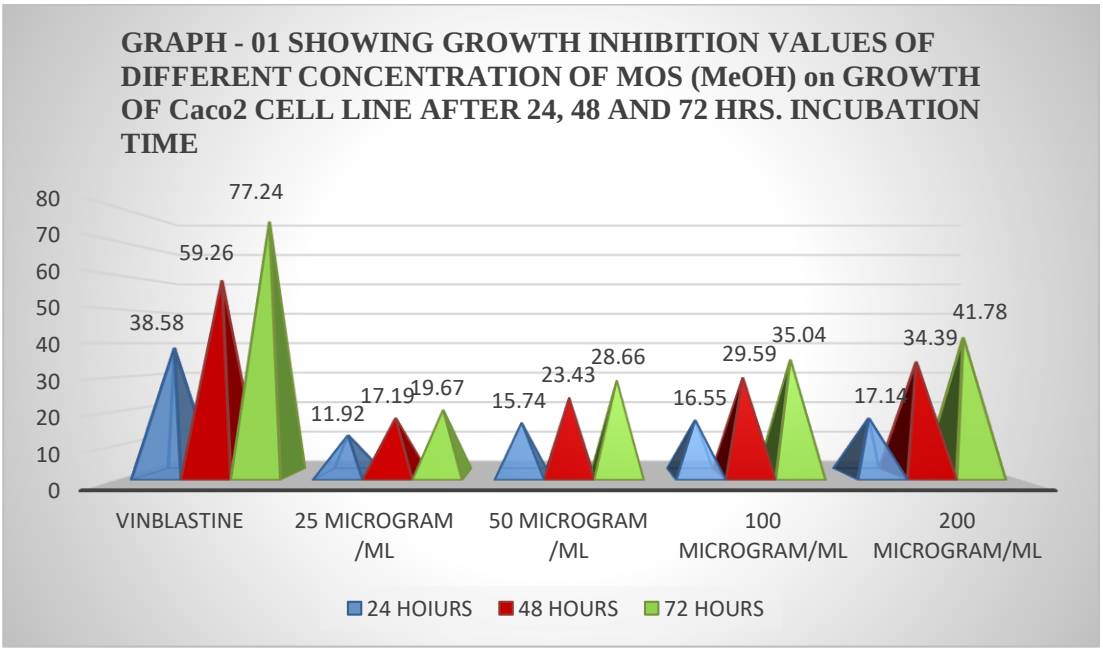
Cellular viability assay

The proliferation rate of CaCo2 (human colon carcinoma) and HepG2 (human liver carcinoma) cells after treatment with different concentrations of extracts were determined using the colorimetric 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.CaCo2 and HepG2 were plated at density of 10 ^{4}cells per well in 96-well flat bottom culture plate and grown in complete growth medium under standard culturing conditions. After 24hrs incubation, cells were treated with different concentration of (MOS.MeOH) methanol extracts at (25, 50, 100 and 200µg/mL) and incubated for24, 48 and 72hrs. At 37 centigrade with 5% CO 2 Subsequently, 5µl of MTT solution (5mg/mL stock) was added to each well and incubated at 37ºC for 4 hr. MTT was reduced by mitochondrial dehydrogenases to the water-insoluble blue compound formazan, depending on the viability of cells (Mosmann, 1983). Then the solution was removed and 100µL of DMSO was added to each well followed by 30min Incubation. The plates were shaken for 2min to ensure total solubility of formazan Crystal's and absorbance were measured at 570 nm with spectrophotometric microplate Reader (Synergy, Biotech, United States).

Observation and Result-

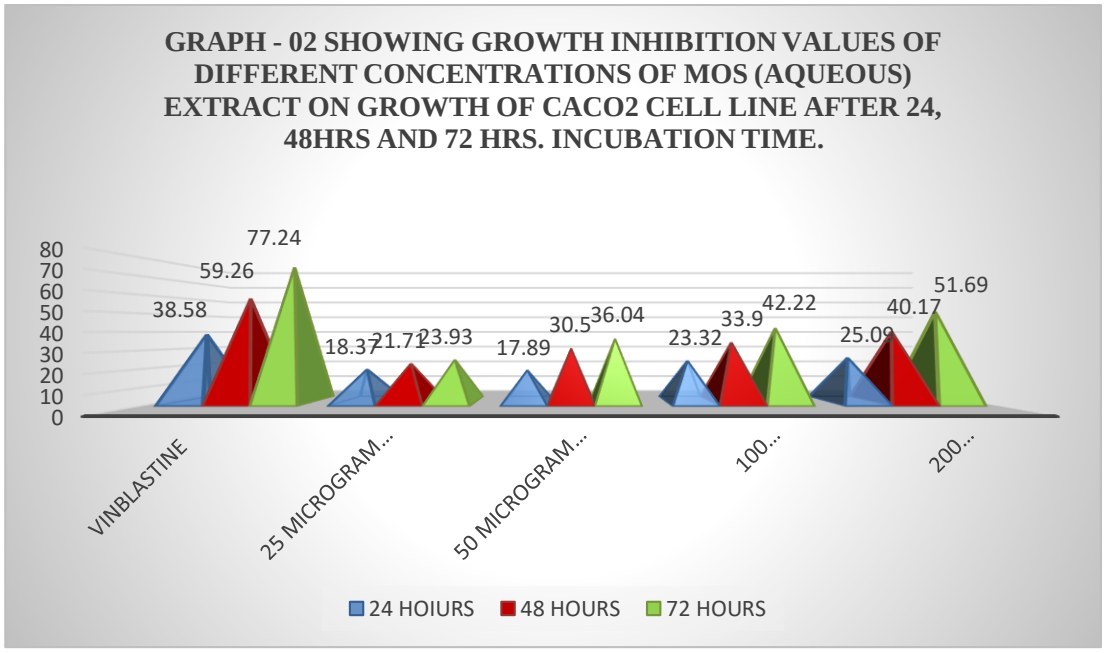
Moringa oleifera seed and Calotropis leaves extract cytotoxicity

MTT assay was performed to examine the effect of different extract of *Moringa oleifera* seed (MOS) and *Calotropis Procera* leaves (CPL) on cellular viability of CaCo2 and HepG2 cancerous cell lines. Results of MTT assay shows that, both compounds showing cytotoxic effects against both the cell lines i.e. CaCo2 and HepG2 at different concentrations (25, 50, 100 and 200 µg/ml) after 24, 48and 72 hrs. incubation.

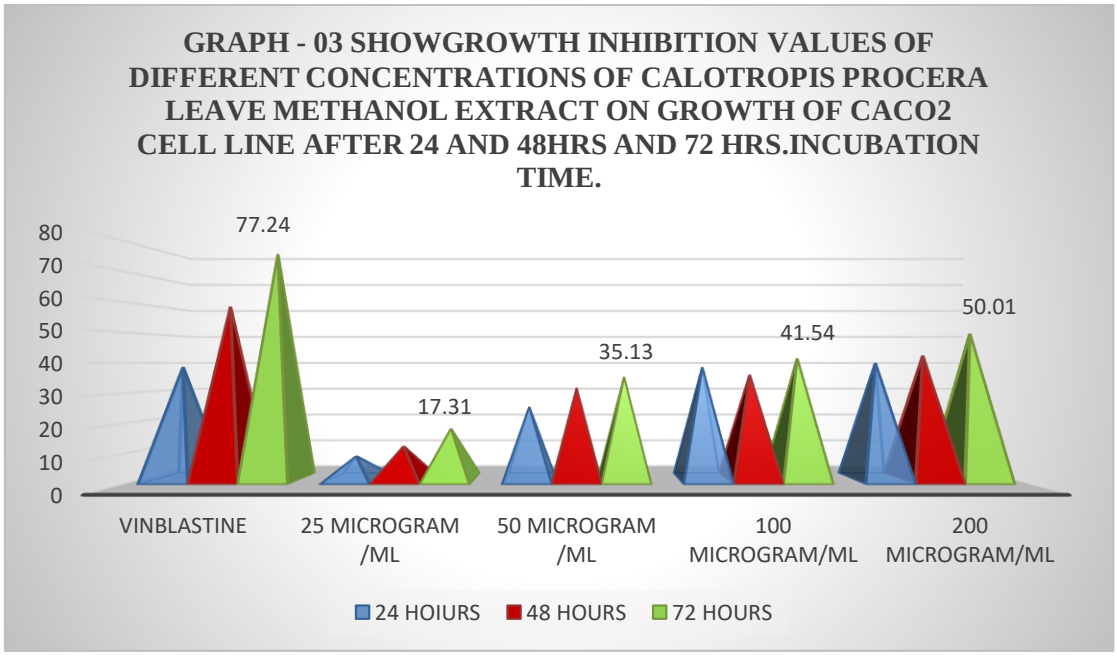


In above study (GRAPH - 01) human Caco2 cell line (colon cancer) are used to measure anticancer activity and cytotoxicity in methanol extract of Moringa seed at four different concentrations of 25,50,100,200µg/ml. Study was carried out for 24,48, and 72hrs. and growth inhibition of cell was measured after 24,48&72hrs of incubation. As compared to the Positive control which is Vinblastine explicit fixation at10µg/ml for 24, 48, & 72hrs.with mean value and standard deviation is 38.58±4.67, 59.26±0.53, 77.24±3.30. As per study the percentage of growth inhibition of Moringa seed methanol extract mean value and standard deviation at the concentration of 25µg/ml within24, 48, &72hrs. And observations are as follows.11.92±2.81, 17.19±3.73, 19.67±4.91. And at the concentrations of 50µg/ml within 24, 48hrs, &72hrs. And observations are as follows.15.74±0.24, 23.43±1.54, 28.66±3.94 and at the concentration of 100µg/ml the observed values are as Follows 16.55±0.88, 29.59±1.42, 35.04±3.08.At the higher concentration of 200µg/ml with in 24, 48hrs, and 72

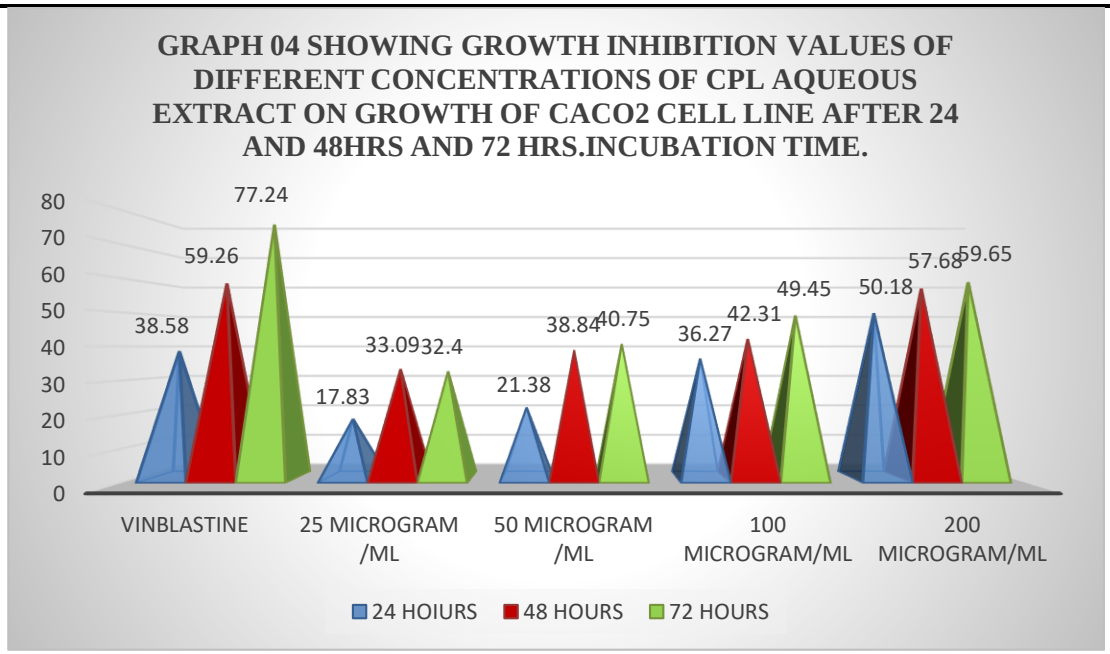
hrs. And observations are as follows.17.14±1.12, 34.39±1.61, 41.78±2.73. But as compare to the positive control the cytotoxicity of selected medium is less.



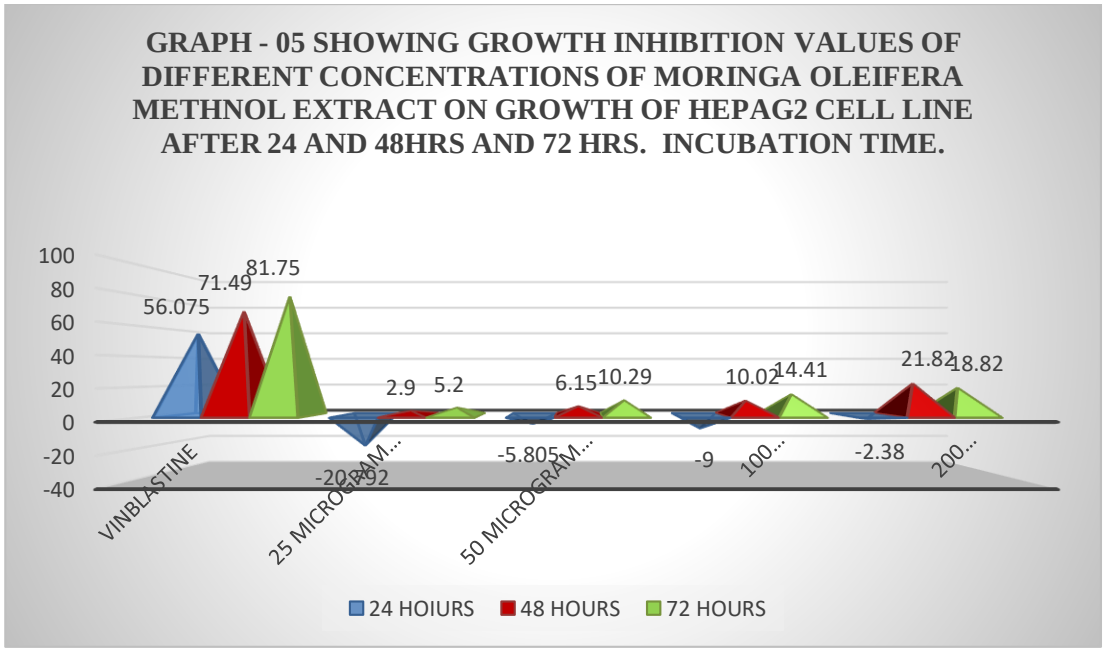
In above study (GRAPH - 02) human Caco2 cell line (colon cancer) are used to measure anticancer activity and cytotoxicity in aqueous extract of Moringa seed extracts at four different concentrations 25µg/ml,50,100, 200µg/ml. Study was carried out for 24, 48, and 72hrs.and growth inhibition of cell was measured after 24, 48 &72hrs.of incubation. As compared to the Positive control which is Vinblastine explicit fixation at10µg/ml for 24, 48, &72hrs.withmean value and standard deviation is 38.58±4.67, 59.26±0.53, 77.24±3.30. As per study the percentage of growth inhibition of *Moringa* seed aqueous extracts mean value and standard deviation at the concentration of 25µg/ml within 24, 48, &72hrs.and observation areas follows.18.37±1.86,21.71±8.2,41.78±2.73. At the concentrations of 50µg/ml within 24, 48, &72 hrs. And value observed are as follows.17.89±0.61, 30.50±1.05, 36.04±0.49.At the concentration of 100µg/ml within 24, 48 and 72hrs. And the observed values are as follows 23.32±3.90, 33.90±1.05, 42.22±1.16. At the higher concentration of 200µg/ml and values with in 24, 48, & 72hrs. As follow. 25.09±3.87,40.17±2.04,51.69±3.95. But as compare to the positive control the cytotoxicity of selected medium is less



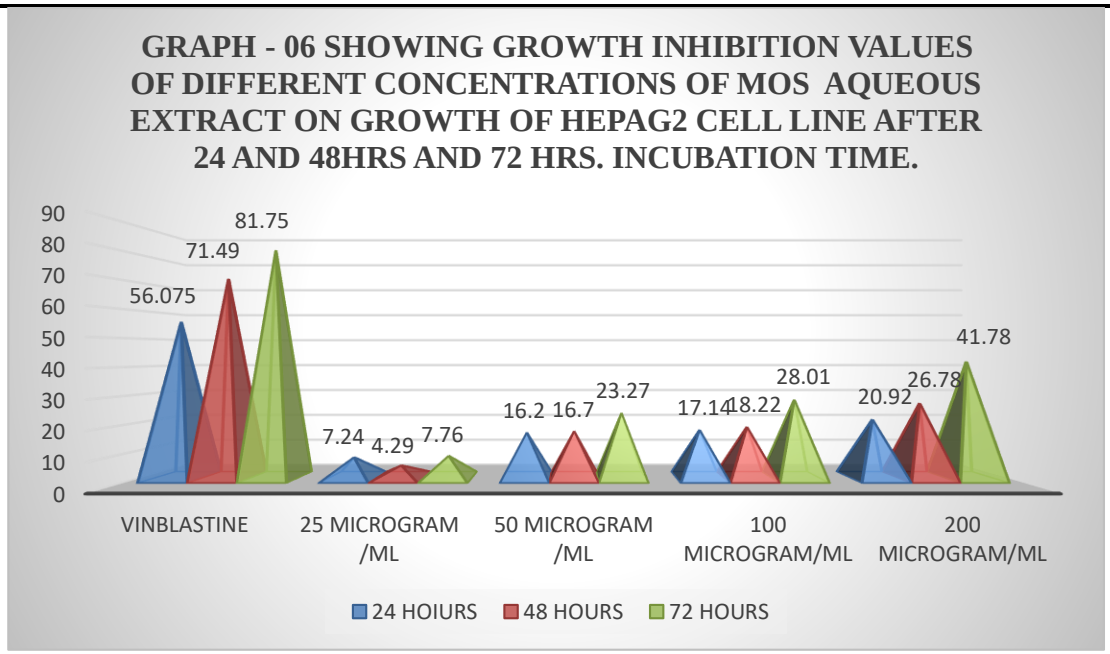
In above study (GRAPH - 03) human Caco2 cell line (colon cancer) are used to measure anticancer cytotoxicity in methanol extract of *Calotropis procera* leaves at four different concentrations25µg/ml,50,100,200µg/ml. Study was carried out for 24,48, and 72hrs. Growth inhibition on cell was measured after 24hrs, 48hrs &72hrs of incubation. Positive control which was Vinblastine. As per study the percentage of growth inhibition of *calotropis* leave methanol extracts mean value and standard deviation at the concentration of 25µg/ml with in 24, 48 and 72 hrs. And the observations are as follows.7.73±4.93, 11.20±1.27, 17.31±1.66 and at concentration of 50µg/ml within same time period the calculated value are as follows. 24.82±1.69, 31.41±2.12, 35.13±1.60 at the concentrations of 100µg/ml. And the observed values are 38.52±2.62, 35.90±1.29, 41.54±1.28. At the concentration of higher value at 200µg/ml the calculated value are as follows39.97±2.61, 42.52±6.64, 50.01±0.96. But as compare to the positive control the cytotoxicity of selected medium is less.



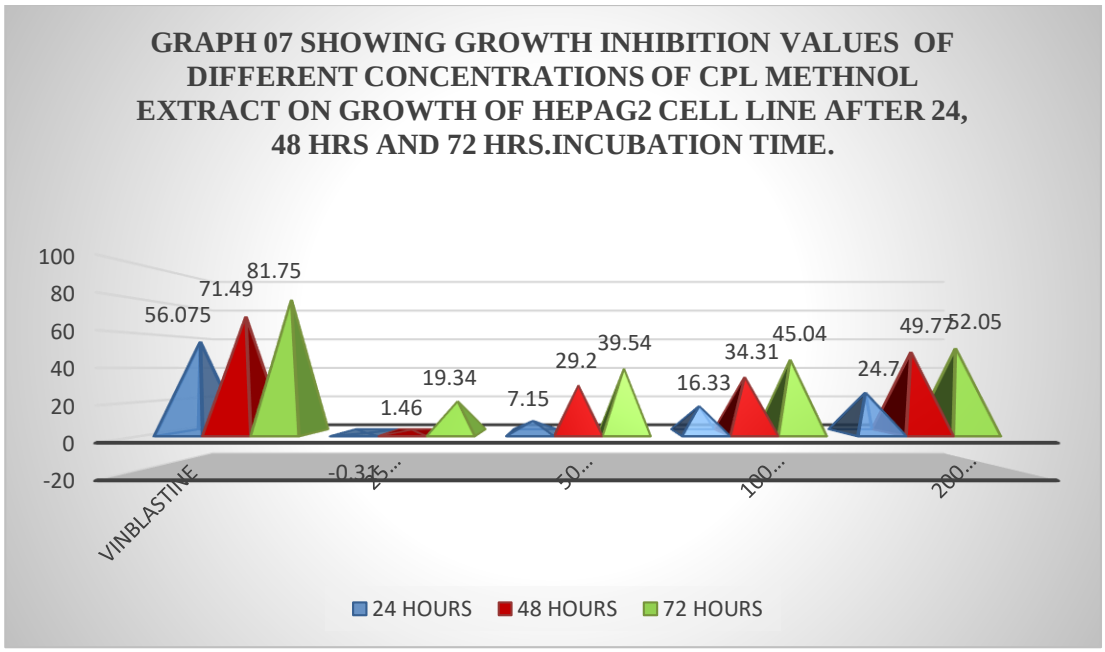
In above study (GRAPH – 04) human Caco2 cell line (colon cancer) are used to measure anticancer activity and cytotoxicity in aqueous extracts of *Calotropis procera* leave aqueous extract at four different concentrations 25µg/ml,50,100,200µg/ml. Study was carried out for 24, 48, and 72hrs. Growth inhibition of cell was measured after 24, 48 &72hrs.incubation.Positive control which was Vinblastine. As per study the percentage of growth inhibition of *calotropis* leave aqueous extracts mean value and standard deviation at the concentration of 25µg/ml within 24, 48, &7 2hrs. And observations are as follows. 17.83±2.89, 33.09±3.67, 32.40±1.55. At 50µg/ml with same time period the calculated values are as follows 21.38±1.69, 38.84±2.99, 40.75±0.88.60, at 100µg/ml the observed values are 36.27±2.74, 42.31±1.51, 49.45±1.54. At the concentration of 200µg/ml within same period the observed values areafollows.50.18±5.34, 57.68±2.15, 59.65±2.79.But as compare to the positive control the cytotoxicity of selected medium is less.



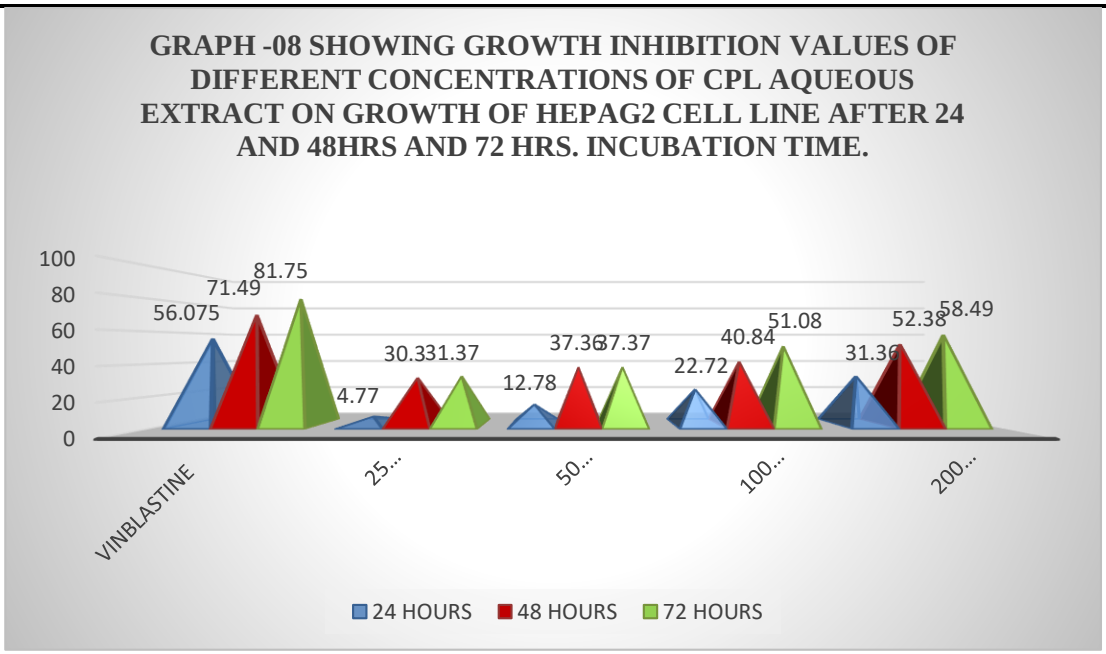
In above study (Graph-05) human HepG2 cell line (liver cancer) are used to measure anticancer activity and cytotoxicity in methanol extract of *Moringa* seed extract at four different concentrations.25µg/ml,50,100,200µg/ml. Study was carried out for 24,48,and 72hrs. And growth inhibition of cell was measured after 24, 48&72hrs of incubation. Positive control which is Vinblastine with explicit fixation at 10µg/ml for 24, 48, &72hrs. with mean worth and standard deviation as (56.07±4.66),(71.49±3.92),81.75±4.90.As per study the percentage of growth inhibition of *Moringa* seed methanol extract mean value and standard deviation at the concentration of 25µg/mlwithin24hrs,48,&72hrs.and observations are as follows. 20.792±6.91, 2.9082±1.93, 81.752±4.90.Andthe concentrations of 50µg/ml the observed values are as follows.-5.805±2.77, 6.15±4.44, 6.15±4.44, 10.29±1.21. At the concentration of 100µg/ml the observed values are -9.00±6.87, 10.02±5.39, 14.41±5.75.At the concentration of 200µg/ml the observed values are 2.38±1.36, 21.82±6.89,18.82±7.94. On Hepg2Cell line methanol extract of *Moringa* seed shows very less cytotoxicity within given period of time. But as compare to the positive control the cytotoxicity of selected medium is less.



In above study (GRAPH - 06) human HepG2 cell line (liver cancer) are used to measure anticancer activity and cytotoxicity in aqueous extracts of *Moringa* seeds extracts at four different concentrations.25, 50,100, 200µg/ml. Study was carried out for 24,48, and 72hrs. And growth inhibition of cell was measured after 24, 48&72hrs.of incubation. Positive control which isVinblastine with explicit fixation at10µg/ml for 24, 48, & 72hrs.with mean worth and standard deviation (56.07±4.66),(71.49±3.92),81.75±4.90.As per study the percentage of growth inhibition of *Moringa* seed methanol extract mean value and standard deviation at the concentration of 25µg/mlwithin24,48,&72hrs and observations are as follows. 7.24 ±2.17, 4.29±3.40, 7.76±5.37. And the concentrations of 50µg/ml the observed values are as follows.16.20±3.05, 16.71±8.24, 23.27±1.76 at the concentration of 100µg/ml the observed values are 17.14±1.66, 18.22±3.44, 28.01±3.48. At the concentration of 200µg/ml the observed values are 20.92±1.36, 26.78±2.89, 36.41±2.79. On Hepg2Cell line methanol extract of *Moringa* seed showed less cytotoxicity within given period of time. But as compare to the positive control the cytotoxicity of selected medium is less.



In above study (GRAPH-7) human HepG2 cell line (liver cancer) are used to measure anticancer activity and cytotoxicity in methanol extract of *CalotropisProcera.* leaves and extract of four different concentrations 25,50,100, 200µg/ml. Study was carried out for 24,48,and 72 hrs. Growth inhibition of cell was measured after 24, 48&72hrs of incubation. Positive control which is Vinblastine with explicit fixation at 10µg/ml for 24, 48,& 72hrs.with mean worth and standard deviation56.07±4.66), 71.49±3.92,81.75±4.90. As per study the percentage of growth inhibition of *Calotropis* leaves methanol extracts mean value and standard deviation at the concentration of 25µg/ml within 24, 48, & 72hrs. And observations are as follows. 0.31±2.24, 1.46±5.26, 19.34±5.05. At 50µg/ml 7.15±3.92, 29.20±1.9, 39.57±4.10. At100µg/ml16.33±2.54,34.31±1.55,45.04±5.39. At 200µg/ml calculated values are 24.70±3.99, 49.77±0.22, 52.05±0.92. Here at the higher concentration of *Calotropis* leave methanol extract shows more cytotoxicity on HepG2 cell line as shown in above figure. But as compare to the positive control the cytotoxicity of selected medium is less.



In above study (GRAPH-8) human HepG2 cell line (liver cancer) are used to measure anticancer activity and cytotoxicity in aqueous extract of *CalotropisProcera*. leaves at four different concentrations25µg/ml,50,100,200µg/ml. Study was carried out for 24,48,and 72hrs. Growth inhibition of cell was measured after 24,48 &72hrs of incubation. Positive control which is Vinblastine with explicit fixation at10µg/ml for 24hrs, 48, &72hrs. with mean worth and standard deviation as follows. 56.07±4.66, (71.49±3.92), 81.75±4.90. As per study the percentage of growth inhibition of *Calotropis* leaves aqueous extract mean value and standard deviation at the concentration of 25µg/ml within 24, 48, &72hrs. And observations are as follows. 4.77±1.04, 30.30±2.03, 31.37±5.25 and at 50µg/ml the observed values are 12.78±2.24, 37.36±6.85, 37.37±5.25. At100µg/ml the values are 22.72±0.15, 40.84±2.48, 51.08±2.26. At the concentration of 200µg/ml and calculated values are31.36±3.24, 52.38±8.20, 58.49±0.18. Here *Calotropis* leave water extract shows more cytotoxicity at higher concentrations of 200µg/ml. But as compare to the positive control the cytotoxicity of selected medium is less.

Discussion on Colon Cancer and liver cancer -Hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome, was originally described based on familial clustering of cancers at several sites including the colorectal, endometrial, stomach, ovary, urethra, brain, small bowl, (Intestine) hepatobiliary tract and colon cancers in HNPCC patients.It tends to occur at younger ages than sporadic colon cancers and are often located in the right colon[x]. In India, the yearly recurrence rates for colon illness and rectal harm in men are 4.4 and 4.1 per 100000, exclusively. The colon cancer development in women is 3.9 per 100000.In primary ten malignancies Colon illness positions eighth and rectal threat positions ninth among men. For women, rectal illness doesn't figure in the primary 10 malignancies, while colon harmful development positions 9[th]. Malignant tumors occurring in the liver can be primary or metastatic. Most of the discussion in this section deals with primary hepatic tumors. Primary carcinomas of the liver are relatively common in India. Most primary Liver cancers arise from hepatocytes and are termed hepatocellular carcinoma. It's progressively basic in individuals with cirrhosis. Cirrhosis implies scarring of the liver because of past harm, for example, scarring due to hepatitis B or C infection or long-term alcohol drinking. Hepatocellular carcinoma will undoubtedly make in men than in women and ends up being logically normal with age. In India, the age-adjusted incidence rate of hepatocellular carcinoma (HCC) for men ranges from 0.7 to 7.5 and for women 0.2 to 2.2 per 100,000 of population per year. The age-standardized mortality rate for HCC in India for men is 6.8/100,000 and for women is 5.1/100,000. The incidence of HCC in cirrhotic in India is 1.6% per year. The incidence of HCC is increasing in India. The high incidence of HCC in India is related to cirrhosis of liver, chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, alcohol consumption and aflatoxin exposure, diabetes mellitus, non-alcoholic fatty liver disease (NAFLD), smoking and tobacco use[xi] [xii]. *Ayurvedic* aspects of Cancer the disease *Arbuda*[xiii] was prevalent during the *Vedic* period. In *Atharvaveda*[xiv], there is reference of *Arbuda* and its management. *Arbuda* is one of the surgical diseases and was explained in detailed by *Acharya Sushruta* in the pioneer of ancient *Ayurvedic* texts that have remarkable similarities with modern interpretation of cancer[xv]. While searching the literature one finds that *Arbuda* is the nearest clinical term correlated with the externally visible cancerous growth. Many diseases can be correlated with *Arbuda* but Cancer is a parallel disease for it. The signs and symptoms of *Arbuda* can be very well explained in terms of Cancer. *Sushruta Samhita*[xvi] and *Charaka Samhita*[xvii], two well-known classics have described cancer as inflammatory or non-inflammatory swelling and mentioned them as either *Granthi* or *Arbuda*. *Acharya Madhava* while describing *Arbuda* opines that the vitiated *Doshas* afflict the *Mamsa* and *Rakta*[xviii] both to produce a swelling. *Sushruta Samhita, Charaka Samhita* as well as *Astanga Hridaya*[xix], which was written much later, give very detailed description about this subject. The *Doshas* get vitiated in any part of the body and afflicting the *Mamsa dhatu* later produce swelling. This lesion is circular, fixed, slightly painful, big in size, broad based, slowly growing and it does not suppurate; same is called *Arbuda*. These clinical symptoms which are parallel for malignant growth are correlated with cancer. There is no particular etiology (*Nidana*) of *Arbuda* has been notice in *Ayurveda* and a large portion of the *Acharyas* guessed the etiological factor of *Arbuda* is like that of *Sopha* and *Granthi*. *Aacharya Vagbhata* referenced that the expanding (*Granthi*) which is small in contrast with that of *Arbuda* should be considered as earlier symptoms of *Arbuda*. *(Poorvaroopa).*

Etiopathogenesis of *Arbuda* depends on *Doshas* hypothesis. Vitiated "*Doshas*" are responsible for the improvement of *Arbuda*. *Acharya Sushruta* has mentioned that because of excessive *Kapha, Arbuda* doesn't decay, which is viewed as the normal and significant factor for any development in the body. Subsequently, it appears to be advocated to hypothesize that overabundance of vitiated *Kapha* in the body may be answerable for the precipitation of malignancy. *Acharya Vagbhatta* has described that there is over formation of *MamsaDhatu* it might prompt different obsessive conditions, for example, *Galaganda*, *Gandamala*, *Arbuda*, *Granthi* and *Adimamsa*. It shows that *Mithya Ahara* and *Mithya Vihara* presumably changes nearby or orderly bio concoction factors including the hemodynamic prompting the formation of *Arbuda*. Disease starts because of metabolic changes. *Vatadosha* is liable for cell division. Disturbance of *Vatadosha* and concealment of *Kaphadosha* or both the *doshas* connecting with each other may bring about expansion of cells. However, the *Ekadesavriddhi* (development at a particular part) is a piece of irregular cell division bringing about amiable or threatening tumors. *AcharyaSushruta* has clarified around six phases in the pathogenesis and all are considered. These are *Sanchaya*-the beginning phase of confined changes (Accumulation of *doshas) Prakopa*- Change of essential developments of pathology into cells (*doshas* gets aggravated) *Prasara*- (Aggravated *doshas* leave their original place and spread). *Sthanasamsraya*- development of disease in that place (aggravated *doshas localized) Vyakti*-Clinical signs and side effects watched (Stage of clinical features) *Bheda*-Stage where separation of development is comprehended based on histopathology [xx] (Type of *doshas* innvolvment is known).

Discussion on current therapy used in Cancer-Current treatment practiced on cancer patients are therapies such as radiation-therapy and chemotherapy medications along with surgical procedures and different treatments to fix a disease, recoil a malignant growth or stop the movement of a malignancy exist. Contingent upon specific circumstance patient may get one treatment or multiple treatments. Cancer treatments may be used as[xxi]

1. **Primary treatment**: The goal of a primary treatment is to completely cure the canceror kill all the cancer cells. Any cancer treatment can be used as a primary treatment, but the most common primary cancer treatment for the most common types of cancer is surgery. If cancer is particularly sensitive to radiation therapy or chemotherapy, patient may receive one of those therapies as primary treatment.
2. **Adjuvant treatment: T**he goal of adjuvant therapy is to kill any cancer cells that may remain after primary treatment in order to reduce the chance that the cancer will recur. Any cancer treatment can be used as an adjuvant therapy. Common adjuvant therapies include chemotherapy, radiation therapy and hormone therapy.
3. **Neo-adjuvant therapy**: It is similar but treatments are used before the primary treatment in order to make the primary treatment easier or more effective but in case of **Palliative treatments** may help relieve side effects of treatment or signs and symptoms caused by cancer itself.

Discussion on *Ayurveda* therapy.The helpful methodology of *Ayurveda* has been partitioned into four classifications as *Prakritisthapanichikitsa* (health maintains), *Roganashanichikitsa* (disease fix), *Rasayanachikitsa* (reclamation of typical capacity) and *Naishthikichikitsa* (profound methodology)[xxii]. Finding the reason for a sickness is the fundamental objective of *Ayurvedic* treatment. *Ayurveda* doctors can analyze a sickness at even starting phases of body's irregularity and their restorative methodology keeps up and parity by providing lacking substances just as decreasing the unnecessary ones.

Sodhanachikitsa (purification process), which disposes of vitiated *doshas*, have been basically utilized for clinical administration of malignant growth.

Samanachikitsa-Which pacifies the vitiated *Dosha* and steadily cures the disease.

Rasayanachikitsa *(*immunotherapy*),* certain toxic plants, mercury like metals and creature items were rendered non-poisonous and innocuous by the utilization of speculative chemistry and are utilized as restoring drugs. Different techniques for treatment incorporate, *Dhatwagni Chikitsa* (revision of metabolic imperfections),*Vyadhipratyanika Chikitsa* and *Lakshanika Chikitsa* (indicative treatment). Cauterisation (*Ksharakarma*) with alkali and other surgeries were performed with natural and mineral medicines. *Arbuda* is extracted totally from its profound root seat and cauterization done to crush any of the rest of the cell particles.[xxiii] Though in *Ayurveda* literature any specific management principle for colon and liver cancer not described but by avoiding causative factors (*Nidanaparivarjana*) and by following *Samshodana Chikitsa* (Purification), *Rasyana Chikitsa* (rejuvenating process) one can increase the life span.

Discussion on observation and Result- In the present experimental study *Moringa* seeds and *Calotropis* leaves extract cytotoxicity and anticancer activity has been observed on the basis of following MTT assays. MTT assay was performed to examine the effect of different extracts of *Moringa* and *Calotropis* leaves on cellular viability of CaCo2 and HepG2 cancerous cell lines. Results of MTT assay showed that, *moringa* methanol extract and *calotropis* extracts show cytotoxic effects against both the cell lines i.e. CaCo2 and HepG2 at different concentrations (25, 50, 100 and 200 µg/ml) after 24, 48 and 72 hrs. Incubation.

On Caco2 cell line- In this study the human colon cell line (Caco2) is used as In-Vitro model to measure anticancer and cytotoxicity activity of methanol and aqueous extract of *Moringa oleifera* seed (MOS.) and *Calotropis procera leaves* (CPL). It was found that *Moringa oleifera* seeds aqueous (MOS. AQ) and *Calotropis* leaves aqueous extract (CPL.AQ) shows more potential as compare to their respective methanol extract on Caco2 cell line. The methanol extract of *Moringa oleifera* seed (MOS.MeOH) was found more potential than methanol extract of *Calotropis proera* leaves (CPL. MeOH). While the aqueous extract of *Calotropis procera* leave (CPL. AQ) had more potential than *Moringa oleifera* seed (MOS AQ). But as compare to the positive control the different medium indicating less cyotoxicity.

On HepG2 cell line-In this study the human liver cell line (HepG2) is used as In-Vitro model to measure anticancer and cytotoxicity activity of methanol and aqueous extract of *Moringa oleifera* seeds (MOS) and *Calotropis procera* leaves (CPL). It was found that *Calotropis procera* leaves aqueous (CPL.AQ) and *Moringa oleifera* seed (MOS. AQ) shows more potential as compare to their respective methanol extract on Caco2 cell line. But the cytotoxic activity of both the extracts of *Calotropis procera* is found more potential then extracts of *Moringa oleifera* on Hepg2 cell line. But as compare to the positive control the different medium indicating less cyotoxicity.

Inhibitory Concentration (IC50 value of Caco2 cell line)- In this study the inhibitory concentration value (IC50) of *Calotropis procera* leaves aqueous extracts in 24hrs,48hrs and 72hrs are 196.53µg/ml,149.47µg/ml and 99.90µg/ml respectively. While in case of *calotropis procera* leaves methanol extract &*Moringa oleifera* seed aqueous (MOS.Aq) the observed IC50 *values* were noted after 72 hours which are 141.07µg/ml & 150.36µg/ml respectively. These are ideal dose to inhibit colon cancerous growth on (Caco2) cell line on different period of time.

Inhibitory Concentration (IC50 value of HepG2 cell line cancer cell line)- In this study the inhibitory concentration value (IC50) of *Calotropis* leave aqueous extracts and *Calotropis* leave methanol extracts in 72hrs were 96.97µg/ml and109.89µg/ml respectively. These are ideal dose to inhibit liver cancerous growth on (Hepg2) cell line on different period of time.

Discussion on probable action of trial drug (*Shigrubeeja*)

***Shigrubeeja* Extract**- The *Sanskrit* synonyms of *Shigru* itself suggests its high nutritional & potential values of *Shigru* itself. *Shigru* word means strong and piercing quality of drug. The other synonyms of *Shigru*like *TeekshnaGandha, Akshheeva* (relieves intoxication) and *Mochaka* helps to cure diseases. By its *Rasa*, Guna, & *Vipaka* it is *Kaphahara &* by its *UshnaVirya*it*Vatashamak.Karma* of *Shigrubeeja* possesses *Katu-Tikta Rasa* and *Katu Vipaka* and *Ushna Virya* which are helpful in *Agnideepana, and Pachana* of *Ama.*This helps in purification of srotas and subside *Sanga* (blockage) and which leads to removal of *Siragranthi* In the pathogenesis of *Arbuda,*innvolment of *Srotas like.Rasa, Rakta, Mams,Medha,Asthi* is seen and kinds of *Srotodushthi* are *Sanga, Siragranthi*. Thus,by cleansing the natural channel i.e (*Srotas*),*Shigrubeeja* rejuvenate *Rasa,Rakta,Mamsa,Medha, Dhatu* and helps in management of *Arbuda*, *Granthi,Apachi,Gulma* and *Shotha*.

Discussion on probable action of trial drug (*Arkapatra)*-

Arka is Content of *Manashiladi Lepa* use as *Pradeha* in *Kushtha.* It is used as content of *Dantaydilepa*. Which is use in *Kaphaja Granthi. Arka Ksheera* as a content of *Krimighnadi vati* used in *Udavarta.*It is also an ingredient of *Nygrodhadhivranaropa Kwatha* which is use in *Varanagataroga. Pratisaraniya Ksharan*irmana *Vidhi* which is indicated in *Kustha, Arbuda, Arsha, Adhijiwa. Arka* is given in the context of *Dushtavrana.* By its *Rasa*, Guna, & *Vipaka*it is *Kaphahara &* by its *Ushna Virya* it is *Vatashamak. Arkapatra* possess *Katu-Tikta Rasa* and *Katu Vipaka* and *Ushna Virya* which are also helpful in *Agnideepana, & Pachana of Ama.* While describing about all the available medicinal formulation of *Arka*it represents the *Karma like Bhedana, Deepana, Krimighna,Vishaghna, Vranah, Vatahra, Sopha, Shvashara.* By improving the pain, and maintain the metabolic process of body. *Arka* is having *Adhobhaghara* properties.This helps in purification of *Mahasrotas* and subside *Sanga* (blockage)and which leads to removal of *Siragranthi.*

Discussion on over all benefits of Anti-cancer study-Taking everything into account, it was found that Moringa oleifera seeds aqueous (MOS. AQ) and Calotropis leaves aqueous extract (CPL.AQ) shows more potential as compare to their respective methanol extract on Caco2 cell line. The methanol extract of Moringa oleifera seed (MOS.MeOH) was found more potential than methanol extract of *Calotropis procera* leaves (CPL.MeOH). While the aqueous extract of *Calotropis procera* leaves (CPL.AQ) had more potential than Moringa oleifera seed (MOS AQ). But as compare to the positive control the different medium indicating less cytotoxicity. While in context of Calotropis procera the human liver cell line (HepG2) is used as In-Vitro model to measure anticancer and cytotoxicity activity of methanol and aqueous extract of Moringa oleifera seeds (MOS.AQ) and *Calotropis procera* leaves (CPL.AQ). It was found that *Calotropis Procera* leaves aqueous (CPL.AQ) and *Moringa oleifera* seed (MOS. AQ) shows more potential as compare to their respective methanol extract on Caco2 cell line. But the cytotoxicity activity of both the extracts of *Calotropis procera* is found more potential than extracts of Moringa oleifera on Hepg2 cell line. But as compare to the positive control the different medium indicating less cytotoxicity.

The IC 50 values of both selected compounds indicate the ideal dose to kill cancer cell with its inhibitory action on different time period. Hence the above selected drugs having anticancer and cytotoxicity properties can be used in the field of management of cancer (*Arbuda)*and more In -Vitro assessments must be done to give more data of its belongings to destructive advancement cell procedure and movement.

Conclusion- A plan for the conclusion and treatment of malignancy is a key part of any general disease control plan. Its primary objective is to fix malignant growth patients or draw out their life significantly, guaranteeing a decent personal satisfaction.
The human colon cell line (Caco2) and Human liver cell line (HepG2) are used as In Vitro model to measure anticancer and cytotoxicity activity of both compounds that are *methanol* and *aqueous* extracts of *Moringa oleifera* seeds (MOS) and *Calotropis procera* leaves(CPL).
On Caco2 cell line it was found that *Moringa oleifera* seeds aqueous (MOS. AQ) and *Calotropis* leaves aqueous extract (CPL.AQ) shows more potential as compare to their respective methanol extract on Caco2 cell line. The methanol extract of *Moringa oleifera* seed (MOS. MeOH) was found more potential than methanol extract of *Calotropis procera* leave (CPL. MeOH). While the aqueous extract of

Calotropis procera leave (CPL.AQ) had more potential than Moringa *oleifera* seed (MOS AQ). But as compare to the positive control the different medium indicating less cytotoxicity.

On HepG2 cell line it was found that *Calotropis procera* leaves aqueous (CPL.AQ) and *Moringa oleifera* seed (MOS. AQ) shows more potential as compare to their respective methanol extract on Caco2 cell line. But the cytotoxicity activity of both the extracts of *Calotropis procera* is found more potential than extracts of *Moringa oleifera* on Hepg2 cell line. But as compare to the positive control the different medium indicating less cytotoxicity.

Inhibitory Concentration (IC50 value of Caco2 cell line)- In this study the inhibitory concentration value (IC50) of *Calotropis procera* leaves aqueous extracts in 24hrs,48hrs and 72hrs are 196.53µg/ml,149.47µg/ml and 99.90µg/ml respectively. While in case of *Calotropis procera* leaves methanol extract & *Moringa oleifera* seed aqueous (MOS.AQ) the observed IC50 values were noted after 72 hours which are 141.07µg/ml &150.36µg/ml respectively. Thus, these doses of above said drugs are found to have the potential in inhibiting colon cancer growth or (Caco2) cell line in different period of times.

Inhibitory Concentration (IC50 value of HepG2 cell line cancer cell line)- In this study the inhibitory concentration value (IC50) of *Calotropis* leave aqueous extracts and *Calotropis* leaves methanol extracts in 72hrs were 96.97µg/ml and 109.89µg/ml respectively. Thus, these doses of above said drugs are found to have the potential in inhibiting liver cancer growth or (HepG2) cell line in different period of times.

Thus In-Vitro Anticancer study of *Moringa oleifera* seeds (MOS) and *Calotropis procera* leaves extracts (CPL) shows cytotoxicity and anticancer effects on Caco2 and Hepg2 cell line in different period of time with their respective IC50 value to inhibit cancerous growth.

Recommendation for further study-

1. Further examination for its utilization on account of particularly preclinical & clinical studies of Colon malignancy and liver malignancy is required.
2. Further study required for isolating active compounds are reasonable for the cytotoxicity.
3. A few constituents from both *Calotropis* procera (*Arka*) and (*Moringa oleifera*) *Shigru* may fill in as a novel antitumor after further definite examination.

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