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RESEARCH ARTICLE

ANTIPROLIFERATIVE POTENTIAL OF ESCITALOPRAM IN DIMETHYLHYDRAZINE (DMH) INDUCED COLON CANCER IN SWISS ALBINO MICE

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Abstract

A term for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems.This research emphasizes on pharmacological evaluation of anti-colon cancer effect of escitalopram in albino mice by using behavioral and hematological models. Animal House, Aryakul College of Pharmacy & Research, Lucknowprovided albino mice of either sex weighing 70-90g. All the animals were divided into 5 groups (n=6); Group 1 (NC): Mice are administered only 0.25% CMC once a week up to 28 days. Group 2 (CC): Mice are administered DMH (40mg/kg, o.d.) once a week up to 28 days. Group 3 (PC): Mice are administered DMH (40mg/kg, o.d.) once a week up to 28 days + Fluorouracil (10mg/kg) once a day for 15 days. Group 4 (T1): Mice are administered DMH (40mg/kg) once a week up to 28 days + Escitalopram (5mg/kg) once a day for 15 days. Group 5 (T2): Mice are administered DMH (40mg/kg) once in a week up to 28 days + Escitalopram (10mg/kg) once a day for 15 days.They were evaluated for parameters like determination of pH, acidity, of ALT, AST & LDH levels and estimation of lipid profile. The results of the current study demonstrate that Escitalopram administration influences ameliorating anticancer effects by altering several processes, including Phase I & II biotransformation. lipid peroxidation, enzymes, antioxidants, and histopatholgical alterations. It concluded that Escitalopram is effective in management of colon cancer in animal model. This study suggests that Escitalopram can also be given in the treatment of colorectal cancer in human beings after successful clinical trials.

Introduction:-

A term for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems.Cancer can manifest almost anywhere in body. When the body requiresregeneration, human cells frequently divide (cell proliferation & multiplication). Tumour can move across different parts of body to produce new tumour orinvadingin neighbouring tissues. Malignant tumour is another formof tumour. Blood cancer i.e.,leukaemia and other cancer types(Baldwin et al. 2016).Colorectal cancer (CRC) is the 3rdcommonest diagnosis &2nd highly deadly malignancy for human beings.

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There are significant genetic and environmental risk factors for CRC. About 5% of CRC are caused by two genetic diseases, Lynch syndrome and familial adenomatous polyposis. An invasive carcinoma requires a build-up of genetic mutations over a period of around 10 to 15 years, either somatic or germline (Xiao et al. 2019; Sokic-Milutinovic, 2019).

There are three possible forms of colon cancer (Cca): spontaneous (70 percent), familial clustering (20 percent), and inherited disorders (10 percent)(Snyder & Hampel, 2019).In US, CRC affects an estimated 135,439 new patients, of whom 95,520 (or 70%) are attributable to Cca, yearly(Celind et al. 2019; Pedersen et al. 2019). Since 2004, the incidence rate of CRC has been dropping by 3% annually while rising by 2% annually among young persons who have undergone screening (younger than 50 years)(Jalilian et al. 2019).

It takes a build-up of genetic abnormalities, either somatic/ germline, for the normal colonic epithelium to develop into a precancerous lesionand finally into invasive carcinoma(Bien et al. 2019).

Dimethylhydrazine (DMH)

In military applications, 1,1-dimethylhydrazine is largely employed as highenergy fuel as well as a rocket propellant & thruster's fuel. Humans who are exposed to 1,1-dimethylhydrazine through acute (short-term) inhalation experience nose and throat irritation, moderate conjunctivitis, nausea, and vomiting. Additionally, it is extremely corrosive and irritant to mucous membranes, eyes, and skin. Chronic (long-term) exposure to 1,1-dimethylhydrazine can harm the liver in people.

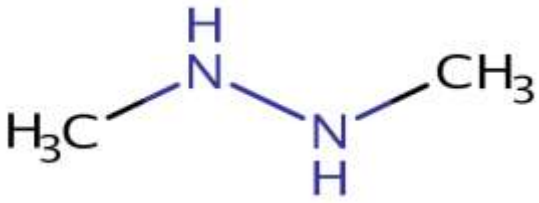

Fig. 1:- Structure of 1,2-Dimethylhydrazine.

C. formula- $C_2H_8N_2$
IUPAC Name- 1,2-Dimethylhydrazine
Physical properties

- flammable
- hygroscopic liquid

BP: 81°C (Lide, 1995)
MP: 9°C

Solubility:
Hydrophilic
Also solubilizein ethanol, diethyl-ether& other organic solvents (Budavari, 1996)
There are two isomers of dimethylhydrazine i.e., 1,2-dimethylhydrazine & 1,1-dimethylhydrazine. They both exist as transparent, colourless liquids (Trochimowicz et al. 1994). 1,1-Dimethylhydrazine is utilised as fuel for rockets and jets, as well as a plant growth regulator and a feedstock for chemical synthesis.

Escitalopram

An inhibitor of selective Serotonin-reuptake is escitalopram. It is the most selective SSRI and the S-enantiomer of citalopram. The use of SSRIs in the cure of anxiety, depression, and other associated diseases is well-known (Sanchez et al. 2014). Escitalopram are also renowned for their off-label uses for the treatment of premenstrual dysphoric disorder, pre-menstrual dysphoric disorder, obsessive-compulsive disorder, panic disorder, posttraumatic stress disorder etc (Baldwin et al. 2016).

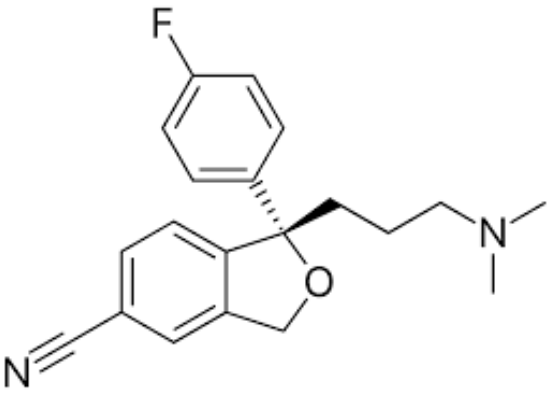

Fig. 2:- Chemical structure of Escitalopram.

The SERT also called as 5-HTT that is seen in presynaptic neurons and is the target of an SSRI's activity(Kasper et al. 2009). A wide range of human behavioural functionsi.e., mood/ appetite&addiction are modulated by serotonin or 5-hydroxytryptamine (5-HT). There are 15 serotonin receptors that have been identified, and each one is also present outside the central nervous system (Berger et al. 2009).

Based on above literature survey, I found that previous researches demonstrate the anti-depressant & anxiolytic potential using human/ animal models. But there was no data on its anti-coloncancer activity of the same.

So, this research emphasizes on pharmacological evaluation of anti-colon cancer effect of escitalopram in albino mice by using behavioral and hematological models.

Materials And Methods:-

Experimental requirements

Escitalopram, Dimethylhydramine (DMH), Swiss albino mice(either sex), distilled waterand ethanol, weighing machine, Rotatory evaporator/Water bath,

ExperimentalDesign

Animal House, Aryakul College of Pharmacy & Research, Lucknowprovided albino mice of either sex weighing 25-30g. The animals are kept in good health, with room temp.(25°C)with 12-hour light-dark environment. The relative humidity is kept at 44–56 percent, and the mice are provided a regular rodent diet and free access to water. The animals were continued to fast but have free access to water until 1 hour prior the experiment (Bhajoni et al. 2016).

Group design

All the animals are divided into 5 groups (n=6) as followings-
Group 1 (NC): Mice are administered only 0.25% CMC once a weekup to28 days.
Group 2 (CC): Mice are administered DMH (40mg/kg) route, i.p.once a weekup to 28 days.
Group 3 (PC):Mice are administered DMH (40mg/kg) once a week up to 28 days+ Fluorouracil (10mg/kg) once a day for 15 days.
Group 4 (T1): Mice are administered DMH (40mg/kg) once a week up to 28 days + Escitalopram (5mg/kg) once a day for 15 days.
Group 5 (T2): Mice are administered DMH (40mg/kg) once in a week up to 28 days + Escitalopram (10mg/kg) once a day for 15 days.
After treatment the mice were sacrificed by cervical dislocation. Stomach and liver were excised-out and thus furthers studies performed using the contents of stomach and liver.

Parameters

pH detection

Gastric content is taken out and kept in contaminated free petri-dish. After, the pH is easily measured by using digital pH meter. It confirms about the acidity level in the rodent.

Acidity
In this procedure, firstly gastric content is taken out separately. The gastric content is titrated against 0.01N NaOH to determine the total acidity level. It confirms the level of acidity and beneficial effect of drug given.

Estimation of ALT, AST & LDH levels
Fresh liver of mice was excised after sacrificing them. It was chopped and homogenized to get the uniform mixture of hepatic cells. Then, it was measured according to the procedure mentioned on kit.The animals were euthanized with an overdose of ether immediately after blood collection, the livers were removed, cleaned in saline, and one lobe of liver was sent for histology. The remaining liver lobes were homogenised, centrifuged, and the supernatant was collected for a study on antioxidants (Nimbakar et al. 2000).

Estimation of anti-oxidant level
The anti-oxidant level was estimated in terms of SOD, Catalase, GSH, Pro C & TBARS.

In a cuvette, carbonate buffer (0.5ml), EDTA (0.1ml) and epinephrine (1ml) were mixed together. The OD of produced adrenochrome was measured at 480 nm for three minutes at 30 second intervals. Standard solutions of 0.01U/ml, 0.1U/ml, 1U/ml& 10U/ml were used to create the SOD calibration curve. The enzyme activity was measured in units per minute per milligramme of tissue (Misra&Fridovich, 1972).

In addition, 1ml of material was combined with 0.2ml sodium dodecyl sulphate, 1.5ml acetic acid in HCl& 1.5mlthiobarbituric acid in hydrochloric acid. The resulting mixture was heated for 1 hour in a hot water bath at 85°C. At 532 nm, the intensity of pink color generated was measured against a blank (Ohkawa et al. 1979).

Estimation of lipid profile
Animals were starved for 16 hours after 28 days, and blood samples from the recto orbital plexus were taken. The serum was then separated to examine the levels of TC, TG, HDL, LDL, VLDL, and antioxidants.

Statistical analysis
Two-tailed t tests were used after ANOVA to assess the statistical data. Values are presented as S.E.M. Sigma Stat pro3.3 will be used to do the statistical analysis. At $P \leq 0.05$, the findings were deemed statistically significant.

Results and Discussion:-

Determination of pH & acidity
In order to determine pH & acidity of gastric content, mice were distributed in 5 groups. Group 1 served as normal control and treated with 0.25% CMC once a weekup to15 days.

Group 2 (Carcinogen control) was fed the DMH (40mg/kg) once a week up to 28 days. Group 3 (Positive control) was given DMH (40mg/kg) once a week up to 28 days + Fluorouracil (10mg/kg) once a weekup to 15 days. Group 4 (T1) was treated with

DMH (40mg/kg) once a week up to 28 days + Escitalopram (5mg/kg) once a dayup to 15 days and Group 5 (T2) was treated with DMH (40mg/kg) once a week up to 28 days + Escitalopram (10mg/kg) once a dayup to 15 days.

CC and PC showed pH as 6.5±0.68 & 7.23±0.72***, respectively whereas T1 & T2 showed pH as 7.51±0.61* & 7.34±0.74**. Both the treated groups significantly increased the pH, thus lowered the acidity in predominant manner. Acidity was noted as 73.48±2.85, 53.04±1.98** in CC & PC groups of mice, respectively. In the same ratio, T1 & T2 showed acidity level as 59.25±2.73* & 50.56±1.51***, respectively that suggests for its potent pH balancing effect of gastric content.

Table 1:- Effect of Escitalopram treatment for 15 days on pH & Acidity.

S. No.	Parameters	NC	CC	PC	T1	T2
1	pH	7.28±0.53	6.5±0.68	7.23±0.72***	7.51±0.61*	7.34±0.74**
2	Acidity	51.67±1.84	73.48±2.85	53.04±1.98**	59.25±2.73*	50.56±1.51***

Significance level was represented by *; P<0.05

n=6; readings were given in Mean± SEM

pH value and colonic acidity value in DMH treated Colon carcinogenic mice

Estimation of enzyme levels

It also significantly decreased the level of LDH when compared with control group.AST was found as 112.45±4.22U/L, 68.76±5.24**U/L, 86.71±3.76*U/L & 61.56±4.35*U/L in CC, PC, T1 & T2, respectively. While ALT was estimated as 135.34±5.97U/L, 69.36±4.56***U/L, 112.34±5.19*U/L & 69.62±2.19***U/L in CC, PC, T1 & T2 respectively. T1 & T2 predominantly lowered the LDH level as 120.29±3.92*U/L & 89.14±5.91**U/L. Thus, it reduced allenzymes level that reduced the frequency of colon cancer.

Table 2:- Effect of Escitalopram treatment for 15 days on enzyme levels.

Biological parameter	NC	CC	PC	T1	T2
AST(U/L)	74.78±2.62	112.45±4.22	68.76±5.24**	86.71±3.76*	61.56±4.35*
ALT(U/L)	68.45±2.56	135.34±5.97	69.36±4.56***	112.34±5.19*	69.62±2.19***
LDH (U/L)	86.23±4.40	135.19±5.60	87.09±4.72***	120.29±3.92*	89.14±5.91**

Significance level was represented by *; P<0.05

n=6; readings were given in Mean± SEM

AST, ALT and LDH level in DMH treated Colon carcinogenic mice

Estimation of anti-oxidant level

Anti-oxidant level was estimated in terms of SOD, Pro C, TBARS, Catalase & GSH.**CC** showed SOD level as1.29±0.98 U/µg of protein, whereas PC, T1 & T2 exhibited SOD level as 5.99±0.91*** U/µg of protein, 2.48±0.78** U/µg of protein & 4.21±0.79** U/µg of protein. CC, PC, T1 & T2 exhibited Catalases as 2.16±0.81nm, 6.63±0.5***nm, 3.91±0.81*nm & 6.69±0.63***nm, respectively. Whereas, TBARS was estimated as 51.49±1.53nm/µg, 32.10±1.12***nm/µg, 43.13±1.51*nm/µg & 34.76±1.28**nm/µg, in CC, PC, T1 & T2 groups, respectively. It also modulated Pro C as 0.99±0.23***µm/µg & 0.41±0.08***µm/µg. It significantly increased the level of GSH when compared with control group. In T1 & T2, GSH was recorded as 5.98±0.08***µm/µg & 6.87±0.10*** µm/µg, respectively.

Table 3:- Effect of Escitalopram treatment for 15 days on anti-oxidant level.

Biochemical Parameter	NC	CC	PC	T1	T2
SOD (U/µg of protein)	5.79±1.13	1.29±0.98	5.99±0.91***	2.48±0.78**	4.21±0.79**
Catalase(nM of H_2O_2/min/µg of protein)	7.95±1.01	2.16±0.81	6.63±0.5***	3.91±0.81*	6.69±0.63***
TBARS(nM/µgof protein)	32.23±1.13	51.49±1.53	32.10±1.12***	43.13±1.51*	34.76±1.28**
ProC(µM/µgof protein)	0.39±0.06	1.98±0.04	0.57±0.10***	0.99±0.23***	0.41±0.08***
GSH(µM/µg	7.48±0.12	2.99±0.07	6.32±0.10***	5.98±0.08***	6.12±0.10***

Significance level was represented by *; P<0.05

n=6; readings were given in Mean± SEM.

AST, ALT and LDH level in DMH treated Colon carcinogenic mice

Estimation of lipid profile

In estimation of lipid profile, mice were distributed in 5 groups. Group 1 served as normal control and treated with 0.25% CMC once a week up to 15 days. Group 2 (Carcinogen control) was fed the DMH (40mg/kg) once a week up to 28 days. Group 3 (Positive control) was given DMH (40mg/kg) once a week up to 28 days + Fluorouracil (10mg/kg) once a week up to 15 days. Group 4 (T1) was treated with DMH (40mg/kg) once a week up to 28 days + Escitalopram (5mg/kg) once a day up to 15 days and Group 5 (T2) was treated with DMH (40mg/kg) once a week up to 28 days + Escitalopram (10mg/kg) once a day up to 15 days.

Table 4:- Effect of Escitalopram treatment for 15 days on lipid profile.

Biological Parameter	NC	CC	PC	T1	T2
TC (mg/dl)	156.80±4.75	230.16±5.93	170.13±2.43***	188.36±3.17***	159.62±2.16***
TG (mg/dl)	99.94±2.37	205.83±5.08	132.63±6.12***	155.52±2.66***	123.64±3.31***
HDL (mg/dl)	60.67±5.41	43.54±4.33	55.38±1.84**	52.46±2.08**	57.18±2.44***
LDL (mg/dl)	76.14±2.28	145.45±1.77	88.22±2.59***	107.79±5.25***	77.71±2.04***
VLDL (mg/dl)	19.98±2.54	41.16±2.28	26.52±1.65***	31.10±1.14***	24.72±2.65***

Significance level was represented by *; P<0.05
n=6; readings were given in Mean± SEM.
AST, ALT and LDH level in DMH treated Colon carcinogenic mice

TC was observed as 170.13±2.43***mg/dl, 188.36±3.17***mg/dl & 159.62±2.16***mg/dl in PC, T1 & T2 groups. Total triglyceride level was also get lowered as 52.46±2.08**mg/dl & 123.64±3.31*** in T1 & T2 respectively. HDL was observed as 52.46±2.08**mg/dl & 57.18±2.44***mg/dl in T1 & T2 in the same order. T1 & T2 also decreased the LDL level as 107.79±5.25***mg/dl & 77.71±2.04***mg/dl, respectively. VLDL also got reduced in test groups i.e., 31.10±1.14***mg/dl & 24.72±2.65***mg/dl.

When DMH was administered, we found lower levels of SOD, GPx, CAT, and GSH, which indicates that the colon's antioxidant-dependent system was totally disrupted. These enzymes' status may have declined as a result of attenuation creation or excessive usage during the generation of free radicals. Oral DEK supplementation increased levels of CAT, GPx, SOD, and GSH in the colon and liver of tumor-bearing mice. This increase may be related to DEK's capacity to decrease lipid peroxidation while also acting as a free radical scavenger. These experiments corroborated our findings that antioxidant activities are recovered in DEK-administered animals, protecting tumorigenesis (Sadeesh et al. 2016). Due to their hydroxyl grouping in "ring and electron-offering effects," DEK could also successfully scavenge free radicals ROS. The cytochrome P450 enzymes' metabolic stimulation of DMH results in the production of active metabolites that are crucial for the development of cancer (Kang et al. 2012).

Tricyclic antidepressants did not significantly reduce the risk of colorectal cancer, which may indicate that confounding by indication accounted for at least some of the risk decrease seen with SSRI use. Only if depression was less prevalent in colorectal cancer patients than in those who did not would this form of confounding be able to explain our findings. However, there is little evidence to support this, and participants in two trials on depression and cancer who experienced depressed symptoms had non-significantly higher rates of colon cancer (Kroenke et al. 2005; Penninx et al. 1998). Additionally, the lower risk of tricyclic use raises the possibility of some form of selection bias, as patients who use antidepressants of any kind are more likely to consult a doctor and receive the diagnoses we selected as controls.

Conclusion:-

The results of the current study demonstrate that Escitalopram administration influences ameliorating anticancer effects by altering a number of processes, including Phase I &II biotransformation. lipid peroxidation, enzymes, antioxidants, and histopatholgical alterations. Escitalopram also functions as an anticancer by reducing inflammation and cell growth in DMH-administered rat colon cancer via regulating cytokine production. Additionally, thorough identification of DEK's mode of action requires molecular research.

We discovered a strong inverse relationship between regular SSRI usage and colorectal cancer risk. Like any observational studies, ours had several limitations, and our findings should be viewed as providing some, but insufficient, support for the concept put forth by the earlier research. Further research on the impact of SSRIs on the risk of colorectal cancer is necessary considering laboratory studies showing that they may suppress colon carcinogenesis.We adjusted for and made stratifications based on NSAID use as determined by chart review and automated data, but there may still be residual confounding if NSAIDs are often purchased over-the-counter but not indicated in the medical record. To document NSAID use, we used both automated pharmacy data and medical

records, but since most use is over-the-counter, there is still a chance of misclassification. It concluded that Escitalopram is effective in management of colon cancer in animal model.

This study suggests that Escitalopram can also be given in the treatment of colorectal cancer in human beings after successful clinical trials.

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Nil.

Conflict Of Interest
Authors have declared for none conflict of interest.

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