ISSN No:-2456-2165

# The Therapeutic Composition AMSAA against Cancer and Reducing the Threat of Mortality

[1]Amitava Mazumder MRSC
MASAA Innovation Pvt. Ltd., India

**Abstract:- The present investigation relates to therapeutic compositions for the treatment of condition or disease in a subject. The present investigation also relates to a method of treating a condition or disease in a subject. The composition AMSAA has shown extremely good efficacy in killing cancer cells of various human organs found in vitro at 5%, 10%, 15% and 20% of its concentration and at the same time reasonable viability of Normal cell lines. Therefore, the current formulation could be taken ahead post trials as effective therapeutic drug to save humans.**

## I. INTRODUCTION

➢ *Background of the Investigation*

The modern healthcare industry is going through a major challenge in the form of various diseases such as cancer. According to global estimates, the number of new cases is expected to increase in the coming decades, reaching a staggering 30.2 million by 2040.

Although traditional medical practices have been known to be moderately effective in treating certain types of cancer, they come with their own limitations that can negatively impact clinical outcomes.

Thus, there is a strong need to develop highly efficacious compositions and methods for treatment of diseases like cancer to overcome the long-standing problems associated with the existing traditional methods and provide a more accessible and affordable treatment.

➢ *Summary of the Present Investigation*

An aspect of the present investigation is to provide a composition comprising:

- Squaric Acid, and/or croconic acid;
- Squaric Acid ester and/or croconic acid ester;
- water; and
- Optionally at least one pharmaceutically acceptable carrier.

➢ *Therapeutic Compositions*

The main embodiment of the present investigation provides a composition comprising squaric acid, and/or croconic acid, squaric acid ester and/or croconic acid ester; water; and optionally at least one pharmaceutically acceptable carrier.

The main embodiment of the present investigation provides the composition, wherein the composition further comprises sodium lauryl sulfate; a base; and a buffer.

The present study provides the composition, wherein the composition further comprises a combination of squaric acid and squaric acid monoamide or squaric acid diamide.

The present study provides the composition, wherein the composition further comprises a combination of croconic acid and croconic acid monoamide or croconic acid diamide.

- *Squaric Acid or Salts*

Squaric acid, also known as 3,4-dihydroxy-3-cyclobutene-1,2 dione, has the following chemical structure.

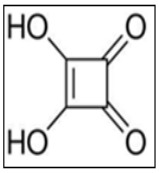


Fig 1 Squaric Acid or Salts

Squaric acid has two acidic protons. The high acidity with pKa of 1.5 for the first proton and pKa of 3.4 for the second proton. It exists in dianion form at pH 7.2 with four-point hydrogen bonding sites and highly stable aromatic species.

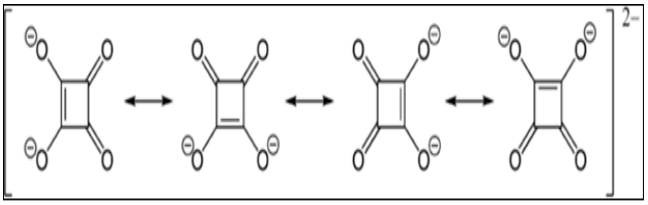


Fig 2 Squaric Acid or Salts

Salt form of squaric or similar acids such as sodium salt, potassium salt or any other possible metallic salt which dissolve in water/polar protic/polar aprotic solvents may also be usedas an active agent.

- *Squaric Acid Esters and Croconic Acid Esters*

The present study provides the composition, wherein squaric acid ester is squaric acid mono ester, squaric acid diester or a combination thereof. Further, diester of the squaric acid comprises two ester groups which may be the same or different.

ISSN No:-2456-2165

- *Sodium Lauryl Sulfate (SLS)*

Another embodiment of the present study provides the composition, wherein the composition also comprises sodium lauryl sulfate.

- *Base and Buffer*

Another part of the present study provides the composition, wherein the composition also comprises base to neutralize the squaric acid or croconic acid and to attain the pH of the composition to the desired range. Further, composition includes but not limited to any base capable of reacting with squaric acid or croconic acid to form squaric acid dianion or croconic acid dianion. The present study provides the composition, wherein the ratio of buffer is 4 to 10.

Yet another embodiment of the present investigation provides the composition, wherein the pH of the composition is from 7 to 9. In certain embodiments, base and/or buffer in the composition are present in an amount sufficient to maintain a pH of the composition from about 7 to about 9.

- *Solvent*

The present investigation provides the composition, wherein the composition also comprises solvent. Any suitable solvent can be used in the present invention such as any solvent which dissolves squaric acid and croconic acid and esters.

Yet another aspect of the present investigation provides the composition, wherein the composition further comprises a solvent selected from a polar aprotic solvent, polar protic solvent, low polar aprotic solvent, a low polar protic solvent, and/or a mixture thereof. In certain embodiments, solvent is a polar aprotic solvent, polar protic solvent, low polar aprotic solvent, a low polar protic solvent, or a mixture thereof which can dissolve sodium salts of active agent.

- *Dosage Forms and Methods of treatment*

The present study provides the composition, wherein the composition also comprises pharmaceutically acceptable carrier or excipient.

Another embodiment of the present study provides the composition, wherein the pharmaceutically acceptable carrier is carrier selected from but not limited to a group comprising a sterile aqueous media, a solid diluent, a filler, an excipient, various non-toxic organic solvent, and/or any combination thereof.

Another aspect of the present investigation provides a method, wherein cancer is selected from prostate cancer, lung cancer, colon carcinoma, cervical cancer, kidney cancer, breast cancer, carcinoma such as adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, transitional cell carcinoma and the like, sarcoma such as chondrosarcoma, ewing sarcoma, leiomyosarcoma, liposarcoma, osteosarcoma and the like, lymphoma such as hodgkin lymphoma, Non-Hodgkin lymphoma, Leukemia, Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Chronic lymphocytic leukemia (CLL), Chronic myeloid leukemia (CML) and the like, Myeloma, Melanoma, CNS tumors such as Astrocytoma, Glioblastoma, Medulloblastoma, Meningioma and the like, Gastrointestinal cancers such as Colorectal cancer, Gastric cancer, Liver cancer, Pancreatic cancer and the like, Gynecological cancers such as Cervical cancer, Ovarian cancer, Uterine cancer and the like, prostate cancer, Lung cancer, Breast cancer, Kidney cancer, Thyroid cancer, blood cancers such as Multiple myeloma, Hodgkin's lymphoma and Non-Hodgkin's lymphoma.

Yet another embodiment of the present investigation provides a method, wherein the cancer is selected from breast cancer, colon carcinoma, kidney cancer, lung cancer, and prostate cancer.

Yet another embodiment of the present investigation provides a method, wherein the subject is a human or animal.

Yet another embodiment of the present investigation provides a method for preventing or treating cancer by administration of a therapeutically effective amount of composition as may be needed on a case to case basis.

The compositions of the present investigation may further comprise a pharmaceutically acceptable carrier, excipient or preservatives. The carriers include but are not limited to, solid diluents or fillers, excipients, sterile aqueous media and various non-toxic organic solvents. Dosage unit forms or pharmaceutical compositions include tablets, capsules, pills, powders, granules, aqueous and non-aqueous oral solutions and suspensions, creams, hard candies, lozenges, troches, sprays, salves, suppositories, gels, pastes, ointments, jellies, lotions, injectable solutions, elixirs, syrups, and parenteral solutions packaged in containers adapted for subdivisioninto individual doses.

## II. EXAMPLES

The invention will now be further illustrated by the following non-limiting examples. The specific examples below are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The components and/or reagents of the present disclosure are commercially available and/or can be prepared according to methods readily available to a skilled person.

- *Example 1: Preparation of Anti-Cancer Therapeutic Compositions*

For the preparation of anti-cancer therapeutic composition, a composition comprising squaric acid, squaric acid dibutyl ester, sodium hydroxide, sodium bicarbonate and sodium laurylsulphate was dissolved in deionized water. This composition was taken as a 100% composition.

ISSN No:-2456-2165

Table 1 AMSAA Composition

| Sr. No. | Components | Concentration (mg per mL) |
|---|---|---|
| 1 | Squaric Acid | 1.745 |
| 2 | Squaric Acid Dibutyl Ester | 0.05 |
| 3 | Sodium hydroxide | 0.65 |
| 4 | Sodium bicarbonate | 0.4 |
| 5 | Sodium lauryl sulphate | 0.5 |
| 6 | Deionized water | |

The therapeutic composition was prepared at various concentrations such as 0.01%, 1.0 %, 5.0%, 10.0%, 15% and 25%.

- *Example 2: The Efficacy of AMSAA at 9uM Comparing with ADR*

- *Example 3: Cell Viability of WI38 while Treated with AMSAA and Efficacy of the Composition AMSAA against PC3 Prostate Cancer cell Line*

- *Example 4: Efficacy of the Composition against other Cancer cell Line, Lymphocyte and Effect of AMSAA on RBC.*

- Other than prostate cancer cell lines, the cancer cell lines for other types of cancer were tested.
- The therapeutic composition was tested on the F549 human lung cancer cell line and compared with the WI38 human normal lung cell.
- The therapeutic composition was tested on the A-498 cell line for kidney cancer.
- The therapeutic composition was tested on the HEK-293 cell line for kidney cancer.
- The therapeutic composition was tested on the Caco-2 cell line forcolon cancer.
- The therapeutic composition was tested on the MDA-MB-231 cellline for breast cancer.
- The therapeutic composition is suitable for the treatment of carcinoma such as adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, transitional cell carcinoma and the like.
- The therapeutic composition is suitable for the treatment of Sarcoma such as Chondrosarcoma, Ewing sarcoma, Leiomyosarcoma, Liposarcoma, Osteosarcoma and the like.
- The therapeutic composition is suitable for the treatment of prostate cancer, Lung cancer, Breast cancer, Kidney cancer, Thyroid cancer.
- Occupational carcinogens due to increased risk of exposure to Radon, radioactive inert gas and asbestos & Diet.

The Anticancer drug screening formulation, containing a chemical entity and other excipients have been used in parallel with Adriamycin at various concentration levels. The normal Lung cancer cell line (Wi 38) and lung cancer cell line A-549 were treated.

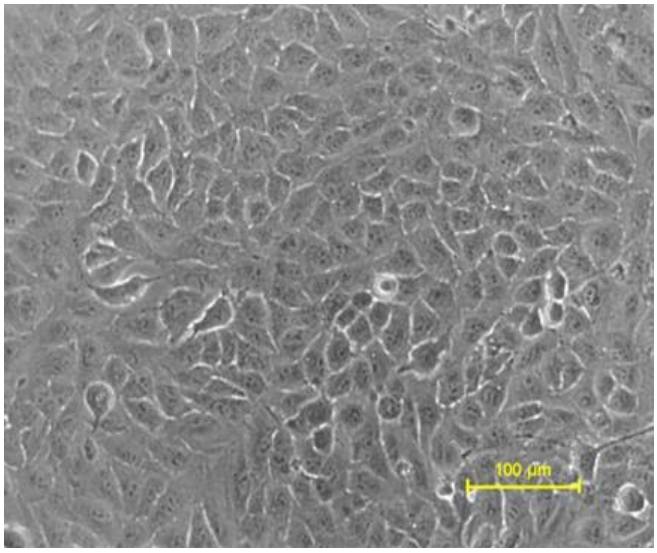

Fig 1 A549 Cancer cell line (ACTREC), Tata Memorial Cancer, India

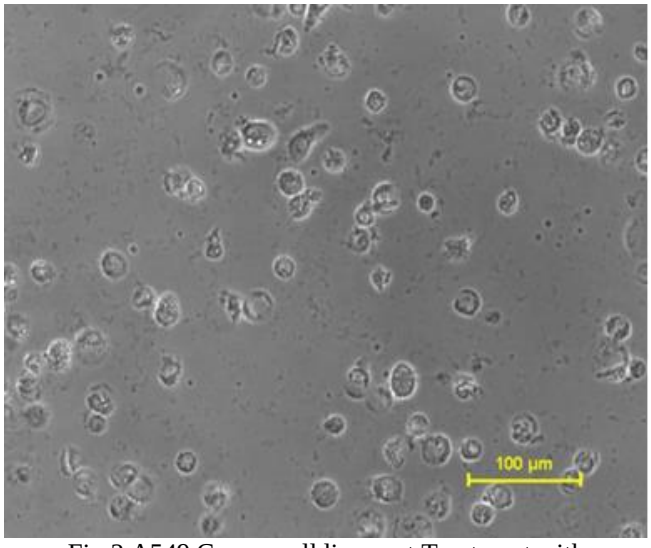

Fig 2 A549 Cancer cell line post Treatment with Adriamycin 9uM (ACTREC), Tata Memorial Cancer, India

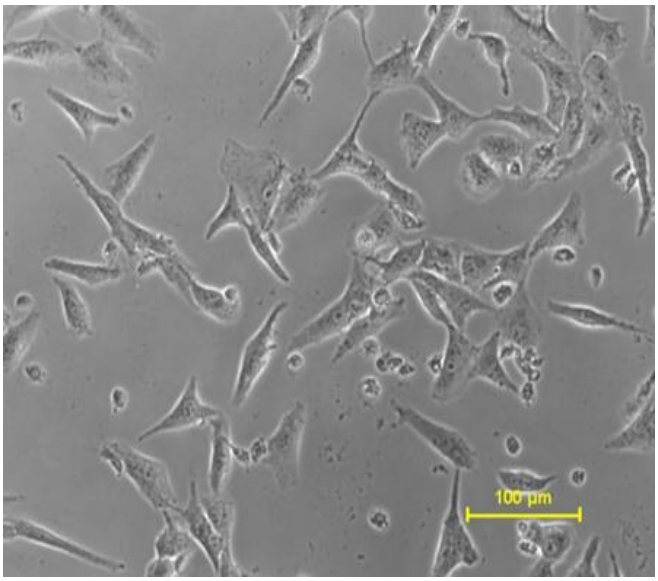

Fig 3 A549 Cancer cell line post Treatment with AMSAA 9uM (ACTREC), Tata Memorial Cancer, India

ISSN No:-2456-2165

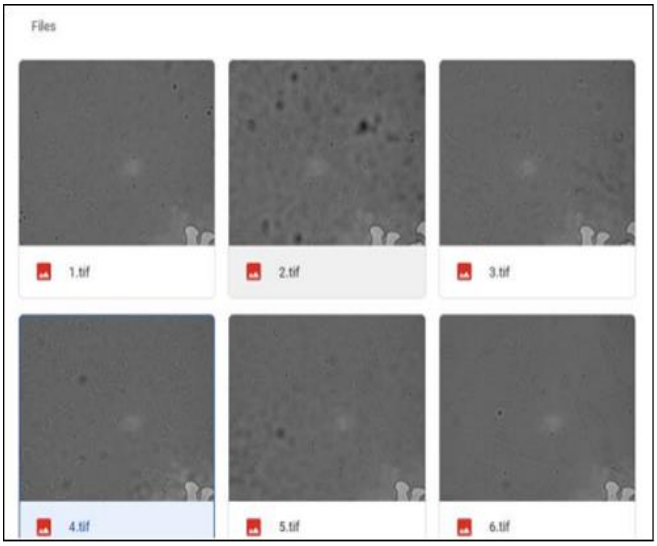

Fig 4 Wi 38 Control and Treated with 0.01% AMSAA, Study Duration 24 hrs.

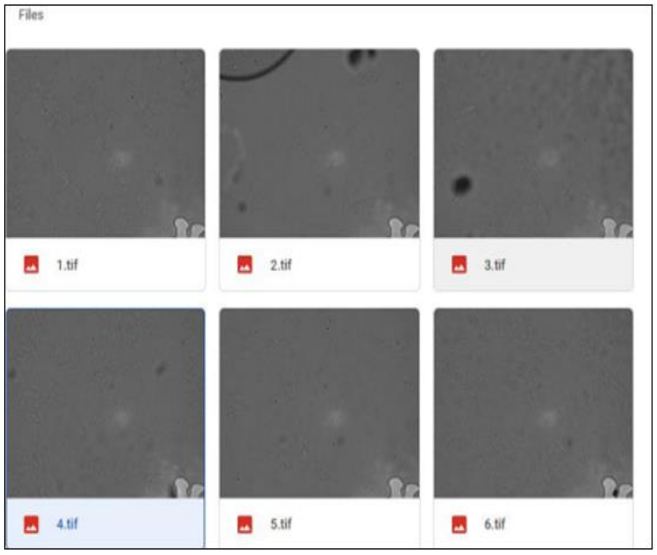

Fig 5 Wi 38 Treated with 1% AMSAA, Study Duration 24 hrs.

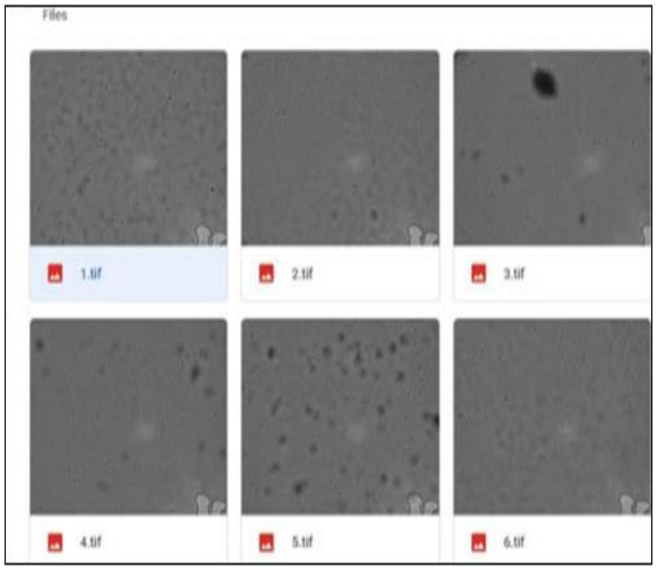

Fig 6 Wi 38 (Control) and Treated with 0.1% AMSAA, Study Duration 24 hrs.

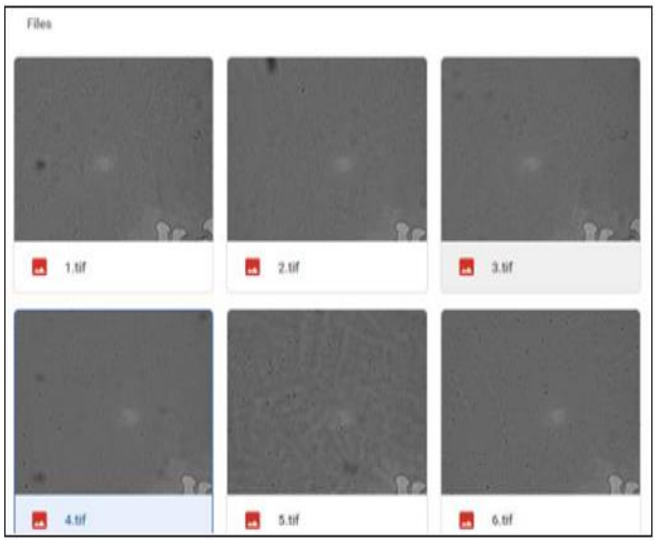

Fig 7 Wi 38 Treated with 1 % AMSAA, Study Duration 12 hrs.

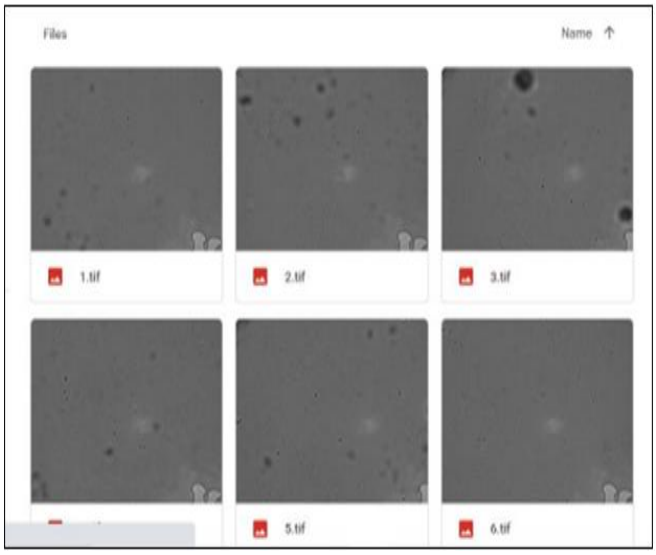

Fig 8 Control and Treated with 0.1 % AMSAA for 8 hrs.

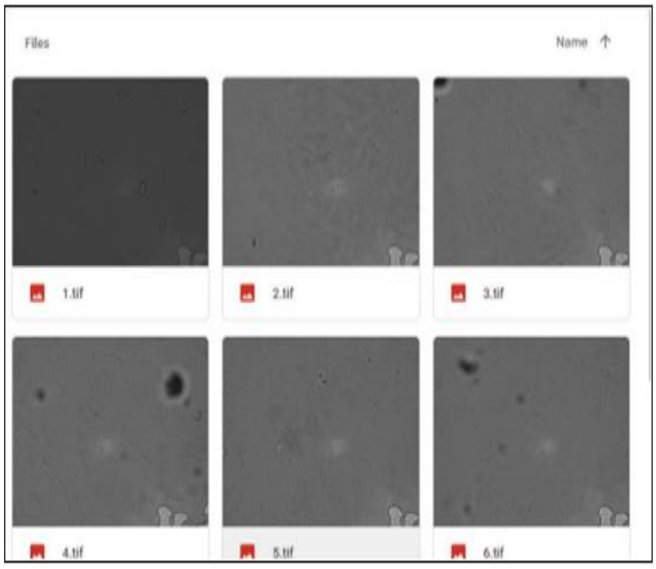

Fig 9 Wi 38 Treated with 1% AMSAA with Study Duration 8 hrs.

ISSN No:-2456-2165

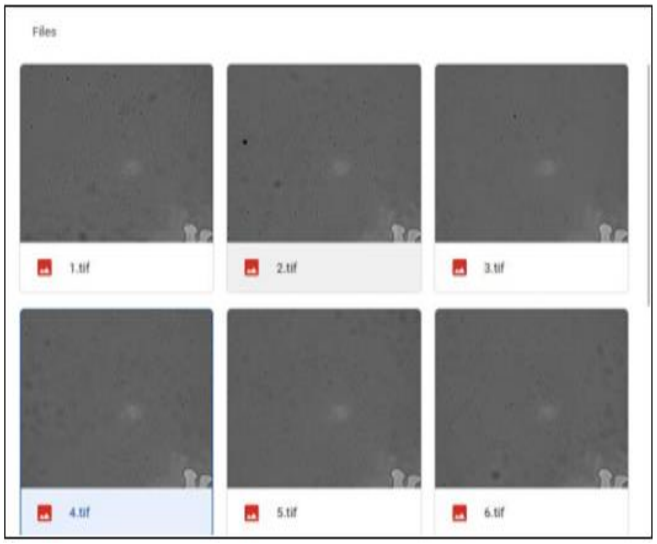


Fig 10 PC3 Control and Treated with 0.1% AMSAA for 24 hrs.

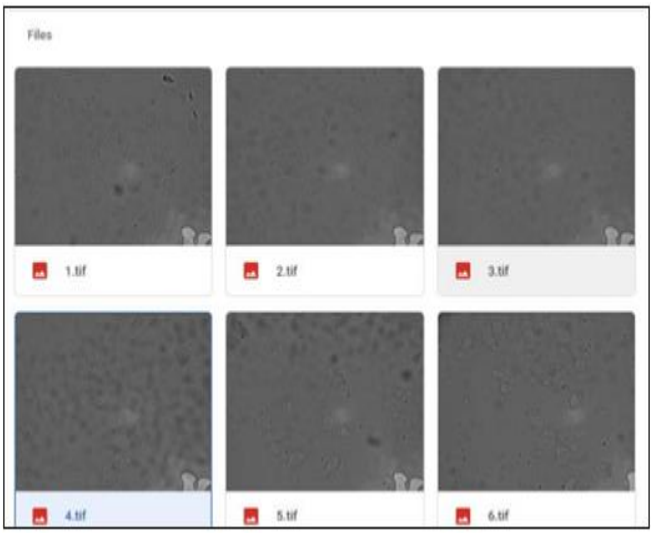


Fig 11 PC3 Treated with 1% AMSAA Treated with Study of 24 hrs.

## III. MTT ASSAY & RESULTS

- *WI 38 Cell Line Treated with AMSAA and ADR*

Table 2 Time Point: 24 Hours after Treatment with AMSAA & ADR

| | Ctrl | 0.1% AMSAA | 1% AMSAA | 5 uM ADR | 15 uM ADR | 50 uM ADR |
|---|---|---|---|---|---|---|
| 1 | | 0.288 0.253 | 0.239 | 0.150 | 0.152 | 0.11 |
| 2 | | 0.282 0.242 | 0.222 | 0.150 | 0.149 | 0.103 |
| 3 | | 0.290 0.244 | 0.240 | 0.152 | 0.151 | 0.11 |
| 4 | | 0.282 0.254 | 0.219 | 0.152 | 0.150 | 0.102 |
| 5 | | 0.285 0.248 | 0.230 | 0.151 | 0.150 | 0.106 |
| % Average Cell viability | 100% | 87% | 81% | 53% | 52% | 37.1% |

Table 3 A-549 Cell Line and Treated with AMSAA & ADR: Time Point: 24 hours after Treatment with AMSAA & ADR

| | Ctrl | 0.1% AMSAA | 1% AMSAA | 5 uMADR | 15 uMADR | 50uMADR |
|---|---|---|---|---|---|---|
| 1 | 0.197 | 0.193 | 0.162 | 0.063 | 0.063 | 0.057 |
| 2 | 0.179 | 0.178 | 0.153 | 0.150 | 0.063 | 0.053 |
| 3 | 0.195 | 0.190 | 0.163 | 0.064 | 0.065 | 0.058 |
| 4 | 0.180 | 0.182 | 0.154 | 0.055 | 0.065 | 0.054 |
| 5 | 0.196 | 0.180 | 0.153 | 0.065 | 0.055 | 0.054 |
| % Average Cancer cell viability | 100% | 91% | 78% | 33% | 28% | 27% |

Table 4 Time Point: 24 hrs. after the Treatment of Cancer cell Viability with AMSAA and ADR

| | Ctrl | 5% | 15% | 30% | 9uM ADR | 15uM ADR |
|---|---|---|---|---|---|---|
| A498 (Kidney cancer) | 100 | 51.79 | 48.20 | 32.37 | 48.2 | 43.1 |
| MCF 7 (Breast Cancer) | 100 | 59.77 | 45.80 | 38.54 | 31.84 | 32.90 |
| Caco 2 (Colon cancer | 100 | 71.11 | 67.77 | 55.35 | 75.55 | 66.66 |
| PC3 (Prostate Cancer | 100 | 89.82 | 52.96 | 41.11 | 43.60 | 34.10 |

Table 5 Lymphocyte Viability by MTT Assay with AMSAA & ADR

| Ctrl | 5% AMSAA | 10% AMSAA | 15% AMSAA | 20% AMSAA | 30% AMSAA | 9uMADR | 15uMADR |
|---|---|---|---|---|---|---|---|
| 0.230 | 0.204 | 0.168 | 0.161 | 0.058 | 0.041 | 0.183 | 0.166 |
| 100% | 88.69% | 73.04% | 70% | 25.2% | 17.8% | 79.6% | 72.17% |

Table 6 WI 38 (Lung Normal Cell) MTT Data Post Treatment with AMSAA & ADR

| Ctrl | 5% AMSAA | 10% AMSAA | 15% AMSAA | 20% AMSAA | 9uMADR |
|---|---|---|---|---|---|
| 0.140 | 0.111 | 0.089 | 0.080 | 0.058 | 0.049 |
| 100% | 79.28% | 63.57% | 57.14% | 41.4% | 35% |

Calculation: 10% AMSAA = 1530.7uM

ISSN No:-2456-2165

## IV. DISCUSSION

The cell membrane rupture, degradation of even nucleus of the cancer cells were found. At 5% AMSAA treatment on PC3 entirely breaks down to powdered form and highly cidal. The complete profile study on Lung, Liver, Kidney, Colon, Prostate, Breast, Lymphocyte have been done at various concentration of AMSAA. 1% to 25% concentration of AMSAA, optimized at 15%, shows excellent results as cidal to cancer cells with much higher viability of normal cells compared to normal therapeutic drug used in 21 days treatment cycle.

## V. CONCLUSION

Safety data shows The Acute, Subacute oral & inhalation GLP OECD studies on Animals show LD 50 Oral > 5000 mg/kg, Non-toxic, Non-irritant to eyes, human skin, inhalation, on Wister rats and no mortality and morbidity in the duration as per OECD guidelines in all 2x, 5x and 10 x repeat dose study (Conclusively NOAEL)as per the Regulatory guidelines. Alternative therapeutic treatment could be very much effective at affordable price with the formulation AMSAA.

## REFERENCES

[1]. Compositions for Pathogen inactivation and Pathogen Reduction Management by Amitava Mazumder ( USPTO Publication no 20220287302)
[2]. Compositions for Pathogen inactivation and Pathogen Reduction Management by Amitava Mazumder (WO2022074667A1 )
[3]. Compositions for Pathogen inactivation and Pathogen Reduction Management by Amitava Mazumder (US 17/436,884)