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ORIGINAL ARTICLE

Anti-cancer activity of Mebendazole, Metformin and Apricoxib in HT-29, MDA-MB-231, Hela and MCF-7cell lines: Preclinical trial

FATIMA RIZVI[1], FARAH ASAD[2], LUBNA SHAUKAT[3], MUHAMMAD ATIF SIDDIQUI[4], FARIDA QADIR[5], ABDUL QADIR[6]
[1]*Professor of Pharmacology, Dow International Medical College*
[2]*Associate Professor of Pharmacology, JSMU*
[3]*Senior Registrar, DIDC*
[4]*Lecturer, DIDC*
[5]*Professor of Pharmacology: KMDC (frfatima121@gmail.com)*
[6]*Senior Lecturer (Pharmacology), UMDC*
Correspondence to Dr. Fatima Rizvi, Email: fatima.rizvi@duhs.edu.pk

ABSTRACT

Aim: To look at anti-cancer effects of a variety of novel drugs on a variety of cancerous cell lines in the quest for a more inexpensive and effective anti-cancer treatment.
Methodology: The research was carried out in conjunction with PCMD at JPMC's BMSI Department of Pharmacology. The experiment lasted about eight months (from April to November 2016). Noninvasive estrogen-dependent tumours, invasive estrogen-independent breast cancer, colorectal cancer, and cervical cancer were all depicted using MCF-7, HT-29, MDA-MB-231and HeLa cell lines, in that order. The MTT assay was used to improve the drugs' anticancer or antiproliferative efficacy in vitro. Using the MTT test, we determined the vitality of all treated tumour cell lines as well as the IC50 values of each chemical against all malignant cell lines.
Results: This study demonstrated thatMetformin significantly decreased the survivability of MCF7, HT-29, MDA-MB-231, and Hela cell lines when compared to Apricoxib and Mebendazole. As a result, comparing the IC50 values of the studied agents for each of the evaluated treated cells backed up this claim. Hence, for Hela(p=0.386), MCF-7 (p=0.083), and MDA-MB-231 (p=0.083) cell lines but pochoc analysis revealed no significant differences in IC50 values of Metformin and Methotrexate.
Conclusion: When compared to Apricoxib and Mebendazole, Metformin has a significant effect on the survivability of the cell lines studied. Metformin, as a result, would have been a wonderful chemotherapeutic addition
Keywords: Cell lines, In vitro, MCF-7, Hela, MDA-MB-231

INTRODUCTION

Cancer is the greatest cause of mortality in the world, and while tremendous progress has been done in medicine, there are still significant difficulties to overcome in order to achieve perfection. As a result, oncological science devotes a significant amount of time and effort to the development of novel and creative medications that can mitigate the significant adverse effects caused by conventional treatments[1].

COX-2 and AMPK have recently emerged as the most fascinating targets[2]. COX-2 expression is associated to tumour development because it inhibits apoptosis, improves angiogenesis, and suffocates anti-tumor activity[3]. As a result, COX-2 inhibitors will play a key role in cancer treatment[4]. By blocking mTOR, AMPK pathway activators can reduce cancer cell proliferation, angiogenesis, and promote cellular death[5].Metformin has been well-known to reduce gluconeogenesis in the liver and improve skeletal muscle uptake of glucose through activated AMPK, a physiological fuel sensor enzyme which maintains bioenergetic balance through being phosphorylated and boosting activities while Adenosine triphosphate production falls and AMP concentration increased. The ATP:AMP ratio shift is utilised as an indicator of resource deprivation[6].

A large number of clinical and epidemiological studies have shown that taking metformin orally lowers the risk of developing and progressing cancers such as pancreatic cancer and breast cancer. Metformin also has anti-cancer action, which is mediated by modulation of the AMP kinase (AMPK)/mammalian target of rapamycin (mTOR) and insulin/IGF-1 signalling pathways. These findings strongly imply that metformin may play a protective role in the formation and progression of numerous human malignancies; however, the detailed mechanism of action of metformin against tumours has yet to be fully explained[7].

Microtubules are also important targets for anti-cancer drugs[8]. Mebendazole, the most commonly used anthelmintic, may prevent tumour cells from polymerizing their microtubules[9].

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In contrast to Western countries, where the occurrence of breast cancer was most prevalent in women over the age of 60, incidences of breast cancer were most prevalent in women of relatively younger age groups in Pakistan, and the majority of those patients were diagnosed at an advanced stage of disease, further worsening the prognosis. Despite the fact that we have numerous anticancer drugs and are working on them, satisfactory cancer control is still lacking due to chemotherapeutic agent resistance. There will always be a strong demand for better, more effective, and safer anticancer medications to help address this problem[10].

Intravenous drug therapy has traditionally been used to treat cancer. However, there has been a steady increase in the number of oral anticancer agents accessible in recent years, presenting apparent advantages in terms of comfort and simplicity of administration, as well as catering to patients' preference for oral treatment[11].

Thus by Utilizing carcinoma cells representative of breast, cervical, and colon malignancies, we evaluated the anticancer capabilities of newer developing affordable medicines in the field of chemotherapy.

MATERIAL AND METHODS

The study's major goal was to see how Apricoxib, Mebendazole, and Metformin affected in vitro atniproliferative effects. This study was conducted in collaboration with PCMD at the pharmacology department of BMSI, JPMC.Preclinical anti-proliferative activity of examined medicines was tested on cell lines representing breast carcinoma (MCF/MDA-MB 231), cervical cancer (Hela), and colorectal adenocarcinoma (HT29) in a dose-dependent manner. We classified the research groups into four categories: Group A (which represents Metformin treaded cells), Group B (which represents mebendazole), Group C (which represents Apricoxib), and Group D (which represents Apricoxib) (represents the Methotrexate treated cells).

Malignant cell cultures were treated with various dose ranges (6 different dilutions) of the investigated medications and incubated for at least 4 days for the study group (or 72 hours). The viability of malignant cell cultures was assessed using the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test after the required incubation duration. We repeat the test quadruply for four days, as described by Cumming et al. 2007[12].

The MTT assay is a calorimetric metabolic assay that assesses the viability of examined cells based on the principle that viable cells can reduce MTT dye into insoluble purplish color formazan, which could be quantified spectrophotometrically[13].

The cytotoxic effects of the medications under investigation were examined using percent viability of each cell line (as determined by MTT test), Absorbance value of test (At) and IC50 values. The IC50 value is the concentration at which the examined cell cultures are 50% inhibited. It's determined by plotting successive dilutions against percent inhibition. IC50 is measured in μM[14].

IBM SPSS version 21.0 had been used to analyze data. The data had first been entered into SPSS, and descriptive statistical analyses were run, with the findings reported as mean and standard deviation. To determine the mean difference of relatively similar parameters between dose - related impacts of experimental medicines, the Kruskul-Wallis statistical tool had been used. Consequently, Mann Whitney test was applied to evaluate parameters among the groups treated. A p-value of 0.05 or less was regarded statistically significant, while 0.01 or less was considered highly significant.

RESULTS

Metformin outperformed Mebendazole and Apricoxib in reducing the percentage of viability of breast and cervical cancer cell lines in preclinical cytotoxic studies.The IC50 values of these studied cell lines further supported that evidence.

As comparison of IC50 values of studied cancerius cell lines shown significant differences between all treated groups ($\chi2(2)=14.118$, $p=0.003$), ($\chi2(2)=13.257$, $p=0.004$) and ($\chi2(2)=13.5$, $p=0.004$) for MCF-7, Hela, MDA-MB-231 respectively. Pairwise comparison revealed Metformin more meritoriously decreasing the feasibilities of MCF-7, Hela and MDA-MB -231 than Mebendazole and Apricoxib at lower concentration.

However for HT-29 Mebendazole reduces the percentage viability at lower concentration than Metformin and Apricoxib, as there was statistically significant differences for IC50 value against HT-29 between treated groups ($\chi2(2)=13.296$, $p=0.004$). Pairwise comparison revealedstatistically significant

Metformin and Apricoxib were shown to have statistically significant differences in IC50 values against HT-29 across the treated groups ($p=0.004$). Metformin and Apricoxib showed statistically significant differences, however there was no difference between the Mebendazole and control groups ($U=3.00$, $p=0.149$).

Table 1: Comparison of percentage decrease of At values of MCF-7 among all treated groups

Percentage decrease N=16	Treatment Groups				p-value
	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	
Dose 0 – 1st Dose	-11.024 ± 1.169	-10.259± 1.600	-10.169 ± 1.038	-14.248 ± 2.363	0.039
Dose 0 – 2ndDose	-21.913 ± 1.583	-22.979± 2.728	-19.717 ± 1.691	-25.346 ± 2.539	0.044
Dose 0 – 3rdDose	-31.638 ± 2.509	-34.239 ± 2.376	-29.402 ± 3.029	-35.048± 3.577	0.078
Dose 0 – 4th Dose	-42.467 ± 2.178	-46.414 ± 3.183	39.362 ± 3.424	-45.307± 3.226	0.044
Dose 0 – 5th Dose	-51.299 ± 1.982	-57.84 ± 5.231	-48.274 ± 3.968	-54.428 ± 3.311	0.046
Dose 0 – 6th Dose	-61.651 ± 1.699	-68.972 ± 5.557	-53.806 ± 0.645	-64.378 ± 3.062	0.014
P-value	< 0.001	< 0.001	< 0.001	< 0.001	

Table 2: Comparison of percentage decrease of At values for MDA-MB-231 among the all treated groups

Percentage decrease (n=16)	Treatment Groups				p-value
	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	
Dose 0 – 1st Dose	-8.197 ± 1.133	-7.434± 1.086	-9.288 ± 1.022	-10.251 ± 1.788	0.104
Dose 0 – 2nd Dose	-16.414 ± 2.087	-16.079± 2.169	-17.834 ± 1.188	-18.599 ± 2.868	0.298
Dose 0 – 3rd Dose	-25.929 ± 3.109	-24.578 ± 3.237	-26.572 ± 1.632	-28.404± 3.489	0.346
Dose 0 – 4th Dose	-33.504 ± 3.763	-32.802 ± 2.989	-35.453 ± 1.496	-36.111± 3.436	0.423
Dose 0 – 5th Dose	-40.923 ± 2.703	-41.505 ± 4.682	-43.850 ± 1.13	-43.046 ± 3.073	0.210
Dose 0 – 6th Dose	-48.453 ± 3.403	-50.329 ± 4.364	-54.165 ± 1.186	-50.628± 3.462	0.192
P-value	< 0.001	< 0.001	< 0.001	< 0.001	

Table 3: Comparison of percentage decrease of At values for HT-29 human colorectal adenocarcinoma among the different dose between the treated groups

Percentage decrease (n=16)	Treatment Groups				p-value
	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	
Dose 0 – 1st Dose	-9.364 ± 1.479	-6.794± 1.367	-8.125 ± 4.227	-11.851 ± 1.588	0.036
Dose 0 – 2nd Dose	-19.286 ± 1.500	-14.631± 2.342	-15.82±5.016	-21.527 ± 1.615	0.044
Dose 0 – 3rd Dose	-28.648 ± 2.781	-21.906 ± 3.339	-22.965±5.76	-31.112± 3.152	0.026
Dose 0 – 4th Dose	-38.259 ± 3.414	-30.126 ± 5.264	-31.019± 7.358	-40.574± 3.628	0.050
Dose 0 – 5th Dose	-47.059 ± 4.358	-37.342 ± 5.904	-38.12 ± 7.788	-49.433 ± 4.655	0.028
Dose 0 – 6th Dose	-58.151 ± 5.659	-45.016 ± 7.778	-54.313±1.684	-60.874± 5.239	0.027*
p-value	< 0.001	< 0.001	< 0.001	< 0.001	

Table 4: Comparison of percentage decrease of At values for hela cell line among the all treated group

Percentage decrease (n=16)	Treatment Groups				p-value
	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	
Dose 0 – 1st Dose	-8.945 ± 1.105	-6.013± 0.538	-1.179 ± 0.262	-11.514 ± 1.00	0.004
Dose 0 – 2ndDose	-18.257 ± 0.644	-11.874± 0.948	-2.097 ± 0.380	-20.352 ± 1.042	0.003
Dose 0 – 3rdDose	-27.178 ± 0.998	-18.034 ± 1.037	-3.300 ± 0.409	-29.228± 1.132	0.004
Dose 0 – 4th Dose	-35.564 ± 0.968	-24.128 ± 1.256	-4.429 ± 0.508	-37.495± 0.976	0.003
Dose 0 – 5th Dose	-43.470 ± 1.047	-30.203 ± 0.630	-5.349 ± 0.547	-45.686 ± 1.089	0.004
Dose 0 – 6th Dose	-54.254 ± 0.905	-36.359 ± 0.977	-6.216 ± 0.701	-56.282± 1.075	0.003
p-value	< 0.001	< 0.001	0.001	< 0.001	

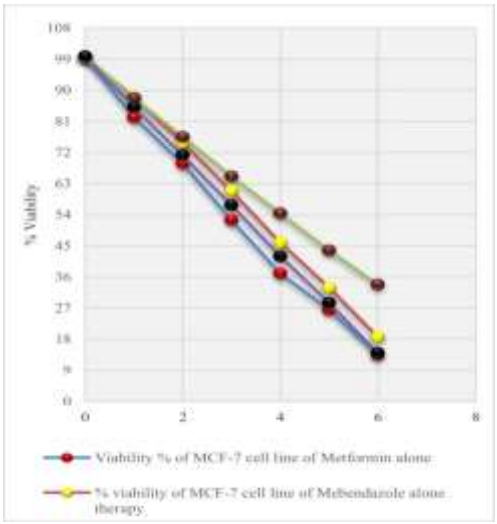

Figure 1: Comparison of Percentage Viabilities of MCF-7 treated cells among treated groups of alone therapies

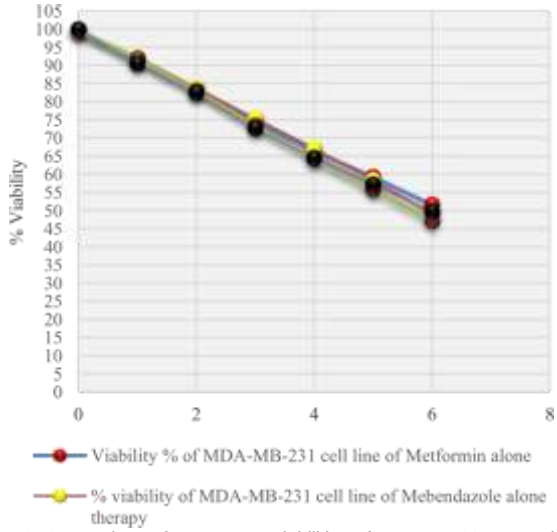

Figure 2: Comparison of percentage viabilities of MDA-MB-231 treated cells among treated groups of alone therapies

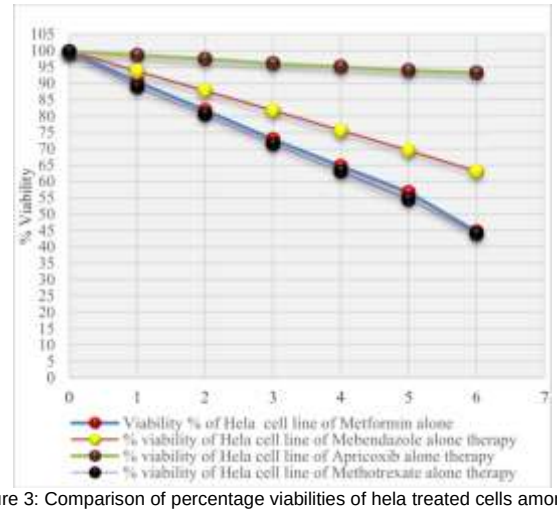

Figure 3: Comparison of percentage viabilities of hela treated cells among all treated groups

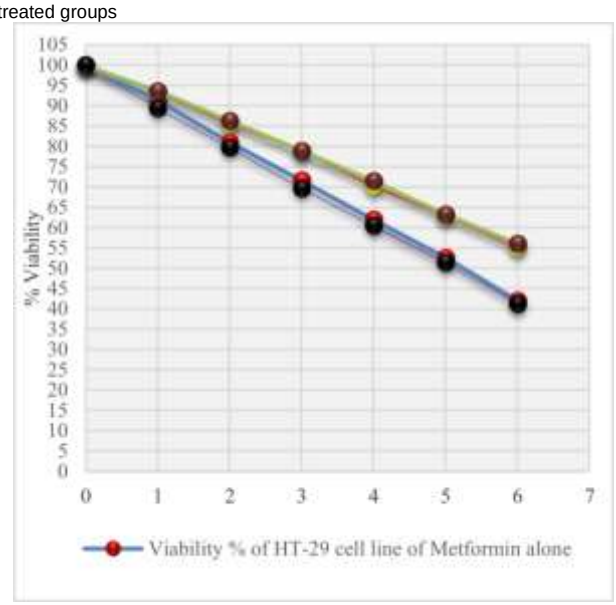

Figure 4: Comparison of percentage viabilities of HT-29 treated cells among treated groups

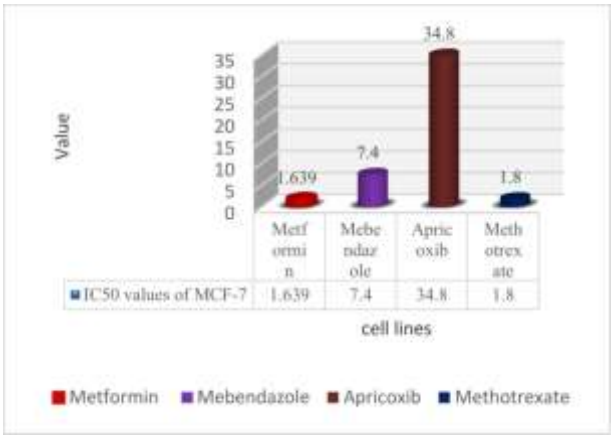

Figure 5: Comparison of IC50 values of all treatment groups against MCF-7

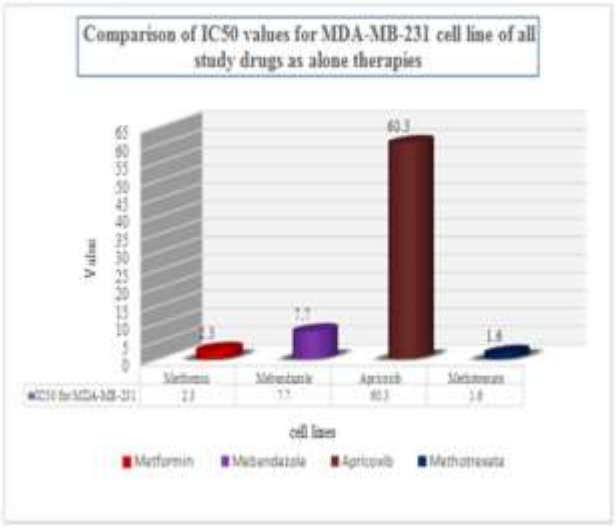

Figure 6: Comparison of IC50 values for MDA-MB-231 cell line of all treated groups

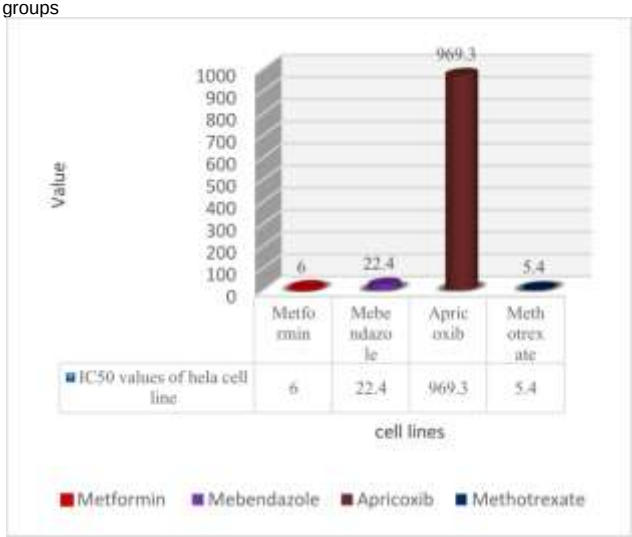

Figure 7: Comparison of IC50 values for helacell line of all treated groups

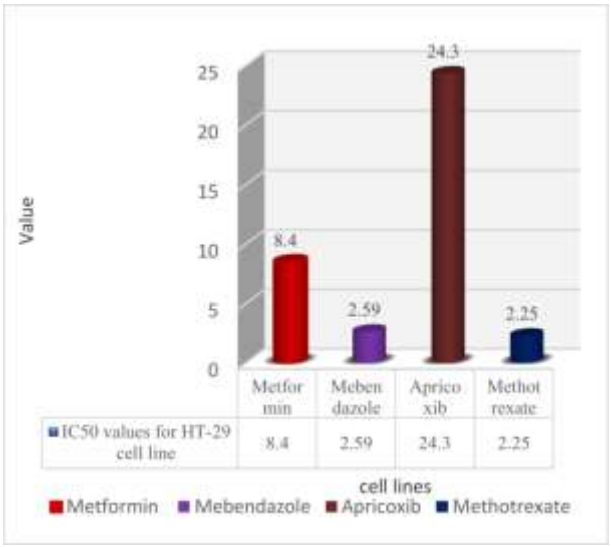

Figure 8: Comparison of IC50 values for HT-29 cell line of all treated groups

DISCUSSION

In the world, cancer is the leading cause of death. In 2005, 7 million people died from cancer, with up to 15 million more cases expected by 2020, the large bulk of which will occur in developing nations as a result of late cancer detection and poor patient compliance[15]. However, the cause of that are underprivileged compliance, financial load and parenteral therapy dangers. As a result, additional therapeutic options for cancerare being researched these days[16].

For scientific purposes, malignant cell lines have been widely used. Metformin, Mebendazole, and Apricoxib were tested in vitro for antiproliferative activity. Metformin's insulin-dependent and insulin-independent activities have been proven to be beneficial in the treatment of certain cancers[17]. Metformin can suppress mTOR pathway via actuating AMPK pathway. By developing cellular proliferation, mTOR plays a crucial role in carcinogenesis[18]. By inhibiting Cyclin D1 and their respective cyclin subordinate protein kinase, AMPK pathway activation can modify the tumour suppressor p53-p21[19].

Mebendazole was an effective anthelmintic medication that disrupted microtubule or tubulin polymerization, causing growth inhibition and cellular programmed death in malignancies[20]. Prostaglandin has another important anticancer effect. By reducing the creation of cancer-promoting prostaglandins, apricoxib can help to slow down the progression of cancer[21].

In this investigation, we found significant differences in percent viabilities of MCF-7 treated cells (as measured by the MTT assay) among all treated groups. While Metformin and Mebendazole have been demonstrated to be as effective as methotrexate in lowering At and % viabilities. However, when comparing the IC50 of the treated groups, Mebendazole lowers MCF-7's proliferative capability in vitro at higher levels than Metformin and Methotrexate.

Similarly, studies of MDA-MB-231 treated cells' viabilities revealed substantial changes across treatment groups. Mebendazole, Metformin, Methotrexate, and Apricoxib groups had mean IC50 values of 7.7±0.4, 2.3±0.5, 1.6 ±0.5, and 60.3 ±5.6 against MDA-MB231 cell line, respectively. Methotrexate hindered MDA-MB-231 proliferation quite efficaciously than Mebendazole as well as Apricoxib at lower doses, but there weren't any significant differences statistically with Metformin. Metformin was found to be just as effective as Methotrexate in lowering MDA-proliferative MB-231's potential in vitro.

These findings were consistent with those of Zakikhaniet al[22], who tested Metformin's pre-clinical anti-cancer effectiveness in different cell lines representative of breast carcinomas and used Rapamycin as their correlativestandard treatment. They accepted that Metformin lowers the viability of studied breast cancer cell lines, whereas Rapamycin does it more effectively.

Mebendazole was as effective as methotrexate in decreasing HT-29 proliferative potential, according to post-hoc analyses.The Ic50 among all treated groups were compared to those of the HT-29 cell line, confirming this conclusion. At lower doses, Mebendazole was much more beneficial than Metformin or Apricoxib in reducing HT-29 cell viability, but not as effective as Methotrexate. Nygren et al (2013)[23] found similar results in their study.

Metformin was more efficacious than Mebendazole as well as Apricoxib in diminishing the % viabilities of the HeLa cell line in our study, but not as efficacious as Methotrexate in reducing the cell proliferation abilities of the HeLa cell line.

In a cervical cancer cell line, Yudhaniet al[24]demonstrated the antitumor efficacy of and compared it to doxorubicin. Metformin inhibits cyclin D1 expression as well as p53, which dramatically reduces the replication of Cell line in a dose-dependent manner.

Metformin is the optimum candidate for additional preclinical models that illustrate its cancer advantages, according to this study, which highlights the benefit of new inexpensive oral medications against cancer.

CONCLUSION

Metformin, when compared to Mebendazole and Apricoxib, can diminish the proliferative potential of cell lines representing adenocarcinoma breast, cervical, and colonic cancer.

Conflict of interest|; Nil

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