ESTROGEN ATTENUATES DIMETHYLHYDRAZINE-INDUCED COLON INJURY IN FEMALE RATS VIA ABROGATION OF OXIDATIVE STRESS AND INFLAMMATIONS

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ABSTRACT:

The involvement of estrogen, the female sex hormone, in a variety of gastrointestinal conditions has been documented. We studied the effect of endogenous and exogenous estrogen (estradiol benzoate, 30μg/kg/day S.C) for 8 weeks on early preneoplastic markers induced by the intraperitoneal injection of 1,2-dimethylhydrazine (20 mg/kg) in female rats. Either in sham rats or estradiol benzoate administered animals, estrogen abrogated tumor markers (CA 19.9 and CEA), decreased damage and inflammatory cells infiltration in colon tissue, attenuated oxidative stress markers (MDA, SOD, CAT, and GSH) and inflammatory mediators (IL-6 and IL-10). In conclusion, the estrogen protects against colon injury by reducing precancerous colonic lesions and oxidative stress. The research sheds new light on the therapeutic benefits of estrogen against colon injury in rats.

Keywords: Estrogen, dimethylhydrazine, colon injury, inflammations and oxidative stress.

1. Introduction:

The large intestine, sometimes referred to as the colon, is a part of the digestive tract. The large intestine is approximately 5 feet long, making up one-fifth of the length of the gastrointestinal tract. It is responsible for processing non-digestible food material after most nutrients are absorbed in the small intestine **(Ratto, 2017).** The colon layers influence the motility of the large intestine **(Ermund *et al.,* 2013)**. Additionally, it utilises a variety of methods to absorb water and electrolytes. The colon is essential for supplying required vitamins through an environment that is conducive for bacterial cultivation **(Azzouz and Sharma, 2018)**.

Colon cancer is the third fatal and fourth most diagnosed cancer worldwide **(Rawla, *et al.,* 2019)**. According to an epidemiological study on colon cancer prevalence, females had a greater incidence of the disease. However, women aged 18‑44 with colon cancer had an improved prognosis compared with men of the same age and women >50 years **(Bustos *et al.,* 2017)**. Moreover, an enhanced survival outcome is connected to colon cancer's up-regulated estrogen receptor beta-1 (ERβ1) expression. Likewise, ERβ1 expression down-regulation is linked to a worse survival outcome **(Konstantinopoulos *et al.,* 2003)**. In postmenopausal women receiving hormone replacement therapy (HRT), the prevalence of colon cancer fell by 30% according to the Women's Health Initiative.

1,2-Dimethylhydrazine (DMH) history begins around 1965 when the Cycas circinalis L. seed's neurotoxicity was tested. Rats fed cycad meal crude extract develop tumors in a variety of organs, such as the intestine, kidney and liver. It was discovered that the tumors in the intestine are caused by the glycoside cycasin, a β-d-glucosyloxyazoxymethane isolated from the crude material, and the first metabolite of aglycone cycasin methylazoxymethanol (MAM) **(Laqueur 1965).** The mentioned carcinogen, MAM, is produced as a result of the metabolic activation of DMH and its metabolite, azoxymethane (AOM), MAM rapidly produces methyldiazonium ion, which has the ability to alkylate macromolecules in the liver and colon and cause DNA mutations which leads to colon cancer **(Venkatachalam *et al.,* 2020)**.

Chronic DMH exposure causes adenomas to develop into adenocarcinomas, which is accompanied by the production of growth factors that promote stromal proliferation and angiogenesis, the activation of proteolytic enzymes that facilitate local invasion, and multiple changes in secretion and membrane-associated glycoproteins **(Van de Goot et al.,2003).**

The antioxidant system is crucial for neutralizing reactive oxygen species (ROS) and reducing cellular damage **(Birben *et al.,* 2012)**. Antioxidants play a key role in direct interaction with ROS, or indirect interference with its accumulation, thus, terminates its harmful effects**, (Pisoschi and Pop 2015)**. Targeting antioxidant systems is now seen to be one of the most effective ways to combat various diseases, particularly colon cancer, as they reduce the accumulative negative consequences of oxidative damage **(Schieber and Chandel 2014)**.

Surgery, radiation and chemotherapy are frequently used as treatment options for malignancies, depending on their stage. Typically, colon cancer is discovered in its

advanced stages, when the patients are already showing signs of distant metastases. Due to the disturbing rise in colon cancer cases, there is an urgent need to investigate novel treatments that could overcome the limitations of current treatments **(Braun and Seymour 2011); (Van Der Stok** *et al.,* **2017).**

Inflammation is a physiological biological reaction that occurs during the infection, damage and toxicity **(Chen** *et al.,* **2018)**. To protect our body, it causes the immune system to release pro-inflammatory mediators, but excessive inflammatory responses are quite harmful. Because it is one of the primary events in cancer, there are strong links between inflammation and cancer inflammatory responses accelerate the development of tumors by inhibiting cellular differentiation and supporting tumor growth **Klampfer, L. (2011).** Inducible nitric oxide synthase (iNOS) overproduction may result in DNA damage, DNA repair deficiencies, and the promotion of malignant growth **(Marques** *et al.,* **2019)**. Elevated IL-6 **(Zeng** *et al*. **2017; Chung** *et al.,* **2006)** and IL-10 levels **(Sullivan** *et al.,* **2022) (Li et al. 2019; Sullivan** *et al.,* **2022)** are characteristics of colon cancer and may serve as a helpful indicator of a bad prognosis in patients and may serve as a possible therapeutic target.

Aim of the work:

The present study was designed to evaluate the effect of endogenous and exogenous estrogen as estradiol benzoate (EB), (30 µg/kg/day S.C) in adult female Wistar albino rats.

Materials and methods:

Adult female Wistar albino rats about (8 weeks old, 150-170 g weight) were obtained from the animal house at the El-Nile Company for Pharmaceuticals & Chemical Industries (Cairo, Egypt). For the purpose of acclimatization of rats to our study animal house, they were kept six per cage for two weeks in controlled housing conditions (room temperature $25\pm 2°C$, humidity (50-70%) and 12/12 h dark-light cycles) and kept free on a standard diet with ad libitum access to food and water. The experiment was conducted in accordance with the ethical guidelines for investigations in laboratory animals, **(Clark** *et al.,* **1997).**

A total of 78 female Wistar albino rats were randomly divided into 8 groups as follows:

Group 1: Intact female rats were administered tap water orally and did not receive any treatment for 8 consecutive weeks and served as a normal control group.

Group 2: Sham-operated female rats were administered tap water orally and did not receive any treatment for 8 consecutive weeks.

Group 3: OVX rats were administered tap water orally and did not receive any treatment for 8 consecutive weeks.

Group 4: Sham-operated female rats were injected with DMH (20 mg/kg i.p., twice weekly) for consecutive 8 weeks.

Group 5: OVX rats were injected with DMH (20 mg/kg i.p., twice weekly) for consecutive 8 weeks.

Group 6: Sham-operated female rats were co-administered Fulvestant (5mg/kg/week S.C) **(Yamamoto et al., 2019)** with DMH (20 mg/kg, i.p. twice weekly) for consecutive 8 weeks.

 Group 7: OVX rats were co-administered Fulvestant (5mg/kg/week S.C) with DMH (20 mg/kg, i.p. twice weekly) for consecutive 8 weeks.

Group 8: OVX rats were co-administered EB (30μg/kg/day S.C) **(Abd El-Lateef et al., 2019)** with DMH (20 mg/kg, i.p. twice weekly) one week before starting DMH and continued for consecutive 8 weeks.

The 8-week study involved treating rats with the designed doses of fulvestrant and estradiol benzoate (EB) beginning one week before colon injury by DMH was induced. EB treatment continued concurrently with DMH treatment throughout the colon injury induction period. After an ovariectomy or SHAM procedure, at four weeks,

At the end of the experimental period, animals were lightly anesthetized using ether and retro-orbital plexus blood samples were collected and processed as in methodology to determine serum parameters. Then, animals were euthanized by cervical dislocation and their colons were rapidly isolated for biochemical analysis and histopathological investigation. The biochemical analysis includes tumor markers as [carbohydrate antigen 19.9(CA 19.9) and carcinogenic embryonic antigen (CEA)] and determination of colon contents of oxidative stress parameters [malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT). Furthermore, the inflammatory mediators (IL-6 and IL-10) in colon tissue were also assessed.

Drugs and Chemicals

1,2 N,N Dimethylhydrazine (DMH) was purchased from Sigma–Aldrich Corporation (Seeize, Germany). Carbohydrate Antigen 19-9 ELISA Kit and Cancer Embryogenic Antigen (CEA) ELISA Kit were purchased from (Roche Diagnostics, Basel, Switzerland). Rat IL-6 (Cat. No: PK-EL-61606R) and IL-10 (Cat.No:PK-EL-61608R) ELISA Kit were purchased from (Promokine, Heidelberg, Germany). Thiopental sodium was purchased from Egyptian International Pharmaceutical Industries Company (EIPICO) (Cairo, Egypt). Isotonic saline was bought from El-Nasr Pharmaceutical Chemicals Company (Abou-Zaabal, Egypt). Povidone-iodine was purchased from the El-Nile for Pharmaceuticals & Chemical Industries Company (Cairo, Egypt). MDA, SOD, CAT, and GSH were purchased from Bio-diagnostic Co., for research kits, Egypt. Any other chemicals or reagents used were of analytical grade.

Surgical procedures:

Animals were injected with thiopental (50 mg/kg, i.p) **(Salman et al., 2012)**, then, the bilateral ovariectomy (OVX) was performed as previously prescribed **(Lasota and Danowska-Klonowska 2004).** After fasting of the animals overnight. An identical procedure was carried out for the female SHAM procedure but without removing the ovaries. Rats were given three weeks to recover before beginning the prescribed treatment, including daily povidone-iodine wound disinfection **(Yousefzadeh *et al.,* 2020).**

Induction of Colon injury:

Colon cancer was induced with DMH, given in a dose of 20 mg/kg/twice weekly, i.p. for 8 consecutive weeks. To ensure colon injury induction in this model, a histopathological study of a colon section isolated 8 weeks after the first DMH dose was performed.

Assessment of the change in body weight, growth rate and % of mortality.

The animals were weighed before and after injection of any drug. Then, the change in body weight, growth rate, % of mortality and % of ACF incidence were calculated as follows:

The change in body weight = (Final body weight-Initial body weight)

Growth rate= (Final body weight-Initial body weight)/ Total No. of experimental days.

% of mortality= (No. of dead rats in each group /Total No. of rats in the same group) X 100

Serum collection and Tissue preparation:

Animals were given light ether anesthesia one week following the last treatment, and blood samples were taken from the retro-orbital plexus using heparinized micro-tubes. The blood was left at room temperature to coagulate, and then centrifuged at 3000 rpm for 10 min using a cooling centrifuge (Sigma 3-30k, USA). The clear supernatant serum layer was removed and placed in a deep freezer set to -80°. Animals were euthanized, and the colons were removed, cleaned from adhering fat and connective tissues, and rinsed in ice-cold isotonic saline shortly after blood was drawn, and separated into two parts. The first part was preserved for histological analysis and kept in 10% neutral buffered formalin solution in normal saline, the second part was immediately flash frozen in liquid nitrogen and kept in a separate location at -80°C for upcoming biochemical and ELISA test.

Serum and tissue biomarkers

ELISA kits and a microplate reader (Spectramax plus 384, molecular devices corporation, Sunnyvale, CA, USA) were used to measure the amounts of serum CEA and CA19.9 in the samples **(Borras *et al.,* 1995); *Ballesta et al. (1995).*** also, ELISA method

was used to assess the oxidative stress parameters in colon tissues. The mentioned test was performed according to the manufacturer's instructions.

Oxidative Stress Parameters

Estimation of Colon Malondialdehyde (MDA) Content, Colon Reduced Glutathione (GSH) Content, Superoxide Dismutase (SOD) and of Colon Catalase Activity

Histopathological examination

Colon tissue samples were fixed at 10% neutral buffered formalin solution in normalsaline.To prevent dehydration, washing was done first in sterilised water and then with successively diluted alcohol solutions. Samples were cleaned in xylene and embedded in paraffin for 24 hours at 56 degrees Celsius in a hot air oven. A sledge microtome was used to prepare paraffin bees wax tissue blocks for sectioning at a thickness of 4 µm. The tissue sections that were produced were then placed on glass slides, deparaffinized, and stained with a standard hematoxylin and eosin (H&E) stain **(Bancroft & Gamble (Eds.). (2008).** before being examined under an electric light microscope for evaluating histopathological abnormalities.

Immunoflurescience examination

Immunofluorescence staining was carried out to assess the expression of the inflammatory mediators (IL-6 and IL-10), as previously described **(Abd-Elbakky *et al.,* 2011).** Primary antibodies used were mouse monoclonal antibodies against IL-6 and IL-10), (Santa Cruz Biotechnology, Inc.; TX, USA). The slides were washed and incubated with conjugated goat anti-mouse for (IL-6 and IL-10), Alexa-conjugated goat anti-mouse for IL-6 and IL-10 secondary antibody. Nuclei were counterstained with a fluorescent stain 4′,6-diamidino-2-phenylindole (DAPI). Tissue sections were finally mounted using Fluoromount® anti-fade mounting media (Sigma Aldrich. co, St. Louis, MO, USA). They were then visualized by Leica fluorescence microscope (Leica DM 5500B, Leica Microsystems, Wetzlar, Germany). At least, 5 fields of each mouse were analyzed and the intensity of immunofluorescence was measured using Image-J software (National Institute of Health, USA).

Statistical analysis

Data comparisons were performed using analysis of variance (ANOVA) test followed by Tukey-Kramer post-hoc *t*-test for multiple comparisons between groups. The levels of significance were accepted at $p \leq 0.05$, all results were graphically displayed as means ± standard deviations (SD).

Results:

Effect of estrogen on body weight, growth rate, and mortality percentage of on DMH-induced colon injury in female rats

Body weight in the DMH-treated groups was considerably lower than the control groups (Normal control, sham-control, OVX-control). DMH treated groups all had lower gain in body weight, lower growth rate, and higher mortality % than the control groups. Moreover, Sham-DMH and OVX-DMH+EB groups trimmed the body weight loss significantly, and had more growth and less mortality rates compared with other DMH-treated groups. The differences between control groups were indefinable

Table 1

Effect of estrogen on body weight, growth rate, and mortality percentage of DMH-induced colon injury in female rats,

	Initial body Weight (g)	Final body Weight (g)	Weight Gain (g)	Growth rate	Nom. Of initial rats/group	Nom. Of dead rats/group	% Of mortality
Normal Control	162.33± 23.37	243.5±29.24	81.17±9.746	1.45	6	0	0
Sham Control	170.33±26.49	255.5±14.28	85.17±4.079	1.52	6	0	0
OVX control	173.55±24.29	260.3±10.33	86.75±1.722	1.55	6	0	0
Sham + DMH	167±14.72	233.8±10.17	66.80±1.695 ^{a}	1.19	12	2	17
OVX+DMH	162.17±17.81	194.6±23.59 ^{a,b}	32.43±3.932 ^{a,b}	0.58	12	4	33
Sham+Fulvestant + DMH	163.33±7.466	196.0±17.59 ^{a,b}	32.67±5.026 ^{a,b}	0.58	12	4	33
OVX+ Fulvestant + DMH	163.83±16.52	196.6±29.24 ^{a,b}	32.77±9.746 ^{a,b}	0.59	12	5	42
OVX + Estradiol + DMH	176.5±19.66	247.1±14.28 ^{a,c,d,e}	70.60±4.079 ^{a,c,d,e}	1.26	12	3	25

Data are presented as means ± SD six rats per group. and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. DMH: dimethylhydrazine, OVX: Ovariectomized, %: percentage.

Growth rate = (Final body weight - Initial body weight) / Total No. of experimental days.

a: significant from OVX-control, b: significant from sham-DMH, c: significant from OVX-DMH, d: significant from sham-DMH+Fulvestrant, e: significant from OVX-DMH+Fulvestrant.

Effect of Estrogen on tumor markers levels in rat serum:

Data in Fig. 1 showed that OVX-DMH group for 8 weeks significantly increased the serum levels of CEA to about 1460%, compared to normal control rats, with significant decrease to 57.46%, when   compared to sham-DMH group Also, co-administration of DMH and Fulvestrant increased the tumor markers serum concentrations as the same way. For OVX-DMH+EB group, a significant regression of tumor markers was noticed when compared to OVX-DMH+Fulvestrant group, with values of 71.7%.

Regarding CA19.9, in the OVX-DMH group significantly increased the serum levels of CA19.9 to about 1855%, compared to normal control rats, with significant decrease to 50.96%, when   compared to sham-DMH group Also, co-administration of DMH and Fulvestrant increased the tumor markers serum concentrations as the same way. For OVX-DMH+EB group, a significant regression of tumor markers was noticed when compared to OVX-DMH+Fulvestrant group, with values of 53.69%, these findings proved the protective role of estrogen on the development of colon injury into colon cancer.
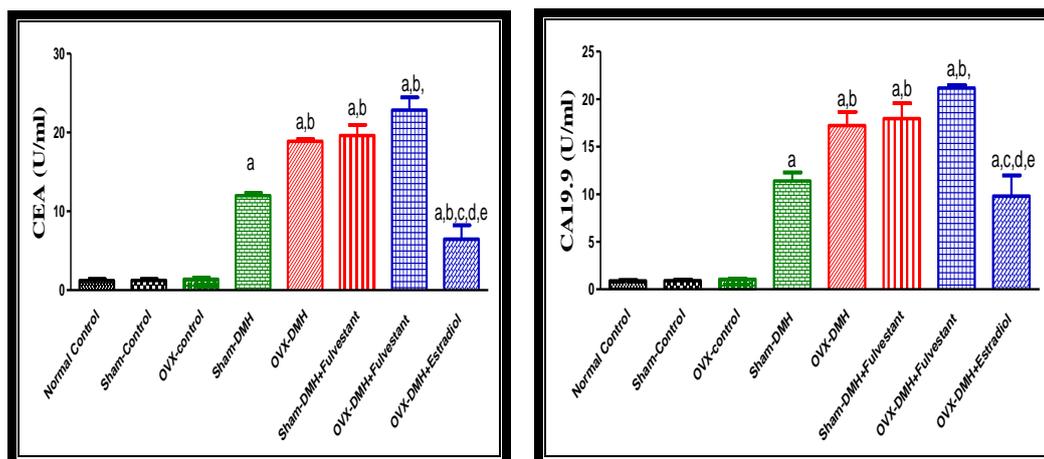
Fig.1: effect of estrogen on tumor markers colon tissue levels of DMH-induced colon injury in female rats; Data are presented as means±SD and statistically analyzed by one-way analysis of variance (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. DMH: dimethylhydrazine, OVX: Ovareictomized CEA: Cancer Embryogenic Antigen, CA19.9: Carbohydrate Antigen 19-9.
[a]: Significant from OVX-control, [b]: Significant from sham-DMH, [c]: Significant from OVX-DMH, [d]: Significant from sham-DMH+Fulvestrant, [e]: Significant from OVX-DMH+Fulvestrant.

Assessment of the colonic lipid peroxidation and oxidative stress tissue content:

The DMH treatment significantly increased the colonic MDA and decreased the colonic CAT, SOD and GSH tissue content when compared to control groups. Beside, the endogenous activity of estrogen in sham-DMH group decreased the colonic MDA and increased the colonic CAT, SOD, and GSH tissue content when compared with estrogen deprived groups (OVX-DMH, sham-DMH+Fulvestrant, and OVX-DMH+Fulvestrant groups). In a similar way, the pre-administration of EB significantly decreased the colonic MDA and increased the colonic CAT, SOD and GSH tissue content when compared to other DMH administered groups **(table.2)**.

Table.2: effect of estrogen on body lipid peroxidation and oxidative stress markers of on DMH-induced colon injury in female rats

	MDA (nmol/g)	SOD (U/gm)	CAT (mmol/g)	GSH (nmol/g)
Normal Control	2.9± 0.32	64.57±4.095	76.23±10.18	121.7±8.324
Sham Control	3.1±0.34	64.75±7.297	74.75±13.22	119.4±10.13
OVX control	3.22±0.43	64.38±1.668	72.72±7.628	122.9±4.255
Sham+DMH	$7.57±0.9^a$	$40.17±7.185^a$	$44.40±3.848^a$	$88.90±8.370^a$
OVX+DMH	$8.88±0.70^{a,b}$	$27.22±7.063^{a,b}$	$25.55±9.768^{a,b}$	$62.22±8.419^{a,b}$
Sham+Fulvestant+DMH	$9.45±0.88^{a,b}$	$21.12±7.158^{a,b}$	$17.78±9.318^{a,b}$	$61.12±11.61^{a,b}$
OVX+Fulvestant+DMH	$11.17±0.79^{a,b,c,d}$	$14.50±5.287^{a,b,c}$	$11.17±0.786^{a,b}$	$46.17±5.781^{a,b}$
OVX+Estradiol +DMH	$4.8±0.78^{a,b,c,d,e}$	$46.47±8.17^{a,c,d,e}$	$44.80±9.498^{a,c,d,e}$	$94.80±10.71^{a,c,d,e}$

Table.2: effect of estrogen on body lipid peroxidation and oxidative stress markers of on DMH-induced colon injury in female rats, Data are presented as means ± SD and statistically analyzed by one-way (ANOVA) test, followed by post hoc Tukey-Kramer test for multiple comparisons between groups. DMH: dimethylhydrazine, OVX: Ovariectomized, MDA: Malondialdehyde, SOD: Superoxide Dismutase, CAT: Catalase, GSH: Reduced Glutathione.

[a]: Significant from OVX-control, [b]: Significant from sham-DMH, [c]: Significant from OVX-DMH, [d]: Significant from sham-DMH+Fulvestrant, [e]: Significant from OVX-DMH+Fulvestrant.

Assessment of pathological changes induced by DMH:

As shown in **(Fig. 2)**, control groups showed normal epithelial mucosa without abnormalities. DMH treated groups revealed gradient degrees of tissue damage. Estrogen-deprived groups (OVX-DMH, sham-DMH+Fulvestrant, and OVX-DMH+Fulvestrant) showed the worst tissue damage picture, with different degrees of inflamed mucosa and inflammatory cell infiltrate, while EB-administered group showed the lowest degree of tissue damage markers
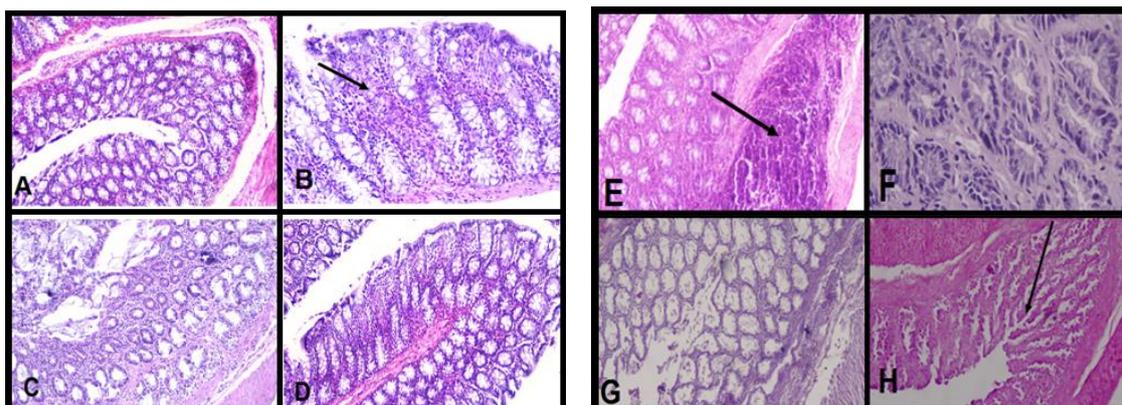

Fig.2: effect of estrogen on pathological changes in colon tissue of DMH-induced colon injury model in female rats, DMH: dimethylhydrazine, A: colonic section of control group(s) lined by blunt looking normal clonic mucosa(x200). b: colonic section of sham-DMH lined by intact epithelial mucosa with moderate degree inlammatory cell infiltrate(200X).c: colonic section of OVX-DMH, sham-DMH+Fulvestrant, and OVX-DMH+Fulvestrant groups lined eroded superficial mucosa with sever degree inflammatory cell infiltrate (200x).D: colonic section of EB administered group lined by intact epithelial mucosa with mild degree inflammatory cell infiltrate(200x).E: Colonic section of Fulvestrant- treated groups lined by inflamed mucosa with sub epithelial Collection of Lymphoid cell aggregate(200x).F: Colonic section of EB administered group lined by mild atypical cells with goblet cell depletion, hyper chromatic nuclei and infrequent mitosis (200x).G: Section of sham-DMH group showed colonic mucosa lined by devitalized ATROPHIC epithelium (200x).H: Section of OVX-DMH showed colonic mucosa lined by devitalized degenerated epithelium (200x).

**Effect of Estrogen on the expression of IL-6 and IL-10 expression in colon tissue:**

Ovariectomized-DMH administered group significantly increased the expression of IL-6 and IL-10 to 137.5 and 150%, respectively, when compared to the normal control rats. And to 61 and 45%, consecutively of the sham-DMH group. Fulvestrant treated groups exhibited a similar expression to OVX-DMH group. In contrast, administration of EB significantly decreased the expression of IL-6 and IL-10 to 33.3 and 29.9% when compared to OVX-DMH+Fulvestrant group
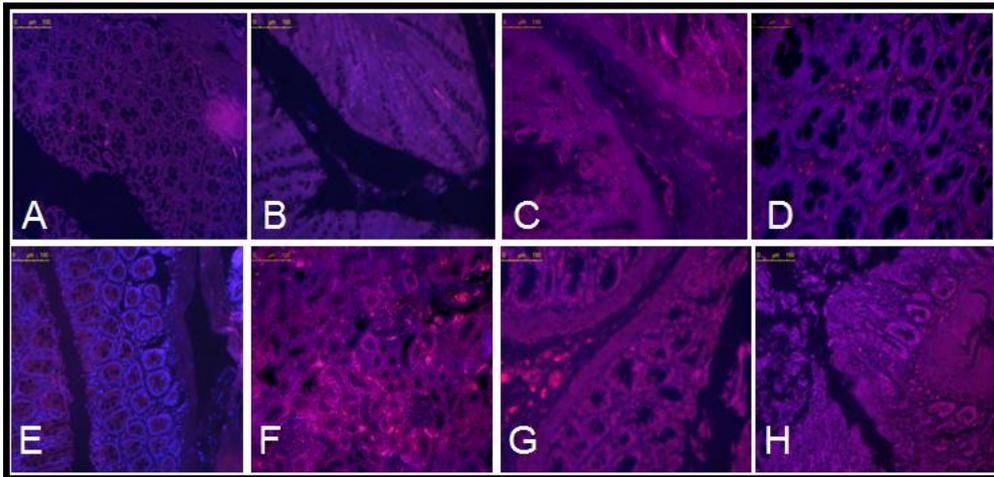
(Fig.3) Immunofluorescence staining of IL-6 on colon tissues.

Fig.3: Immunofluorescence staining of IL-6 on colon tissues exposed to DMH in the presence or absence of estrogen

A: Normal Control, B: Sham Control, C: OVX control, D: Sham+DMH, E: OVX+DMH, F: Sham+Fulvestant+DMH, G: OVX+Fulvestant+DMH, H: OVX+Estradiol +DMH
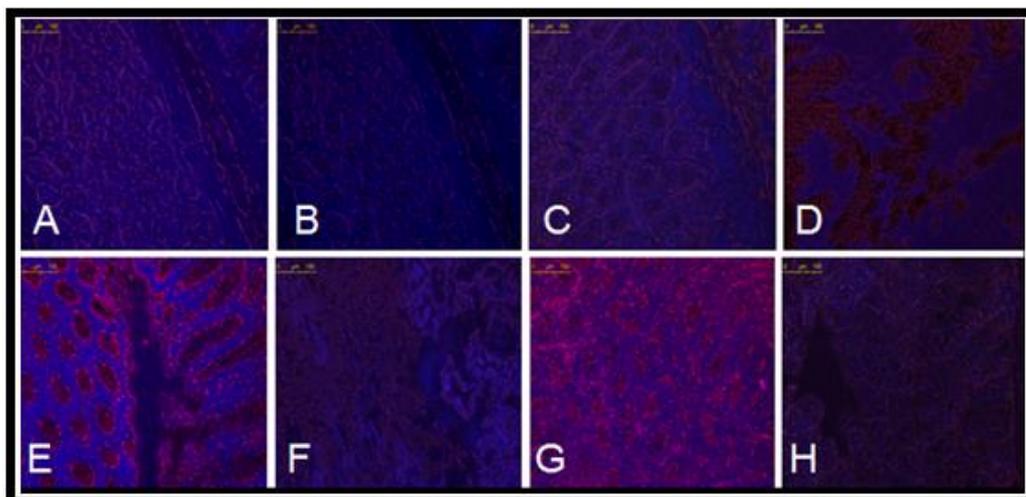
(Fig.4) Immunofluorescence staining of IL-10 on colon tissues

Fig.4: Immunofluorescence staining of IL-10 on colon tissues exposed to DMH in the presence or absence of estrogen
A: Normal Control, B: Sham Control, C: OVX control, D: Sham+DMH, E: OVX+DMH, F: Sham+Fulvestant+DMH, G: OVX+Fulvestant+DMH, H: OVX+Estradiol +DMH
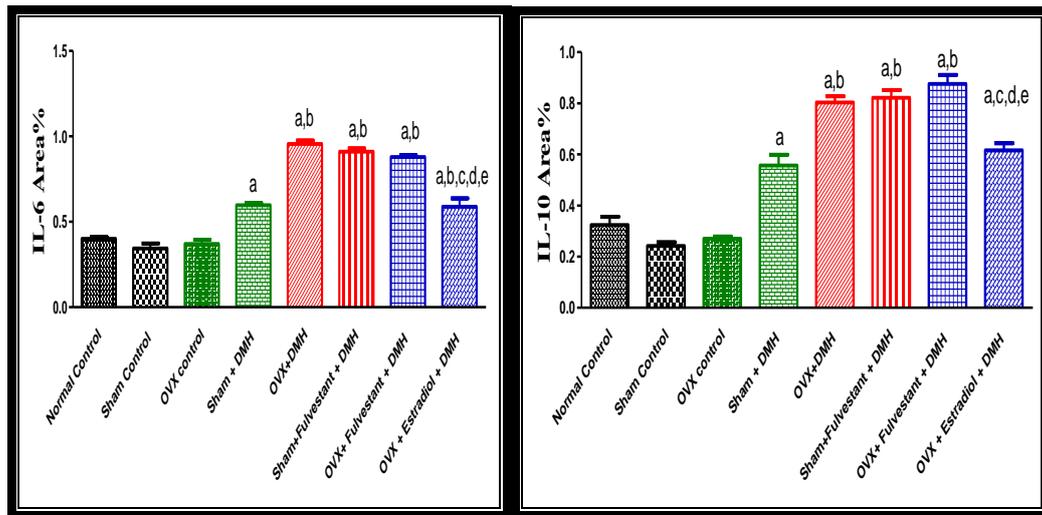

Fig.5: Effects of DMH in the presence or absence of estrogen. (A, C, E) Immunofluorescence staining of

IL-6 and IL-10 respectively on colon tissues exposed to DMH for 8 consecutive weeks in the presence (4th and 8th column) or absence (5th, and 6th and 7th columns) of estrogen. Corresponding histograms of fluorescence intensities of the captured pictures were blotted (1st and 4th column). Quantitative analysis of the fluorescence intensity of protein expression (red color for IL-6 and IL-10) obtained from 5 fields from each mouse section was performed using ImageJ software (B, D, F). Statistical analysis of normally distributed variables were tested by parametric one-way ANOVA followed by post hoc Tukey HDS test for multiple comparisons, The expression was located in colon epithelium. DMH: dimethylhydrazine, OVX: Ovariectomized, IL-6: Interlukin-6, IL-10: Interlukin-10.

[a]: Significant from OVX-control, [b]: Significant from sham-DMH, [c]: Significant from OVX-DMH, [d]: Significant from sham-DMH+Fulvestrant, [e]: Significant from OVX-DMH+Fulvestrant.

Discussion:

Colon cancer (CC) is a primary cause for cancer-related fatalities and its prevention is of great importance. The potent carcinogen 1,2-Dimethylhydrazine (DMH),

which was used in this study, is a widely used in vivo model. **(Fiala *et al.,* 1977)**. It is a common alkylating chemical used to cause malignant neoplasms in rodent colons. As a result of the DMH's metabolic activation, methyl free radicals are produced, which are known to cause oxidative stress. In the presence of a metal ion, the DMH also produces a hydroxyl radical or hydrogen peroxide, which may contribute to the beginning of lipid peroxidation. **(Kawanishi and Yamamoto, 1991)**. The commonest diagnostic for colorectal cancer patients is the CEA, which is also the most fully studied tumor-associated antigen from a biochemical and clinical perspective. **(Ogata *et al.,* 2009)**. Additionally, it has been noted that CA19-9 is an important predictor for colorectal cancer. **(Shibutani *et al.,* 2014)**. Consequently, combining these two tumour markers may result in the development of a more accurate biomarker for colorectal cancer. **(Verazin *et al.*, 1990; De Salvo *et al.*, 1997; Hara *et al.*, 2011)**.

According to our research, the body weight growth was slightly reduced in the sham+DMH group, significantly reduced in the entirely estrogen depleted groups when compared to the normal control groups, and restored in the OVX-DMH-EB treated group when compared to the estrogen deficient group. These results are in   agreement with study of **(Hassan *et al.,* 2021)**, Likewise, compared to the control group, the sham+DMH, OVX+DMH, sham+DMH+fluvestrant, and OVX+DMH+fluvestrant groups all had significantly higher plasma levels of CEA and CA19-9, which was primarily caused by an increase in the production of these molecules by cancerous cells, these results are in approval with a previous study **(Muthu and Vaiyapuri, 2013)** . These findings demonstrate the anticancer action of EB through a reduction in CEA and CA 19-9 release, which leads to a decrease in malignant cells.

The histopathological study, which showed dysplasia, hyperplasia, hyperchromaticity, ulceration, and erosion in the colon's lining epithelium as well as dense lymphocytic infiltration in the submucosa in all DMH administered with sham or OVX with fluvestrant or without it in treated rats, similarly confirmed our findings. When DMH and EB were administered simultaneously to OVX rats, the amount of hyperplasia, dysplasia, and mucosal ulceration was reduced, and there was some residual hyperchromasia in the colon, the obtained results are in agreement with study carried out with **(Parks *et al.,* 2011).**

In the environment of inflammatory areas, inflammatory mediators like IL-6 and IL-10 form a positive feedback loop to cause cellular and DNA damage by encouraging cell proliferation, ultimately resulting in the spread of cancer. It is believed that IL-6 and IL-10 are crucial in controlling the expression of the genes involved in inflammation, proliferation, and apoptosis. **(Shen and Tergaonkar, 2009)**. In this study, production of IL-6 and IL-10 may reflect the degree of inflammation in all DMH treated groups. Thus, the anti-inflammatory effect of EB might be linked with reduced IL-6 and IL-10 in DMH treated rats, which is one of the significant mechanisms for prevention of colorectal inflammation and consequently cancer by EB.

Lipid peroxidation is a result of oxidative stress, and treatment with all DMH-treated groups was associated with a substantial rise in the level of MDA, a lipid peroxidation product **(Liang *et al.,* 2014)**. MDA is a mutagen and a tumour promoter, as has already been established.**(Seven *et al.,* 1999)**. The development of colorectal cancer

can result from lipid peroxidation's induction of DNA mutation and cell proliferation **(Yau, T. M. 1979).** The current study demonstrated that all DMH-treated rats had considerably higher MDA contents in their colon tissue as compared to control rats. As DMH could induce oxidative stress **(Wongjaikam et al., 2014),** Lipid bilayers and reactive oxygen species easily interact, producing MDA, hydroperoxides, and hydroxyl radicals as byproducts. Reactive oxygen species decompose and loosen cell membranes, allowing them to enter intracellular molecules and cause mutations that are conducive to the growth of cancer. **(Wongjaikam et al., 2014).** Furthermore, our study showed decreased colon catalase, superoxide dismutase (SOD) and GSH antioxidant contents after DMH injection in ratsContrarily, our data revealed that administering EB to OVX+DMH rats reduced the amount of MDA present in the colon while increasing the levels of the antioxidants catalase, SOD, and GSH. The EB effect might be caused by free radicals that are reactive receiving electron donations, which would slow down radical chain reactions. (**El-Sayed *et al.,* 2016).** The obtained results are in agreement with the previous study of (**Hassan *et al.,* 2021**).

In conclusion, the present study indicates that estrogen has a protective effect against colon injury Our study proves that inhibition of oxidative stress and inflammation may be two important mechanisms for prevention of colon inflammation and cancer.

Nevertheless, further investigations are required to examine such an effect in human subjects.

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الإستروجين يقلل من إصابة القولون الناجم عن ثنائي ميثيل هيدرازين ويخفف الإجهاد التأكسدي والالتهابات في إناث الجرذان

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الملخص:

تم توثيق ارتباط هرمون الاستروجين ، هرمون الجنس الأنثوي ، بالعديد من أمراض الجهاز الهضمي. قمنا بدراسة تأثير الاستروجين الداخلي والخارجي (استراديول بنزوات ، 30 ميكروغرام / كغ / يوم تحت الجلد) لمدة 8 أسابيع على دلالات ما قبل الورم المبكرة الناتجة عن الحقن داخل الصفاق لـ 1،2 ثنائي ميثيل هيدرازين (20 مجم / كجم) في إناث الجرذان. سواء في الجرذان الزائفة أو الحيوانات التي تم اعطائها استراديول بنزوات ، قام الإستروجين بتحسين علامات الورم مستضد الكربوهيدرات ومستضد السرطان الجنيني وقلل من الضرر وتسلل الخلايا الالتهابية في أنسجة القولون ، وتحسين علامات الإجهاد التأكسدي (مالونداي الدهايد, جلوتاثيون, كاتلاز و سوبر أوكسيد ديسميوتاز)والوسطاءالإلتهابيين (انترلوكين 6،10). في الختام ، يحمي الإستروجين من إصابة القولون عن طريق الحد من آفات القولون السرطانية والإجهاد التأكسدي. يلقي البحث ضوءًا جديدًا على الفوائد العلاجية للإستروجين ضد عطب القولون لدى الجرذانز

الكلمات المفتاحية: الإستروجين ، ثنائي ميثيل هيدرازين ، إصابة القولون ، الالتهابات والأكسدة.