BIOCHEMICAL STUDIES ON THE EFFECT OF TURMERIC ON BREAST CANCER OF RATS

By

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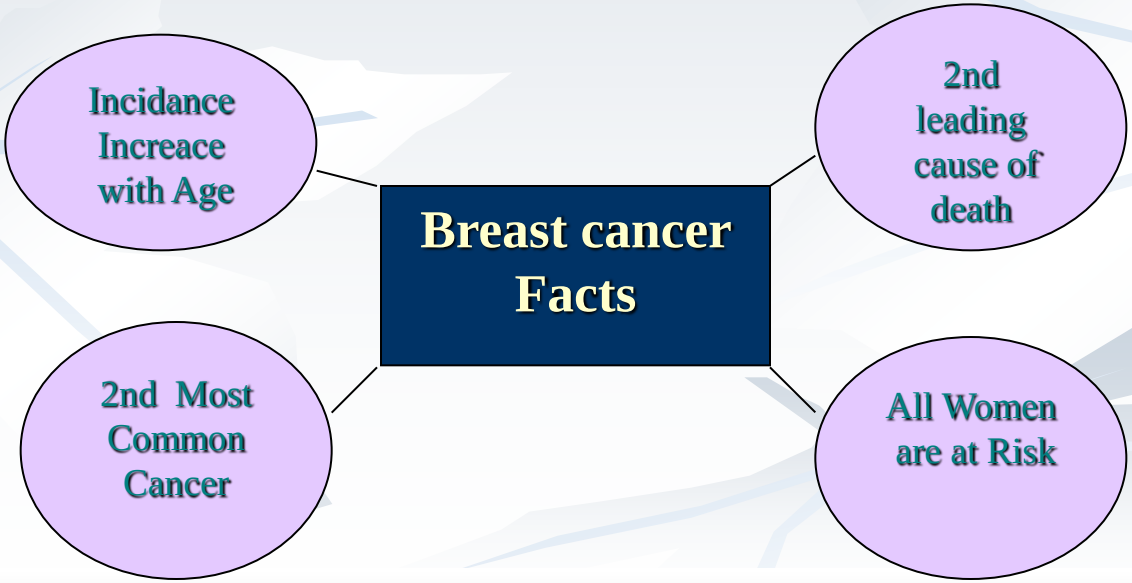
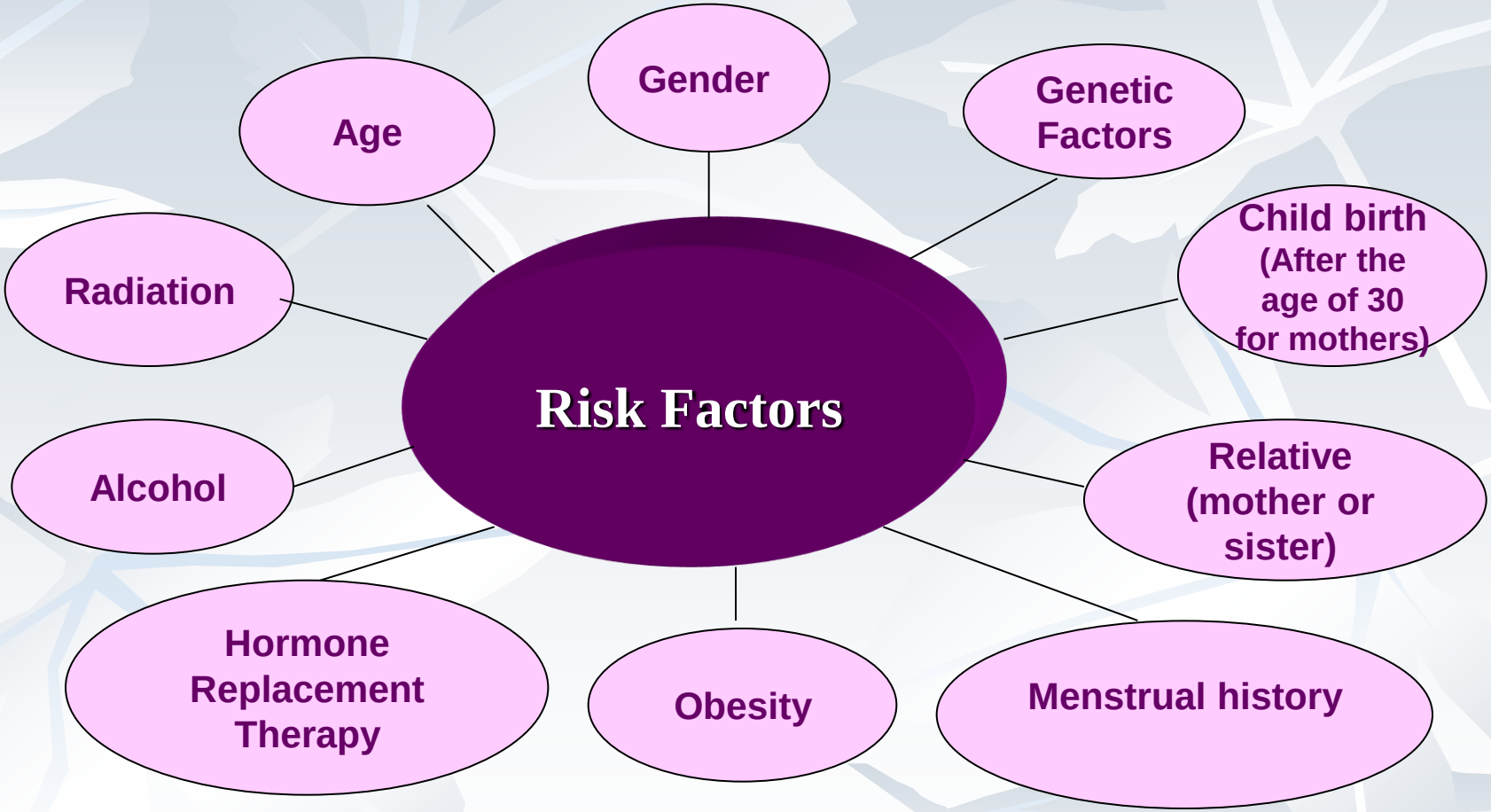
- The incidence of breast cancer has been increasing in the worldwide.
- In Egypt the most common cancers are breast cancer for females 18.9% of all cancer cases .
- The incidence of breast cancer and death rates generally increase with age.



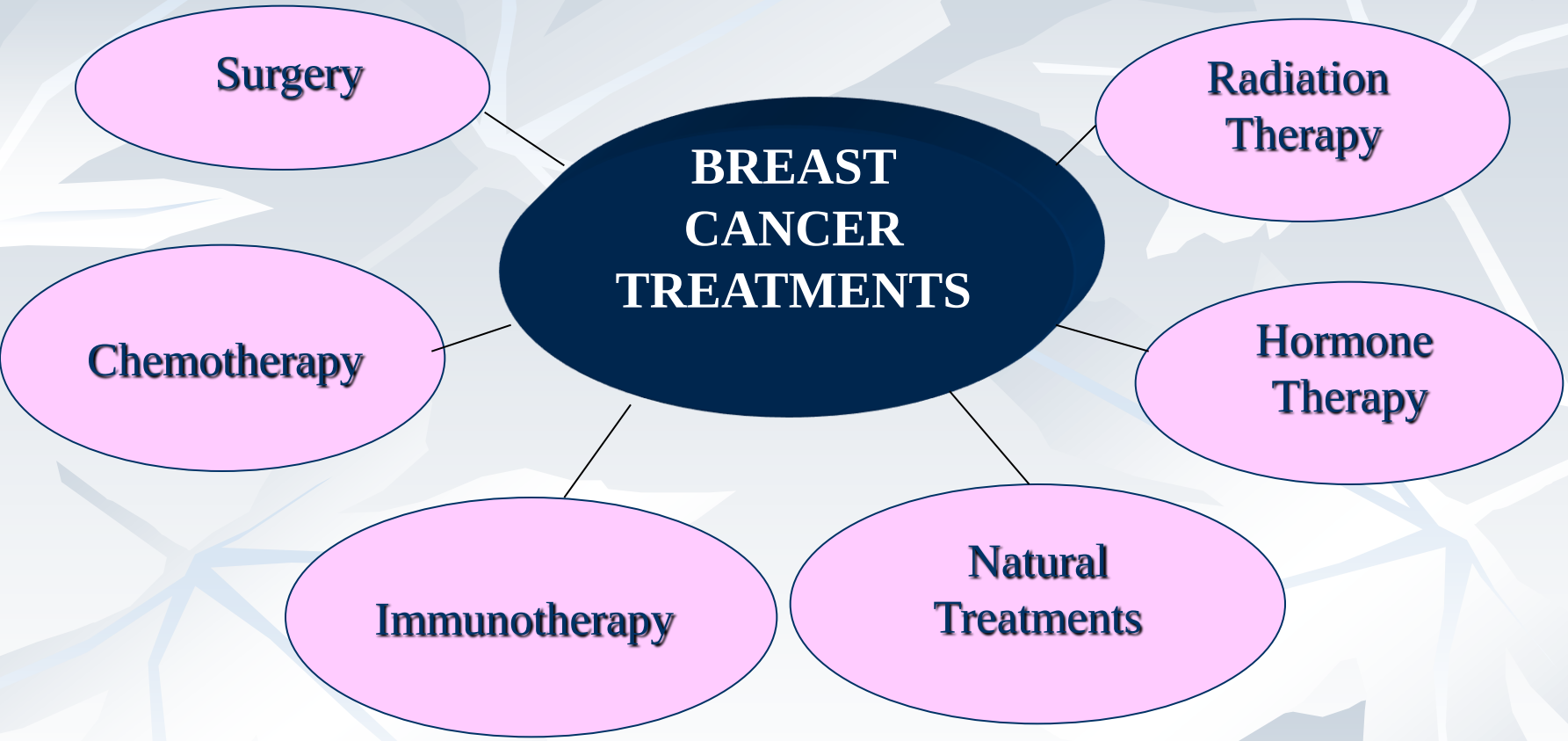
Breast Cancer Risk Factors



Diagnosis

- Clinical Breast Examination (CBE)
- Mammography
- Monthly breast self-exam (BSE)
- T-scan
- Magnetic resonance imaging
- Sentinel lymph node biopsy (SLNB)
- Ultrasound

Breast Cancer Treatments



Turmeric (*Curcuma longa L.*)

- Turmeric is a medicinal plant extensively used in many centuries for different diseases.
- Turmeric is used as a food additive (spice)
- Turmeric has good therapeutic effects include antioxidant, anti-inflammatory, anticarcinogenic and antimicrobial, hepatoprotective , thrombosuppressive, cardiovascular(protection against myocardial infarction), hypoglycemic and antiarthritic.

- Curcumin is a major active component of turmeric, the yellow-pigmented fraction of turmeric contains curcuminoids. The major curcuminoids present in turmeric are demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III), and the recently identified cyclocurcumin.

Curcumin

- **Inhibit:**

-The generation of reactive oxygen species (ROS) like superoxide anions, H2O2 and nitrite radical generation

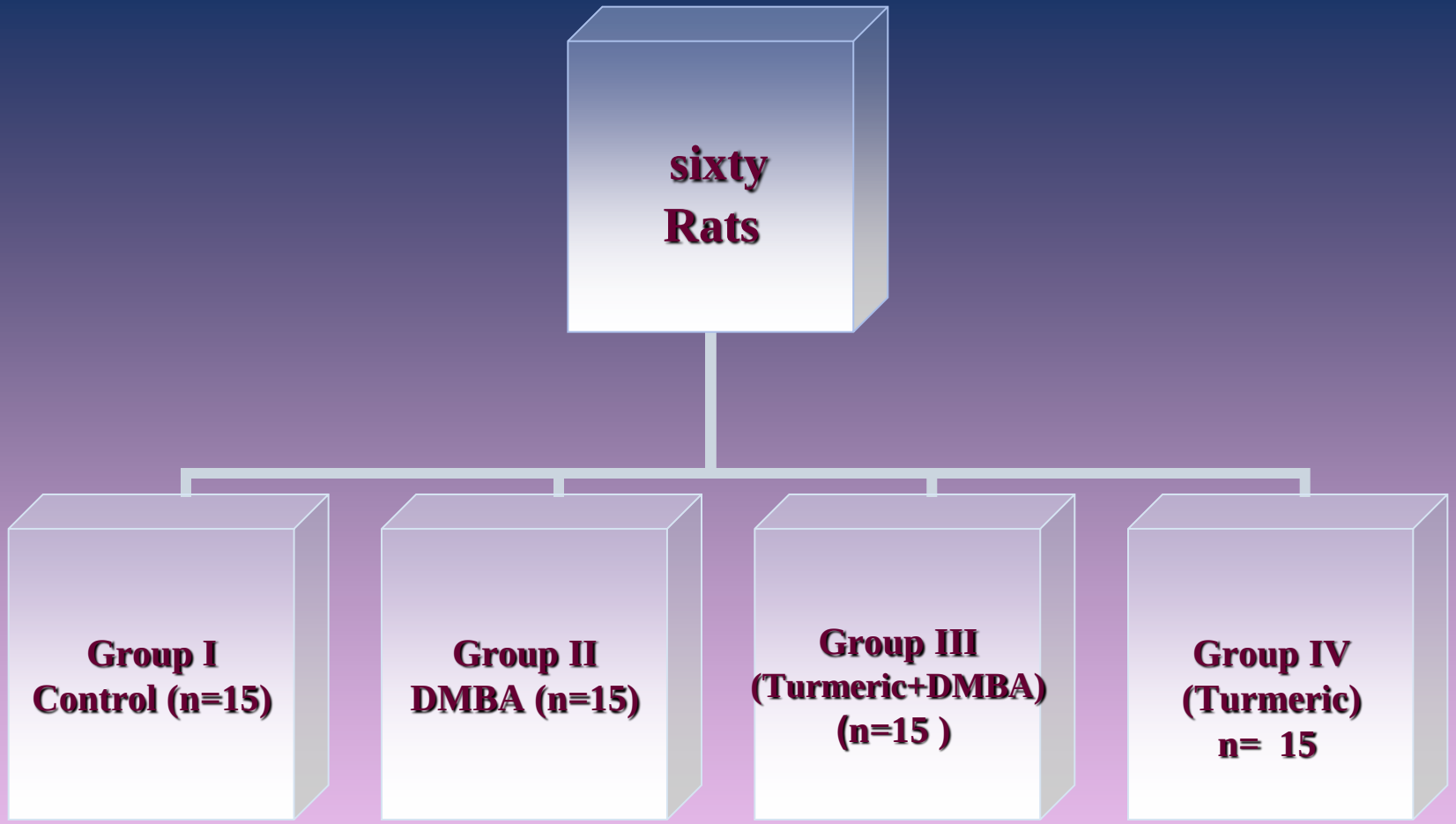
-Proliferation of tumor, transformation of cell from normal to tumor, invasion, metastasis and suppression inflammation.

- **Induction of apoptosis.**

Aim of the Work

The purpose of this study is to investigate the protection of powdered turmeric against breast cancer induced by 7,12- dimethylbenz[a]anthracene (DMBA) in female rats .

MATERIALS AND METHODS


sixty
Rats
Group I
Control (n=15)
Group II
DMBA (n=15)
Group III
(Turmeric+DMBA)
(n=15)
Group IV
(Turmeric)
n= 15

Routine laboratory investigations including:

Markers for tumorigenicity

1.Total sialic acid (TSA)
2. Carcinoembryonic Antigen (CEA)

Markers of endocrine derangement

1. Prolactin
2. Estradiol

Markers for oxidative stress

1. Nitric oxide (NO),
2. Malondialdehyde (MDA)
3. Total antioxidant activity

Histopathological examinations of the mammary glands

RESULTS

Table 1: The body weight (g) of rats in the different groups

Parameters / Groups	Body weight (g)		Increase rate of body weight %
	At start	At sacrifice	
Group I n Range Mean ± S.D.	 15 93 -128 118.6 ± 9.9	 15 185-205 194.93 ± 6.4	63. 58%
Group II n Range Mean ± S.D. p^a value	 15 100 – 130 119.1 ± 7.5	 10 129-170 148.4 ± 15.42 <0.001	27.82%
Group III n Range Mean ± S.D. $P^{(a)}$ value $P^{(b)}$value	 15 108 – 132 123.6 ± 6.7	 12 157-185 169. 7 ± 9.2 <0.001 <0.001	37.3%
Group IV n Range Mean ± S.D. $P^{(a)}$ value $P^{(b)}$ value	 15 115 – 125 120.2 ± 5.8	 13 185-215 199.39 ± 10.17 N.S. <0.001	65.88%

$P^{(a)}$ value versus control group (group I)
$P^{(b)}$ value versus DMBA treated group (group II)
Significant at P value < 0.05, N.S. non significant

Figure 1: Mean ± S.D. for rat's body weight at start and at sacrifice

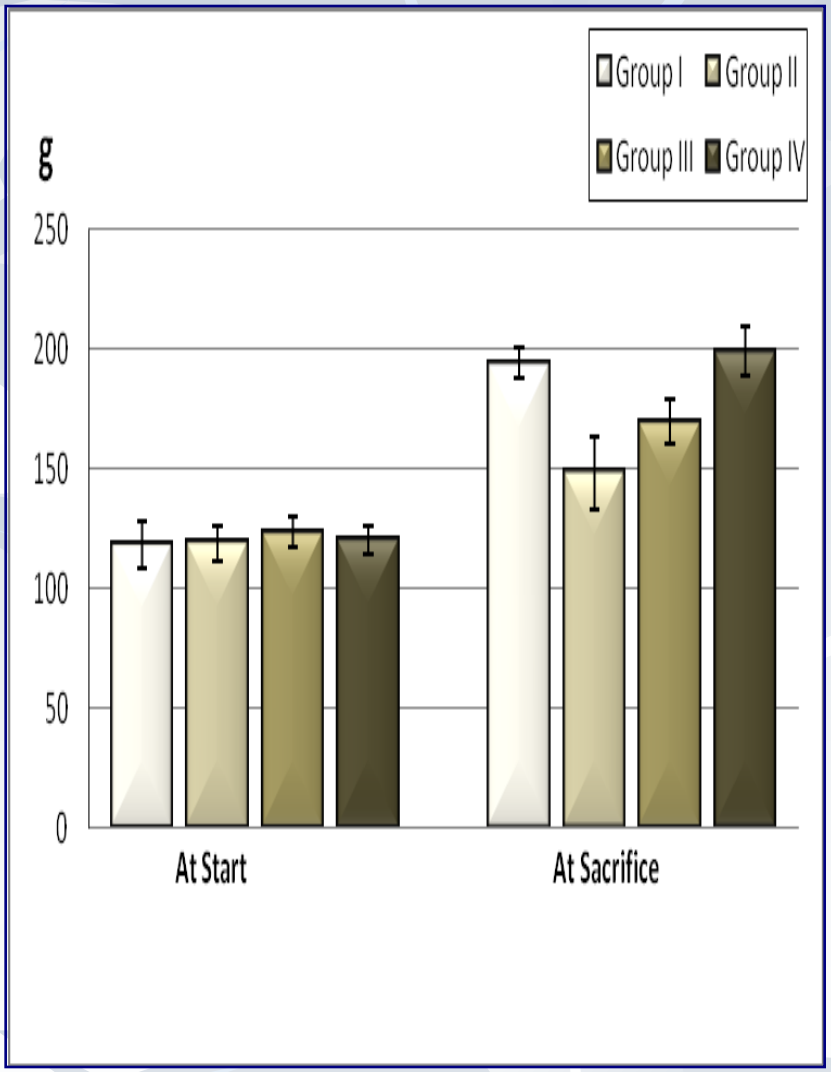


Table 2: Rat Serum Total Antioxidants Level (mM/L) in the different groups

Group / Parameter	Group I (control) n=15	Group II (DMBA) n=10	GroupIII (Turmeric+DMBA) n=12	Group IV (Turmeric) n= 13
Range	2.3 -2.9	1.55 -2.36	2.2 -2.83	1.83 - 2.84
Mean± S.D	2.76 ± 0.26	2.07±0.29	2.52 ± 0.18	2.96 ± 0.35
Percentage change		-24.93%	-8.7%	7.25%
P (a) value *P* (b) value		<0.001	0.01 <0.001	0.09 <0.001

P(a) value versus control group (group I)
P(b) value versus DMBA treated group (group II)
Significant at *P* value < 0.05, N.S. non significant

Figure 2: Mean ± S.E. For Rat Serum Total Antioxidants level In the Different Groups

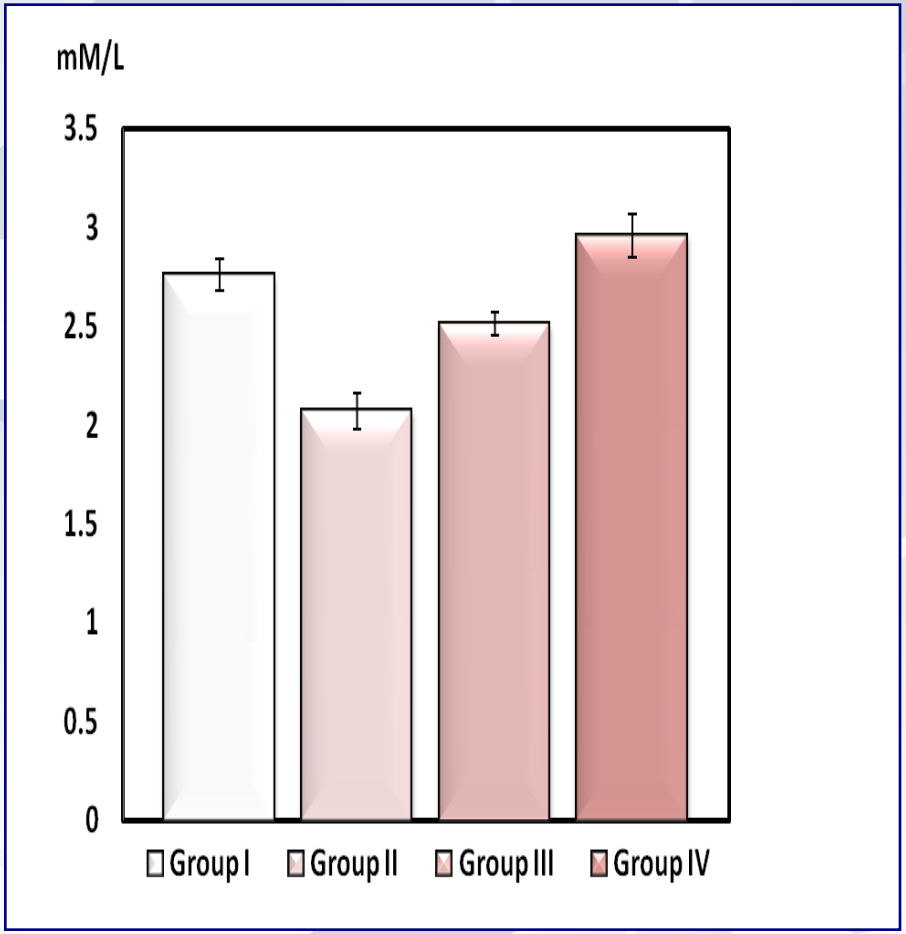


Table 3: Rat Serum Malondialdehyde Level (n mol/ml) in the different groups

Group / Parameter	Group I (control) n=15	Group II (DMBA) n=10	Group III (Turmeric+DMBA) n=12	Group IV (Turmeric) n= 13
Range	3.213 - 6.97	5.875 - 9.878	4.317 - 8.93	3.2 - 5.99
Mean± S.D	4.91 ±1.23	7.91±1.84	6.36 ± 1.43	4.6 ± 0.95
Percentage change		61.1%	29.53%	-5.5%
P (a) value		<0.001	0.009	0.5
P (b) value			0.02	<0.001

P(a) value versus control group (group I)
P(b) value versus DMBA treated group (group II)
Significant at P value < 0.05, N.S. non significant

Figure 3: Mean ± S.E. For Rat Serum Malondialdehyde level In The Different Groups

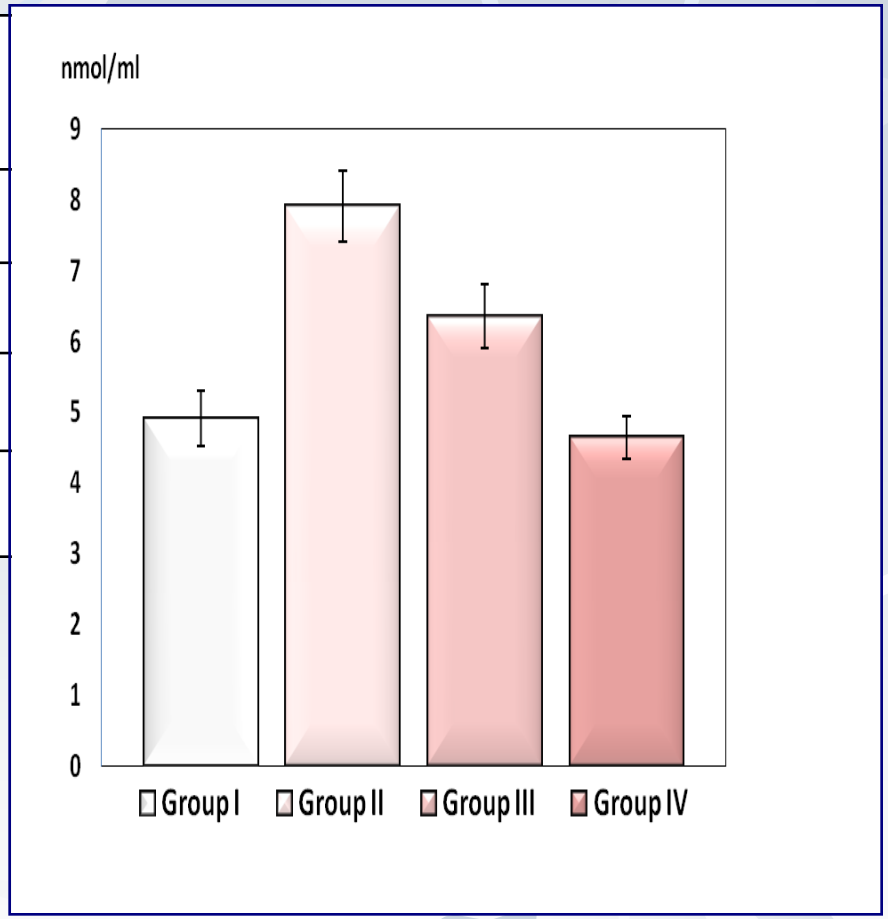


Table 4: Rat Serum Nitric Oxide (µmol/L) Level in the different groups

Group / parameter	Group I (control) n=15	Group II (DMBA) n=10	Group III (Turmeric +DMBA) n=12	Group IV (Turmeric) n= 13
Range	42 -87	120 -188	80 -110	49 – 91
Mean± S.D	66.66± 10.78	144.2± 22.18	91.92 ± 9.30	67 ± 14.24
Percentage change		116.32%	37.89%	0.51%
$P^{(a)}$ value $P^{(b)}$ value		<0.001	<0.001 <0.001	0.9 <0.001

P(a) value versus control group (group I)
P(b) value versus DMBA treated group (group II)
Significant at P value < 0.05, N.S. non significant

Figure 4: Mean±S.E. For Rat Serum Nitric Oxide level In The Different Groups

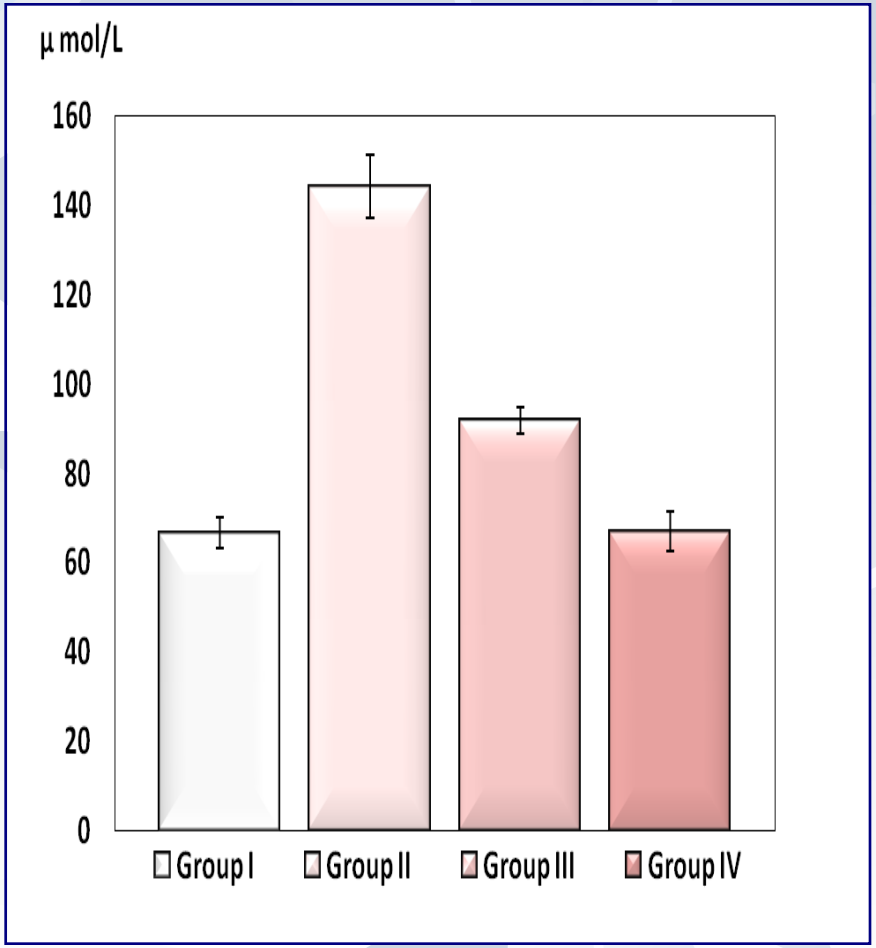


Table 5: Rat Serum Total Sialic Acid Level (ng/dL) in the different groups

Group / parameter	Group I (control) n=15	Group II (DMBA) n=10	Group III (Turmeric+DMBA) n=12	Group IV (Turmeric) n= 13
Range	54.9 - 78.3	87 - 144	63 - 114	45 – 87
Mean± S.D	64.68± 8.68	101.58±18.59	81.75 ± 14.8	62.77 ± 11.54
Percentage change		57.05%	26.39%	-2.95%
P (a) value *P* (b) value		<0.001	<0.001 0. 01	0.62 <0.001

P(a) value versus control group (group I)
P(b) value versus DMBA treated group (group II)
Significant at *P* value < 0.05, N.S. non significant

Figure 5: Mean ± S.E. For Rat Serum Total Sialic Acid level In The Different Groups

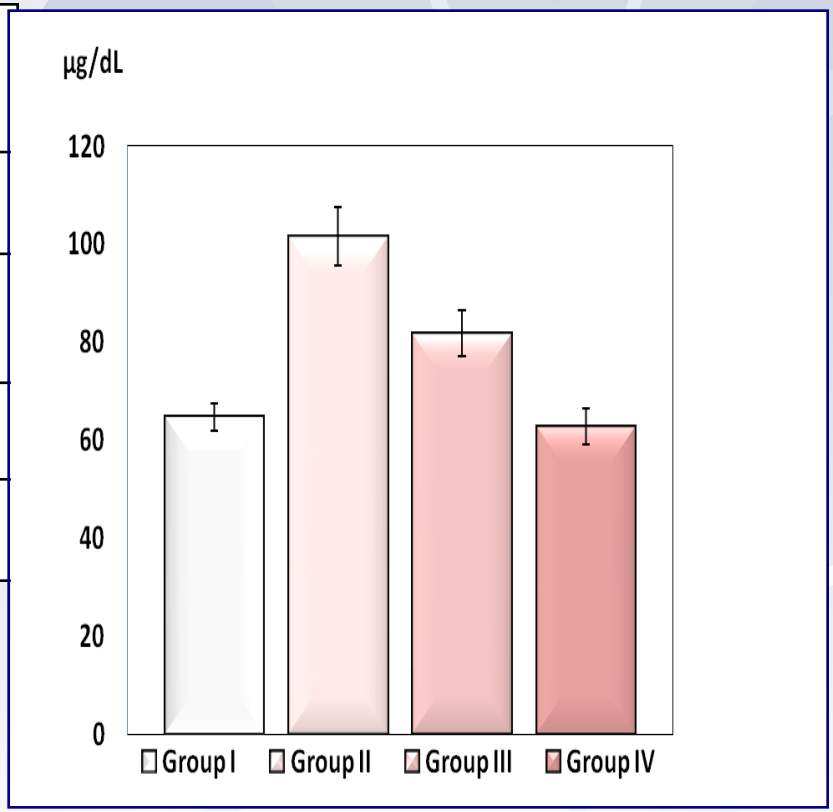


Table 6: Rat Serum CEA Level (ng/ml) in the different groups

Group / parameter	Group I (control) n=15	Group II (DMBA) n=10	Group III (Turmeric+DMBA) n=12	Group IV (Turmeric) n= 13
Range	**1.22- 3.29**	**6.13- 10.24**	**3.24 - 9.13**	**1.12 - 3.28**
Mean± S.D	**2.39±0.75**	**7.84 ± 1.27**	**5.52 ± 1.6**	**1.96 ± 0.77**
Percentage change		**228.03%**	**130.96%**	**-17.99%**
P **(a) value**		**<0.001**	**<0.001**	**0. 139**
P **(b) value**			**0.001**	**<0.001**

$P^{(a)}$ value versus control group (group I)
$P^{(b)}$ value versus DMBA treated group (group II)
Significant at *P* value < 0.05, N.S. non significant

Figure 6: Mean ± S.E. For Rat Serum CEA Level In The Different Groups

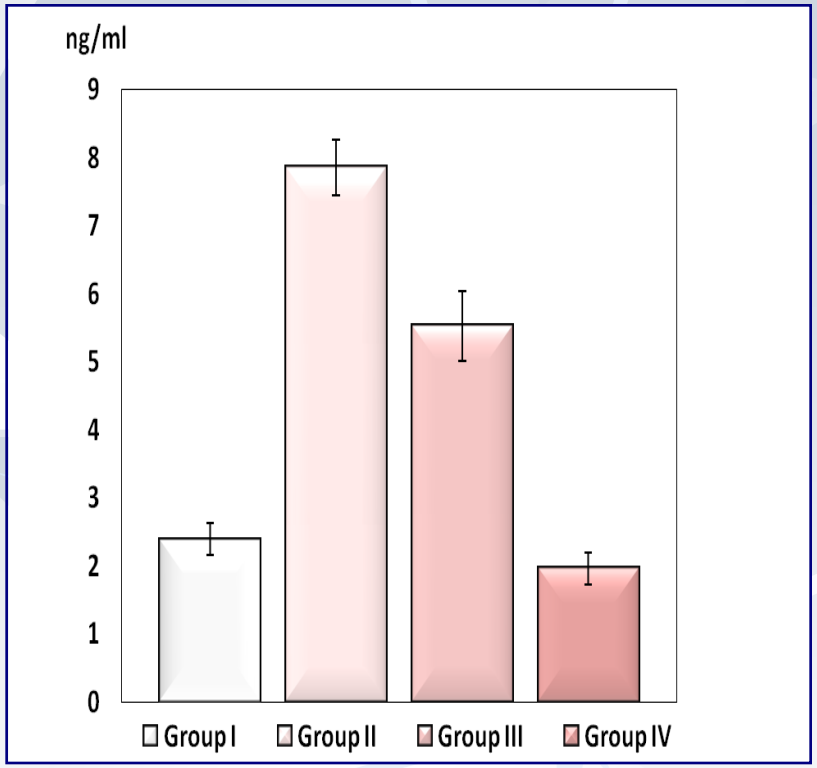


Table 7: Rat Serum Prolactin Level (ng/ml) in the different groups

Group / parameter	Group I (control) n=15	Group II (DMBA) n=10	Group III (Turmeric+DMBA) n=12	Group IV (Turmeric) n= 13
Range	1.6 - 2.6	2.3 – 4.5	1.6 - 3.6	1.3 – 2.9
Mean± S.D	2.12± 0.5	2.97±0.64	2.55± 0.56	1.92 ± 0.48
Percentage change		40.09%	20.28%	-9.43%
P (a) value *P* (b) value		0.001	0.02 0.11	0.2 <0.001

P(a) value versus control group (group I)
P(b) value versus DMBA treated group (group II)
Significant at *P* value < 0.05, N.S. non significant

Figure 7: Mean ± S.E. For Rat Serum Prolactin In The Different Groups

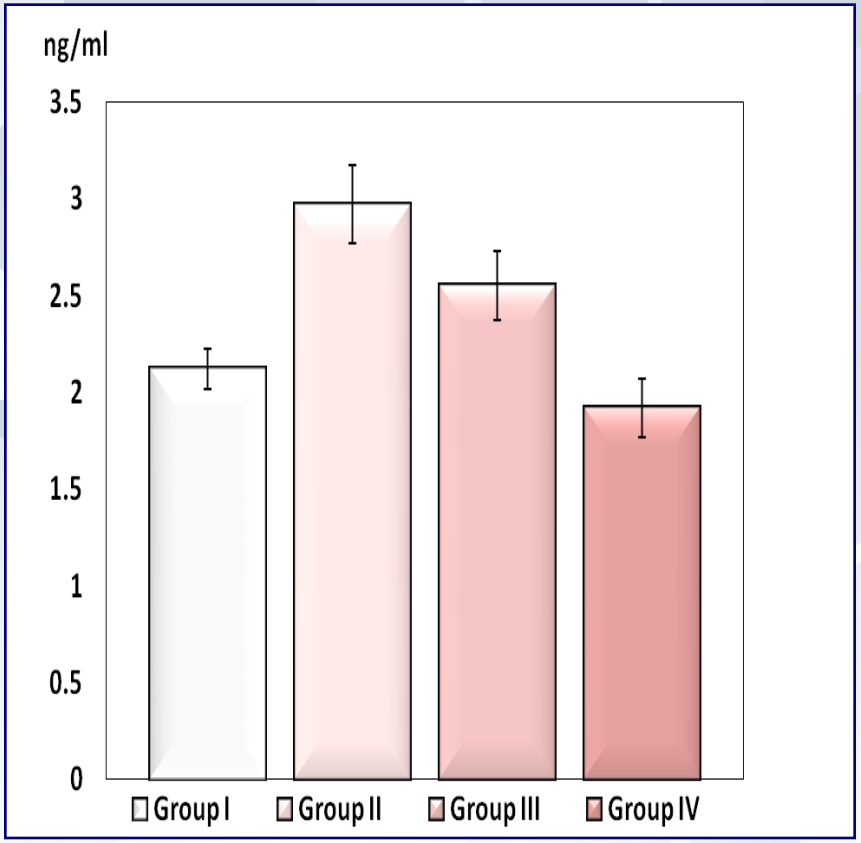
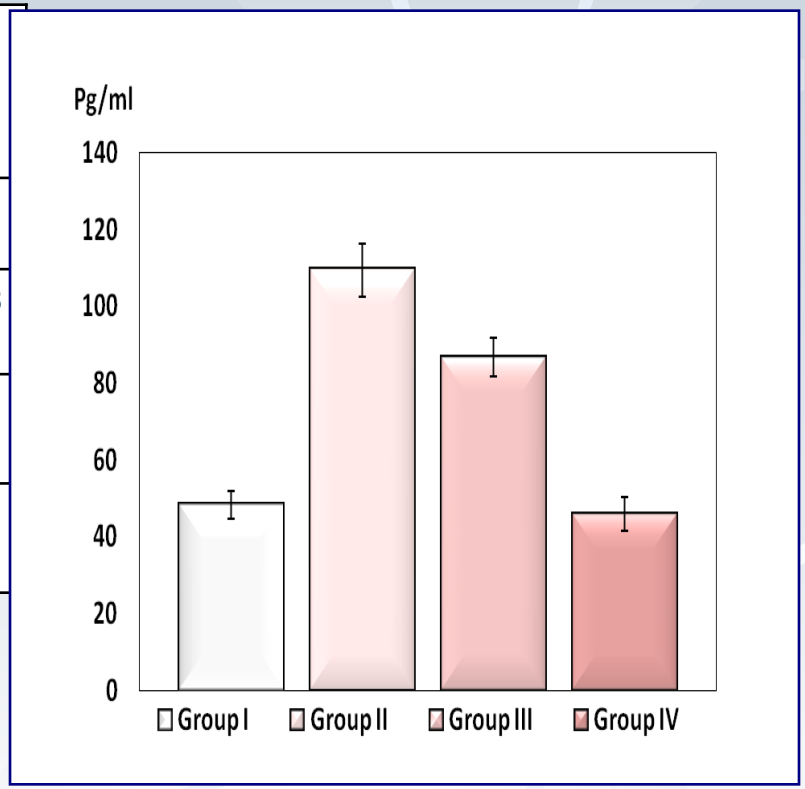


Table 8: Rat Serum Estrogen Level (Pg/ml) in the different groups

Group / parameter	Group I (control) n=15	Group II (DMBA) n=10	Group III (Turmeric+DMBA) n=12	Group IV (Turmeric) n= 13
Range	33 - 67	69 - 145	57- 109	24 - 68
Mean± S.D	48.3 ±15.81	109.4 ±22.07	86.75 ± 15.54	45.92 ± 13.48
Percentage change		126.5%	79.6%	-4.92%
P (a) value *P* (b) value		<0.001	<0.001 0. 01	0. 61 <0.001

***P*(a) value versus control group (group I)**
***P*(b) value versus DMBA treated group (group II)**
Significant at *P* value < 0.05, N.S. non significant

Figure 8: Mean ± S.E. For Rat Serum Estrogen level In The Different Groups

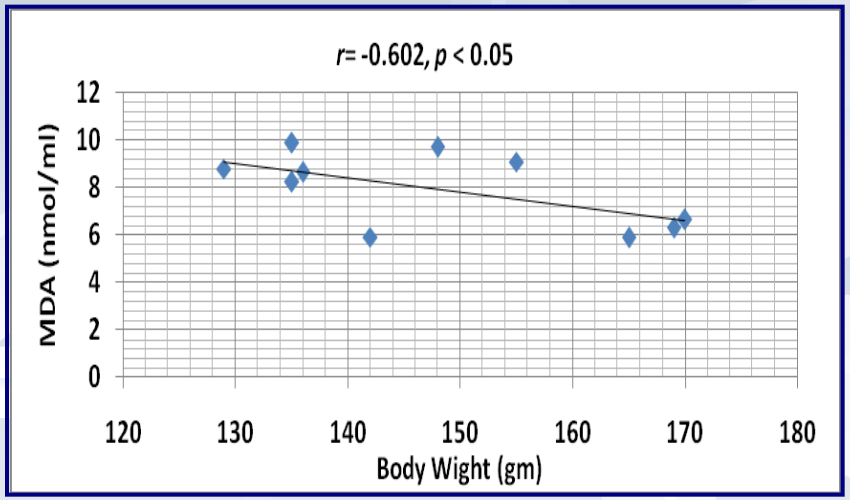


Correlation between all parameters in DMBA group

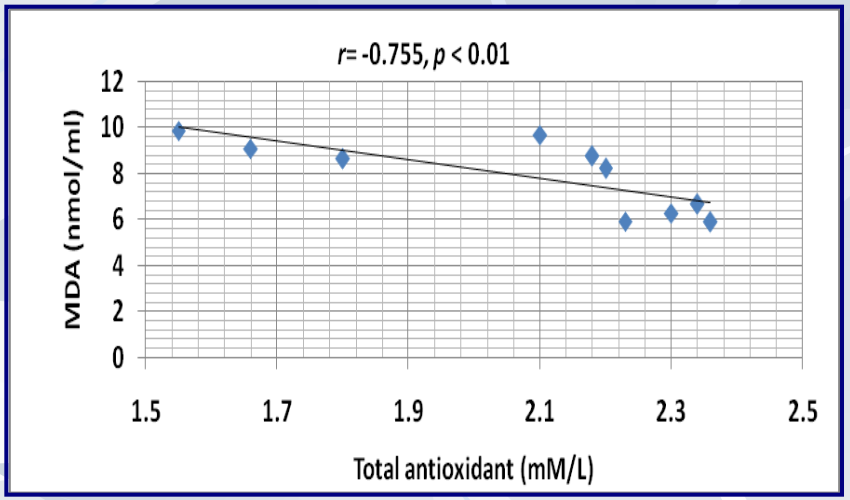
	Body Wight	Total antioxidant	MDA	NO	TSA	CEA	Prolactin
Body Wight							
Total antioxidant	***r*= 0.444** ***p*= N.S**						
MDA	**r= -0.602** ***p* < 0.05**	***r*= -0.755** ***p* < 0.01**					
NO	***r*= -0.386** ***p*= N.S**	***r*= -0.654** ***p* < 0.02**	***r*= 0.887** ***p* < 0.001**				
TSA	***r*= -0.515** ***p*= N.S**	***r*= -0.279** ***p*= N.S**	***r*= 0.685** ***p* < 0.02**	***r*= 0.849** ***p* < 0.001**			
CEA	***r* =-0.041** ***p*= N.S**	***r*=-0.805** ***p* < 0.01**	***r*= 0.698** ***p* < 0.02**	***r*= 0.630** ***p* < 0.05**	***r*= 0.245** ***p*= N.S**		
Prolactin	***r*= -0.300** ***p*= N.S**	***r*= -0.717** ***p* < 0.02**	***r*= 0.584** ***p* < 0.05**	***r*= 0.551** ***p*= N.S**	***r*= 0.126** ***p*= N.S**	***r*= 0.922** ***p* < 0.001**	
Estrogen	***r*= -0.226** ***p*= N.S**	***r*= -0.752** ***p* < 0.01**	***r*= 0.868** ***p* < 0.001**	***r*= 0.775** ***p* < 0.01**	***r*= 0.457** ***p*= N.S**	***r*= 0.914** ***p* < 0.001**	***r*= 0.857** ***p* < 0.001**

r= Correlation coefficient, r (+) = Positive correlation, r (-) = negative correlation, N.S. = Non significant, Significant at p value <0.05.

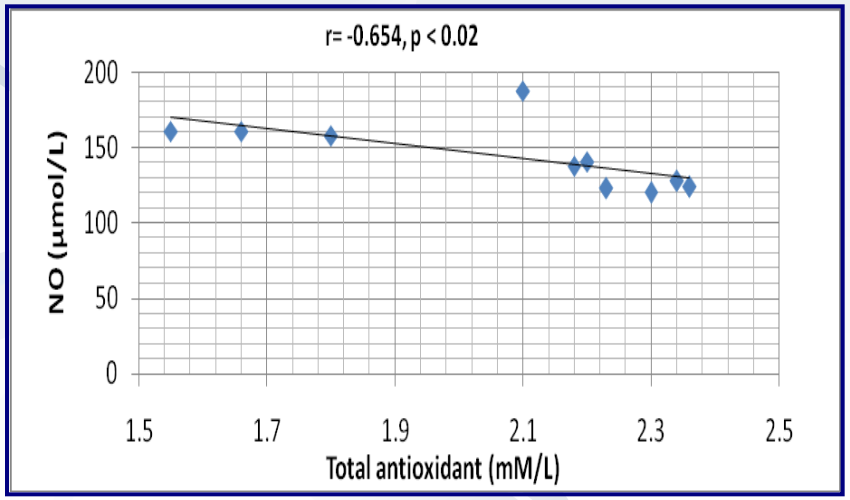
(1)


r= -0.602, p < 0.05
MDA (nmol/ml)
12
10
8
6
4
2
0
120
130
140
150
160
170
180
Body Wight (gm)

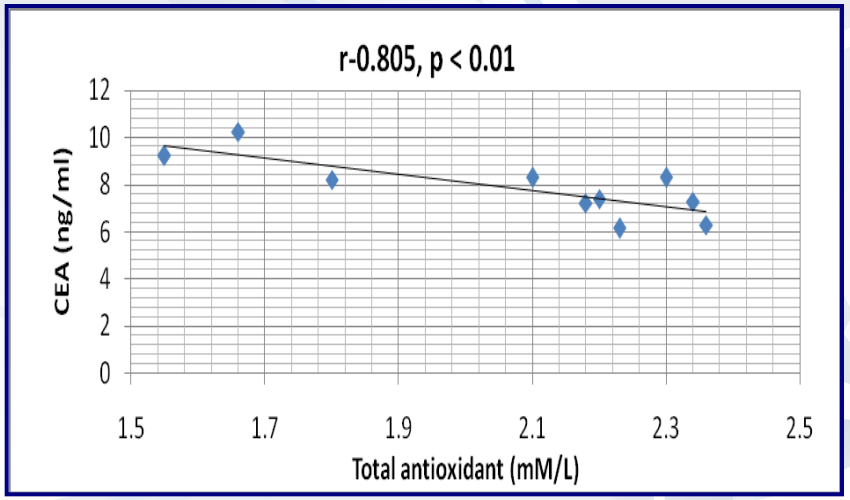
(2)


r= -0.755, p < 0.01
MDA (nmol/ml)
12
10
8
6
4
2
0
1.5
1.7
1.9
2.1
2.3
2.5
Total antioxidant (mM/L)

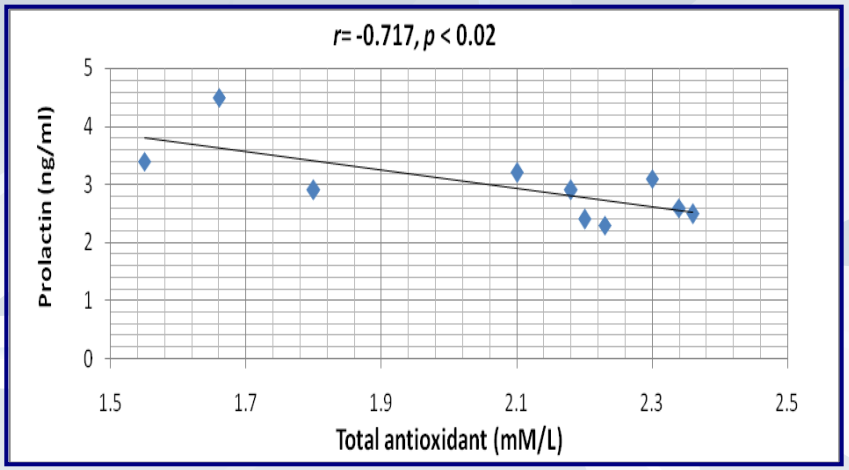
(3)


r= -0.654, p < 0.02
NO (µmol/L)
200
150
100
50
0
1.5
1.7
1.9
2.1
2.3
2.5
Total antioxidant (mM/L)

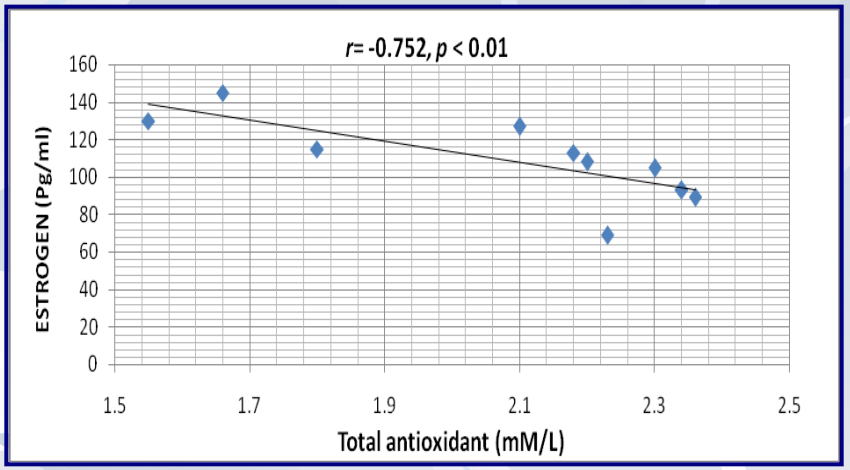
(4)


r-0.805, p < 0.01
CEA (ng/ml)
12
10
8
6
4
2
0
1.5
1.7
1.9
2.1
2.3
2.5
Total antioxidant (mM/L)

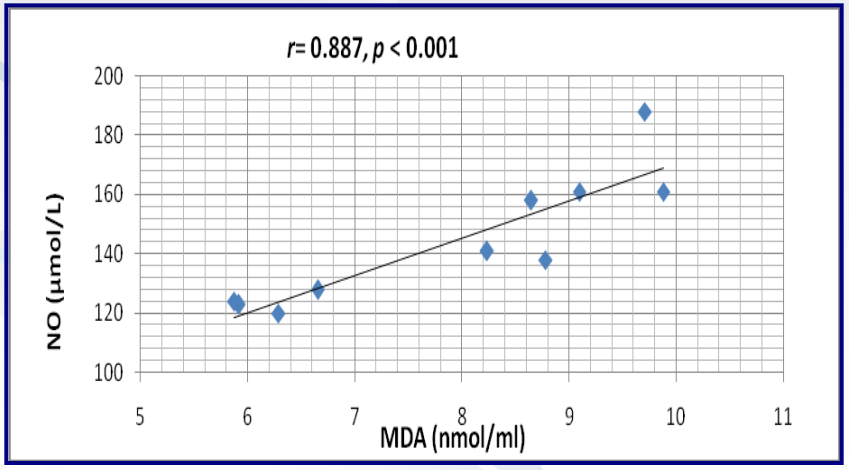
(5)


r= -0.717, p < 0.02
Prolactin (ng/ml)
0
1
2
3
4
5
1.5
1.7
1.9
2.1
2.3
2.5
Total antioxidant (mM/L)

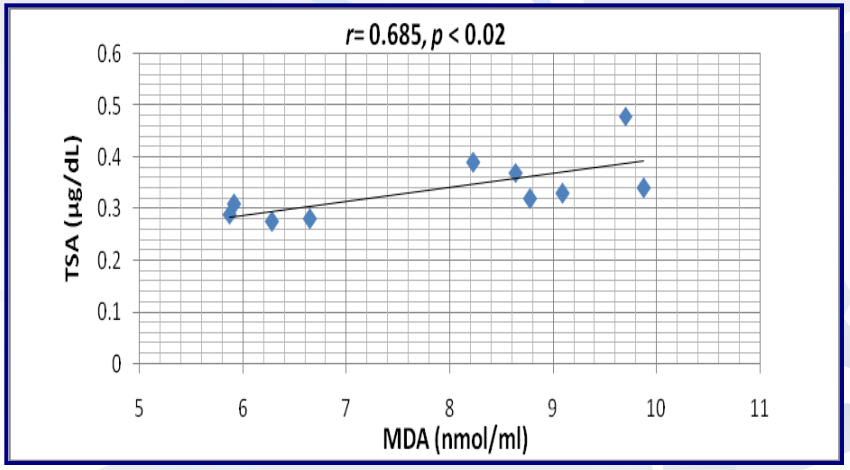
(6)


r= -0.752, p < 0.01
ESTROGEN (Pg/ml)
0
20
40
60
80
100
120
140
160
1.5
1.7
1.9
2.1
2.3
2.5
Total antioxidant (mM/L)

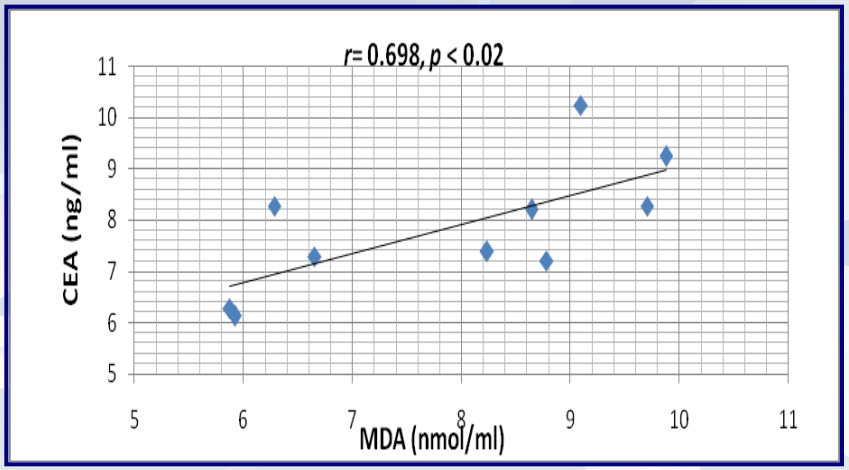
(7)


r= 0.887, p < 0.001
NO (µmol/L)
100
120
140
160
180
200
5
6
7
8
9
10
11
MDA (nmol/ml)

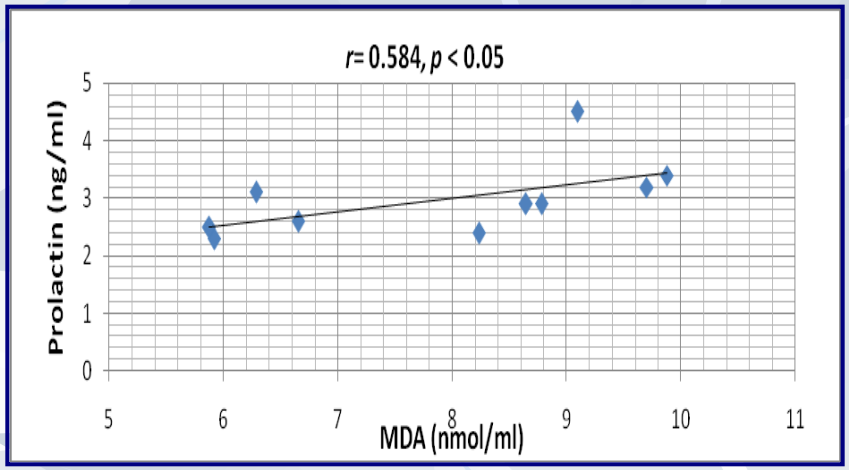
(8)


r= 0.685, p < 0.02
TSA (µg/dL)
0
0.1
0.2
0.3
0.4
0.5
0.6
5
6
7
8
9
10
11
MDA (nmol/ml)

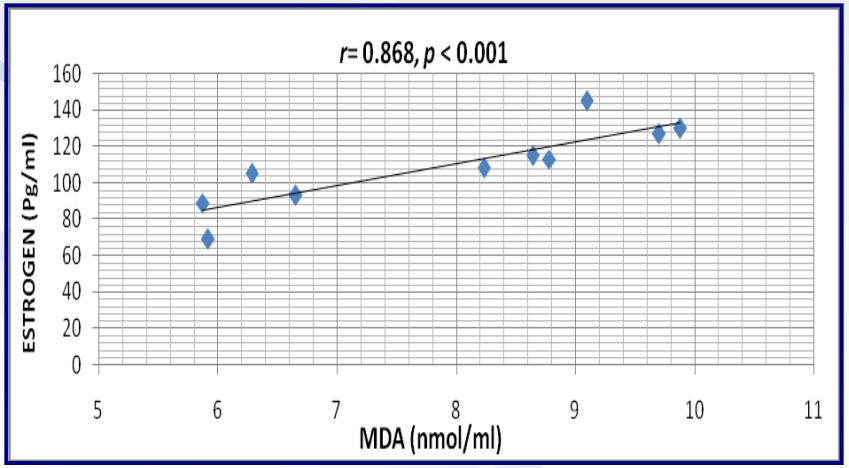
(9)


r= 0.698, p < 0.02
CEA (ng/ml)
MDA (nmol/ml)

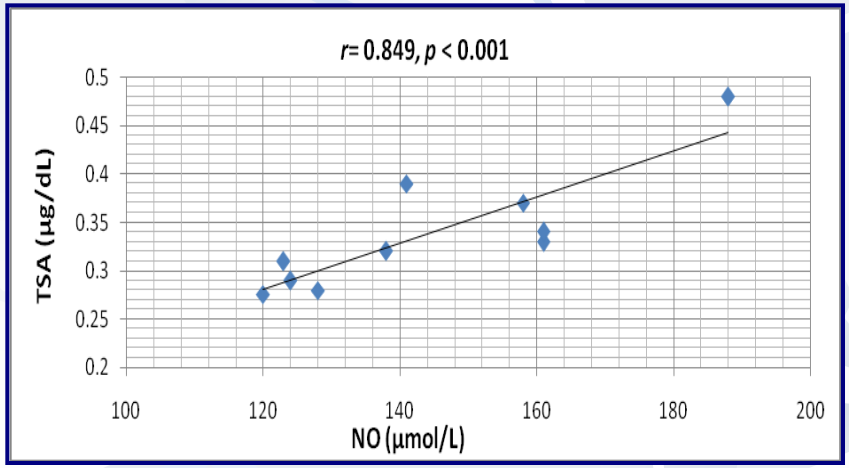
(10)


r= 0.584, p < 0.05
Prolactin (ng/ml)
MDA (nmol/ml)

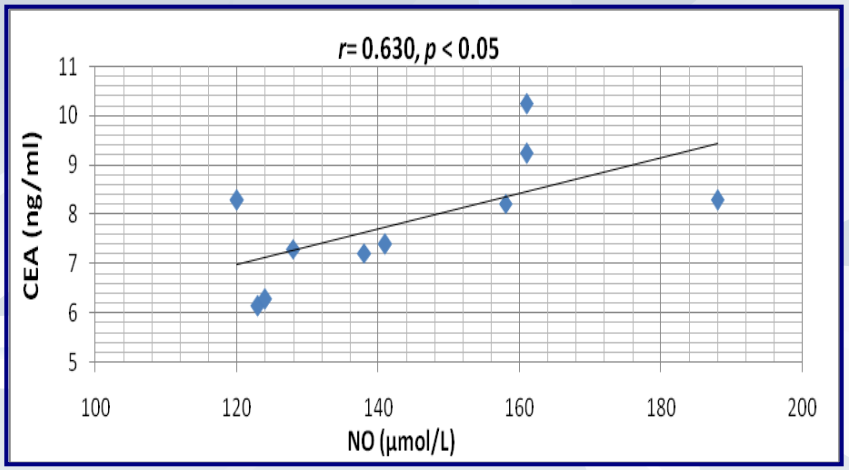
(11)


r= 0.868, p < 0.001
ESTROGEN (Pg/ml)
MDA (nmol/ml)

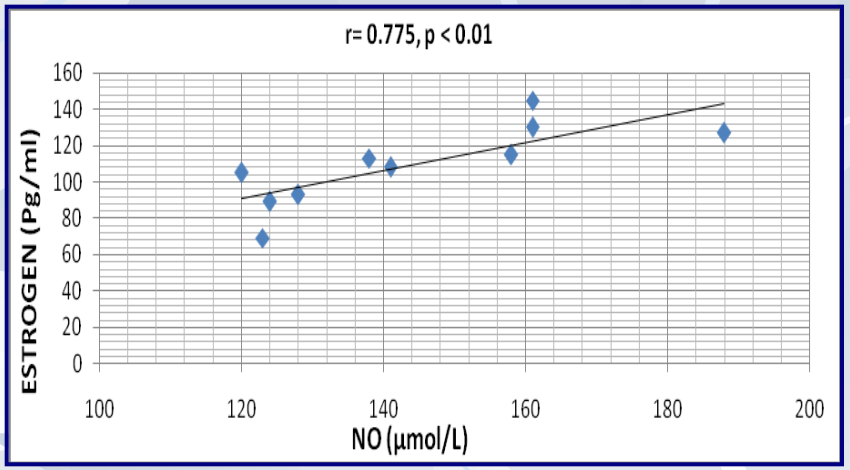
(12)


r= 0.849, p < 0.001
TSA (µg/dL)
NO (µmol/L)

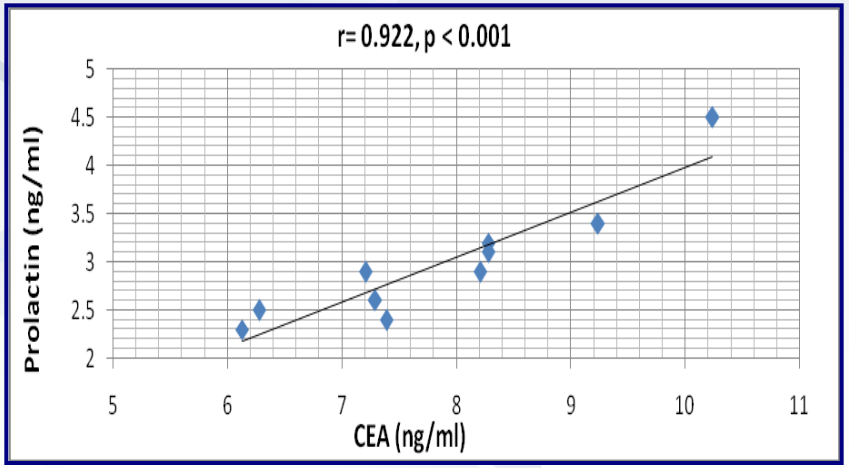
(13)


r= 0.630, p < 0.05
CEA (ng/ml)
NO (µmol/L)
11
10
9
8
7
6
5
100
120
140
160
180
200

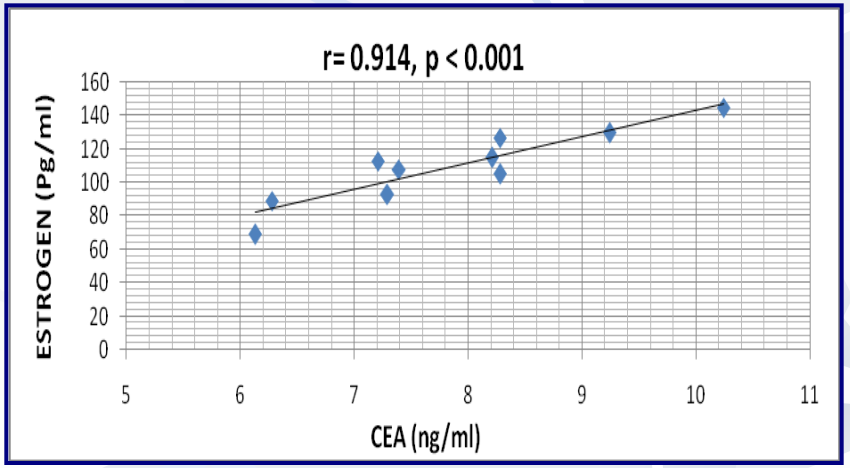
(14)


r= 0.775, p < 0.01
ESTROGEN (Pg/ml)
NO (µmol/L)
160
140
120
100
80
60
40
20
0
100
120
140
160
180
200

(15)


r= 0.922, p < 0.001
Prolactin (ng/ml)
CEA (ng/ml)
5
4.5
4
3.5
3
2.5
2
5
6
7
8
9
10
11

(16)

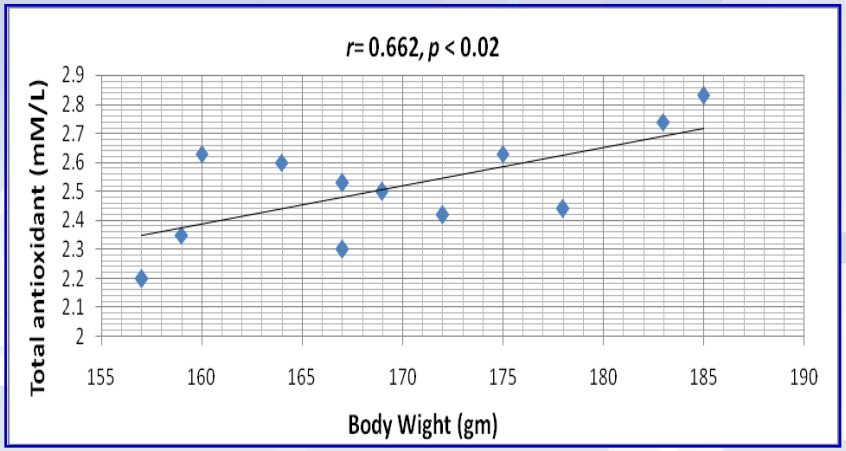

r= 0.914, p < 0.001
ESTROGEN (Pg/ml)
CEA (ng/ml)
160
140
120
100
80
60
40
20
0
5
6
7
8
9
10
11

Correlation between all parameters in TU+DMBA(group III)

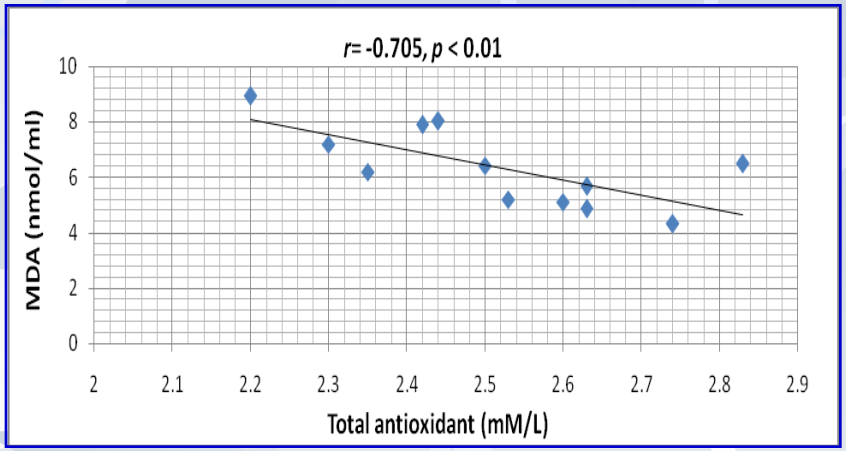
	Body Wight	Total antioxidant	MDA	NO	TSA	CEA	Prolactin
Body Wight							
Total antioxidant	***r*= 0.662** ***p* < 0.02**						
MDA	***r*= -0.222** ***p*= N.S**	***r*= -0.705** ***p* < 0.01**					
NO	***r*= -0.265** ***p*= N.S**	***r*= -0.538** ***p* < 0.05**	***r*= 0.638** ***p* < 0.02**				
TSA	***r*= -0.335** ***p*= N.S**	***r*= -0.574** ***p* < 0.01**	***r*= 0.7403** ***p* < 0.01**	***r*= 0.900** ***p* < 0.001**			
CEA	***r*= -0.227** ***p*= N.S**	***r*= -0.454** ***p*= N.S**	***r*= 0.652** ***p* < 0.02**	***r*= 0.464** ***p*= N.S**	***r*= 0.630** ***p* < 0.02**		
Prolactin	***r*= -0.267** ***p*= N.S**	***r*= -0.336** ***p*= N.S**	***r*= 0.492** ***p*= N.S**	***r*= 0.499** ***p*= N.S**	***r*=0. 505** ***p*= N.S**	***r*= 0.336** ***p*= N.S**	
ESTROGEN	***r*= -0.392** ***p*= N.S**	***r*= -0.366** ***p*= N.S**	***r*= 0.626** ***p* < 0.02**	***r*= 0.635** ***p* < 0.02**	***r*= 0.677** ***p* < 0.01**	***r*= 0.316** ***p*= N.S**	***r*= 0.810** ***p* < 0.001**

r= Correlation coefficient, r (+) = Positive correlation, r (-) = negative correlation, N.S. = Non significant, Significant at p value <0.05.

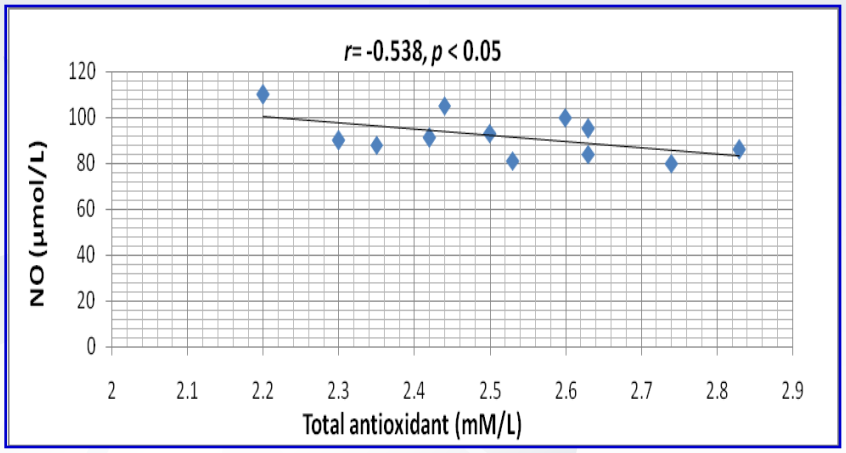
(17)


r= 0.662, p < 0.02
Total antioxidant (mM/L)
Body Wight (gm)

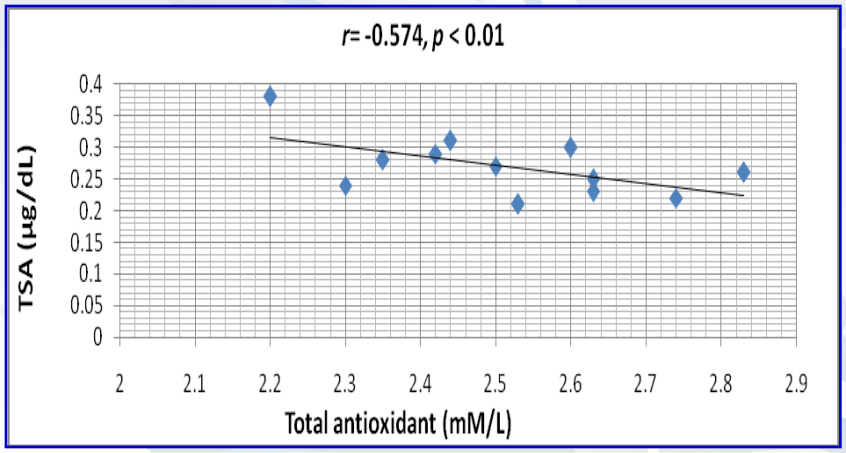
(18)


r= -0.705, p < 0.01
MDA (nmol/ml)
Total antioxidant (mM/L)

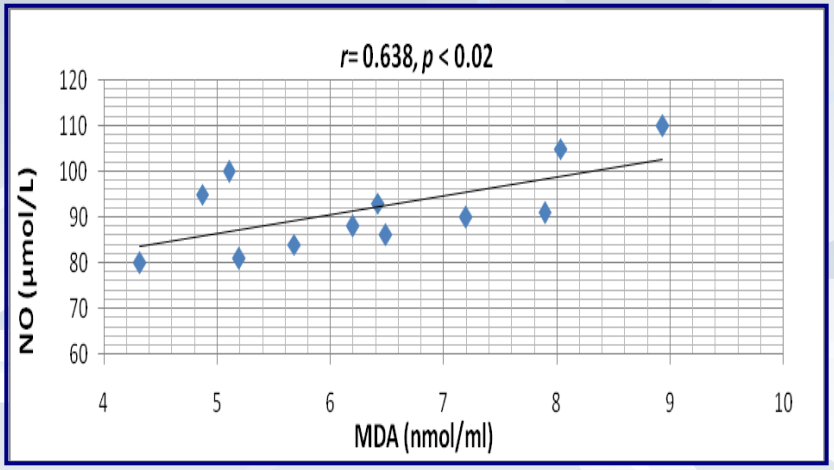
(19)


r= -0.538, p < 0.05
NO (µmol/L)
Total antioxidant (mM/L)

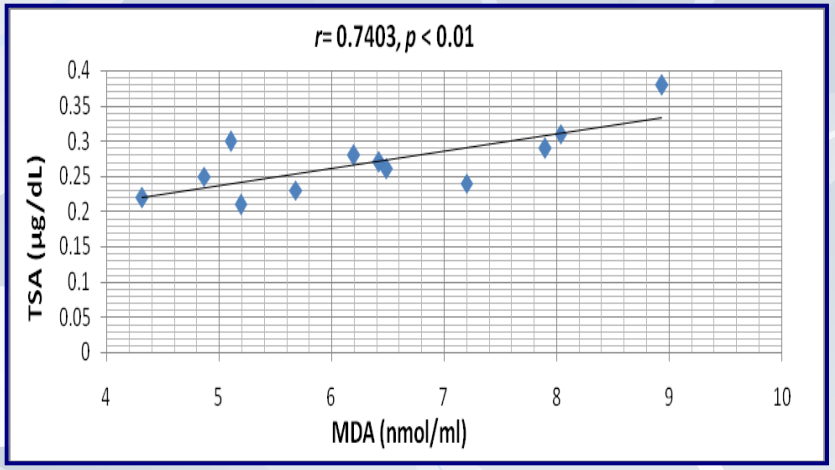
(20)


r= -0.574, p < 0.01
TSA (µg/dL)
Total antioxidant (mM/L)

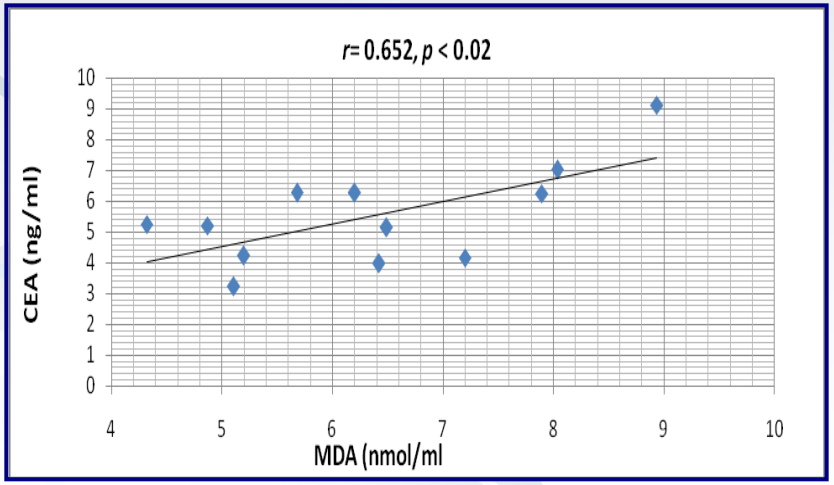
(21)


r= 0.638, p < 0.02
NO (µmol/L)
120
110
100
90
80
70
60
4
5
6
7
8
9
10
MDA (nmol/ml)

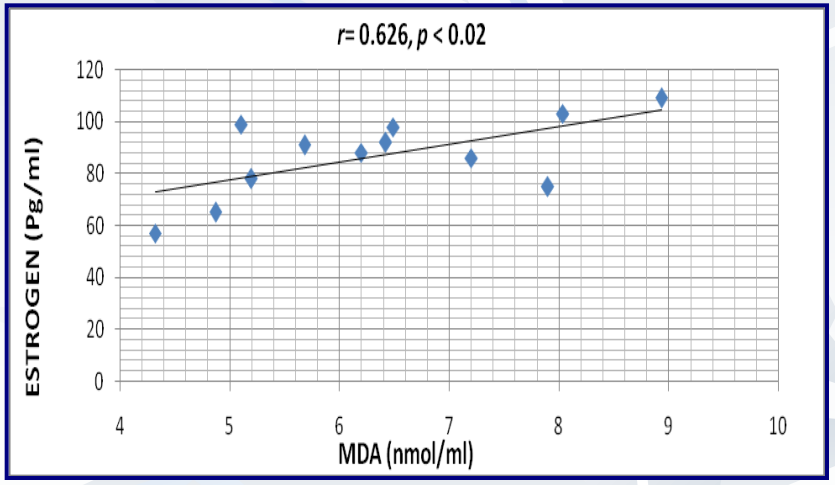
(22)


r= 0.7403, p < 0.01
TSA (µg/dL)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
4
5
6
7
8
9
10
MDA (nmol/ml)

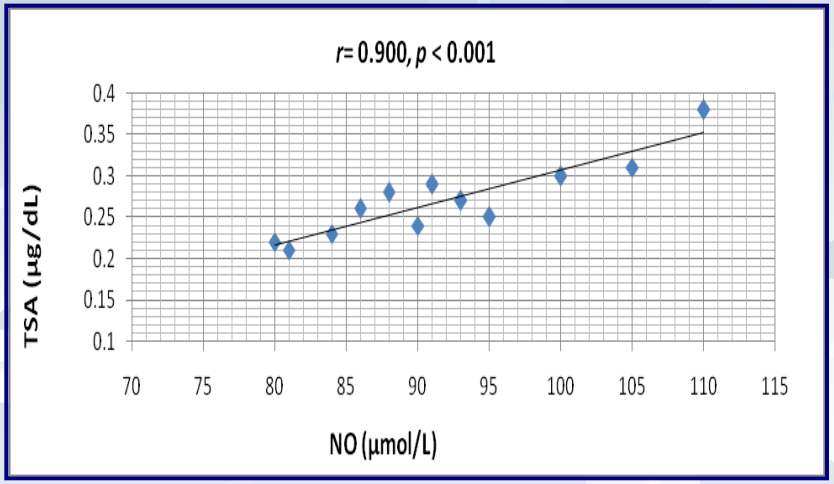
(23)


r= 0.652, p < 0.02
CEA (ng/ml)
10
9
8
7
6
5
4
3
2
1
0
4
5
6
7
8
9
10
MDA (nmol/ml

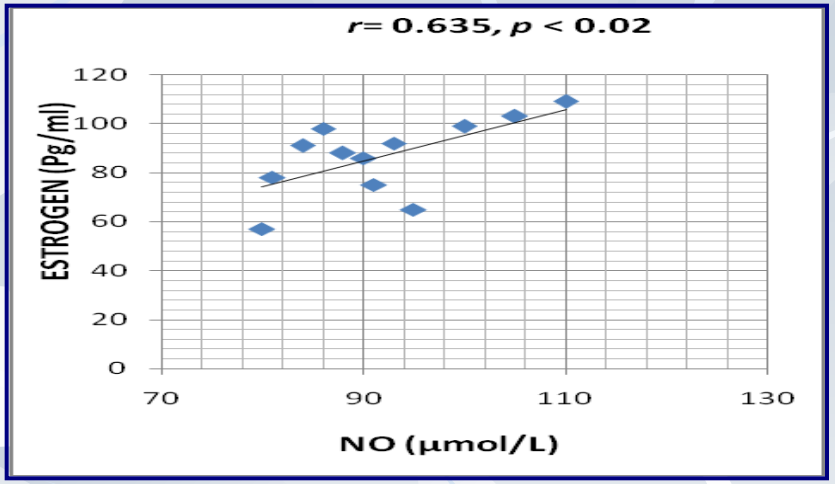
(24)


r= 0.626, p < 0.02
ESTROGEN (Pg/ml)
120
100
80
60
40
20
0
4
5
6
7
8
9
10
MDA (nmol/ml)

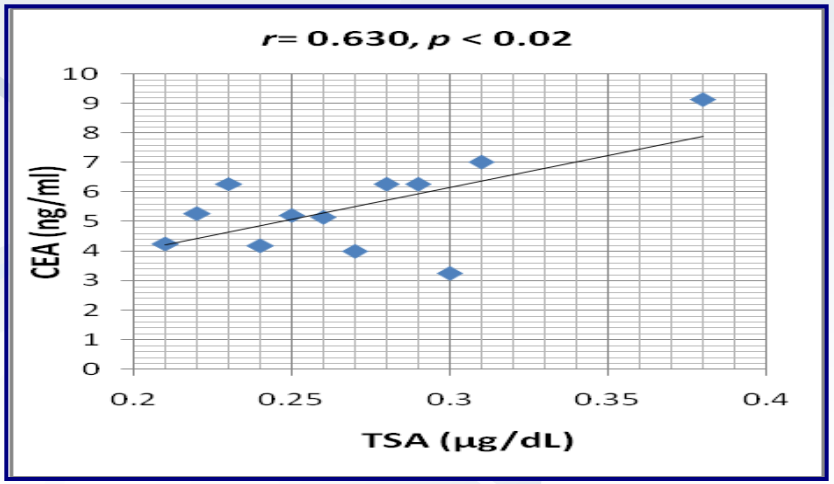
(25)


r= 0.900, p < 0.001
TSA (µg/dL)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
70
75
80
85
90
95
100
105
110
115
NO (µmol/L)

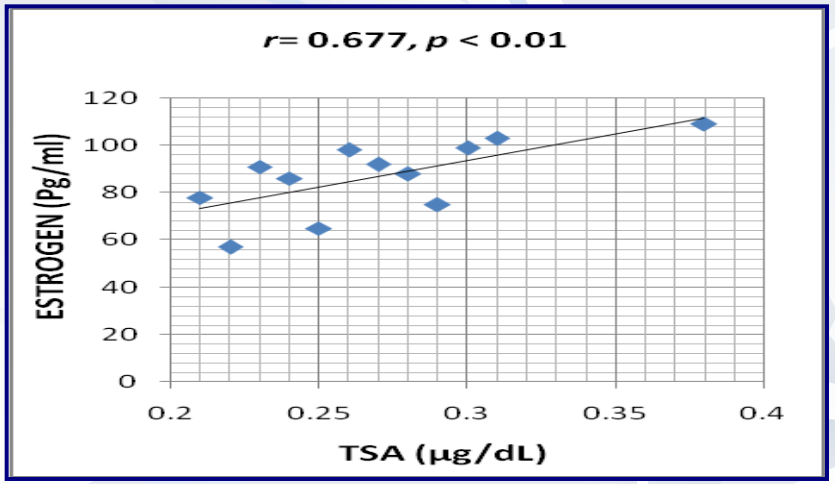
(26)


r= 0.635, p < 0.02
ESTROGEN (Pg/ml)
120
100
80
60
40
20
0
70
90
110
130
NO (µmol/L)

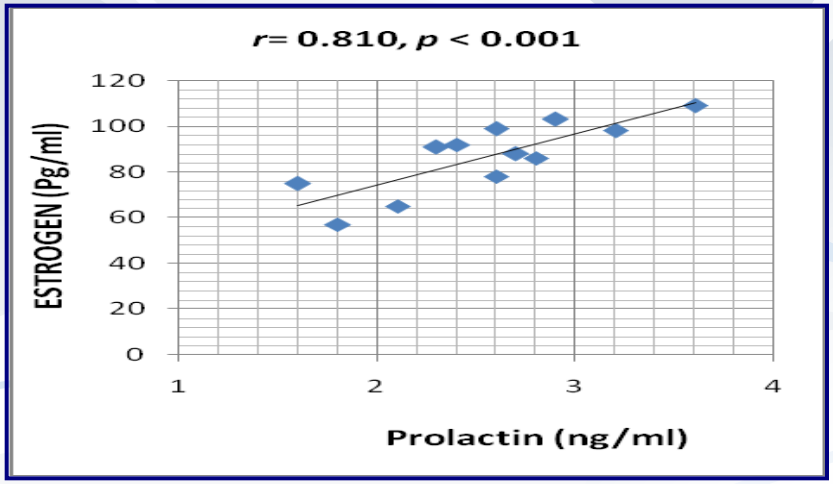
(27)


r= 0.630, p < 0.02
CEA (ng/ml)
10
9
8
7
6
5
4
3
2
1
0
0.2
0.25
0.3
0.35
0.4
TSA (µg/dL)

(28)


r= 0.677, p < 0.01
ESTROGEN (Pg/ml)
120
100
80
60
40
20
0
0.2
0.25
0.3
0.35
0.4
TSA (µg/dL)

(29)

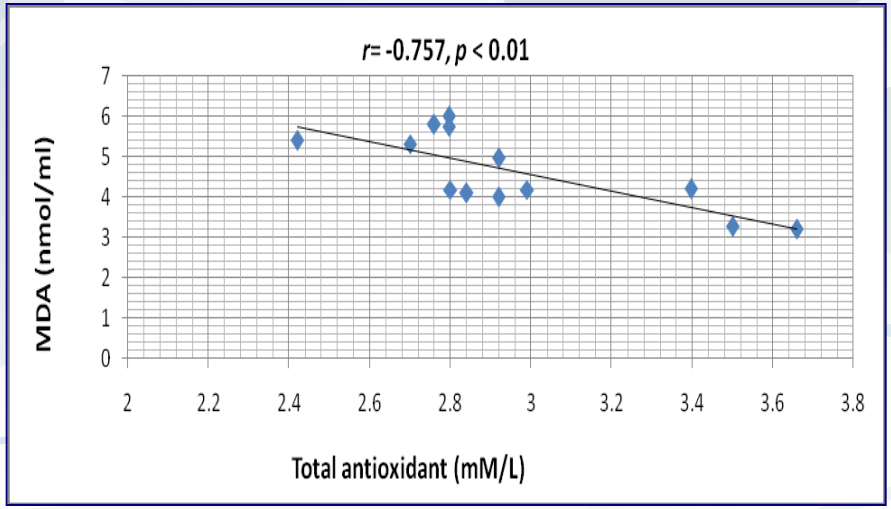

r= 0.810, p < 0.001
ESTROGEN (Pg/ml)
120
100
80
60
40
20
0
1
2
3
4
Prolactin (ng/ml)

Correlation between all parameters in Turmeric (group IV)

	Body Wight	Total antioxidant	MDA	NO	TSA	CEA	Prolactin
Body Wight							
Total antioxidant	***r*= 0.115** ***p*= N.S**						
MDA	***r*= 0.334** ***p*= N.S**	***r*= -0.757** ***p* < 0.01**					
NO	***r*= -0.071** ***p*= N.S**	***r*= -0.489** ***p*= N.S**	***r*= -0.326** ***p*= N.S**				
TSA	***r*=-0.193** ***p*= N.S**	***r*= 0.243** ***p*= N.S**	***r*= 0.142** ***p*= N.S**	***r*= -0.239** ***p*= N.S**			
CEA	***r*= 0.395** ***p*= N.S**	***r*= 0.184** ***p*= N.S**	***r*= -0.023** ***p*= N.S**	***r*= -0.006** ***p*= N.S**	***r*= 0.043** ***p*= N.S**		
Prolactin	***r*= 0.161** ***p*= N.S**	**r= -0.006** ***p*= N.S**	***r*= 0.200** ***p*= N.S**	***r*= -0.022** ***p*= N.S**	***r*= 0.007** ***p*= N.S**	***r*= 0.142** ***p*= N.S**	
ESTROGEN	***r*= -0.336** ***p*= N.S**	***r*= -0.494** ***p*= N.S**	***r*= -0.228** ***p*= N.S**	***r*= 0.236** ***p*= N.S**	***r*= -0.011** ***p*= N.S**	***r*= 0.142** ***p*= N.S**	***r*= 0.210** ***p*= N.S**

r= Correlation coefficient, r (+) = Positive correlation, r (-) = negative correlation, N.S. = Non significant, Significant at p value <0.05.

(30)


r= -0.757, p < 0.01
MDA (nmol/ml)
0
1
2
3
4
5
6
7
2
2.2
2.4
2.6
2.8
3
3.2
3.4
3.6
3.8
Total antioxidant (mM/L)

Histopathological examinations of the mammary glands

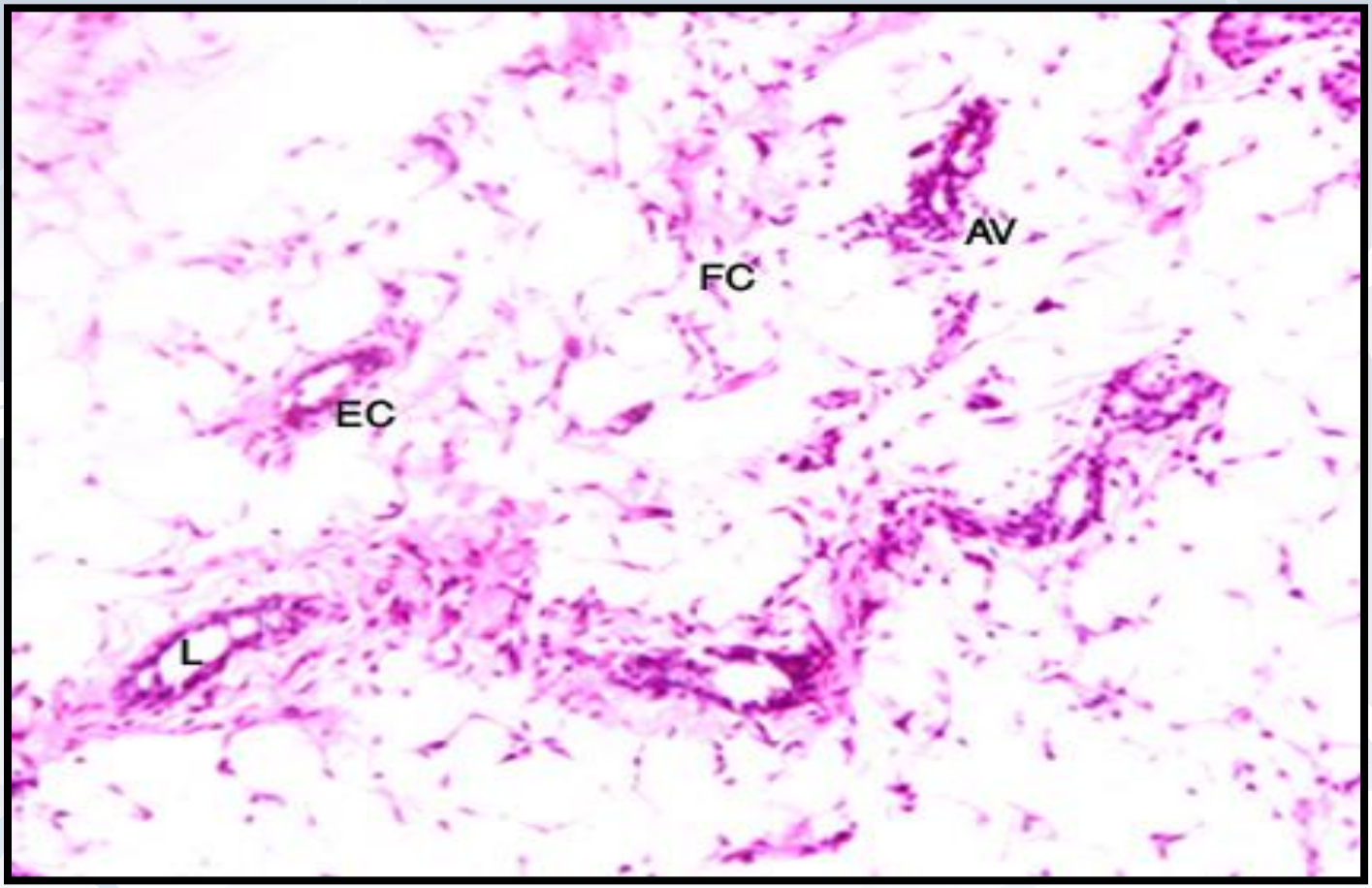


Fig.1 : Light micrograph showing mammary gland epithelia of control rat that consisting of two layers of epithelial cells (EC) surrounding the lumen (L) of the alveoli (AV) embedding in adipose tissue (fat cell FC). Haematoxylin and eosin (H&E X 100).

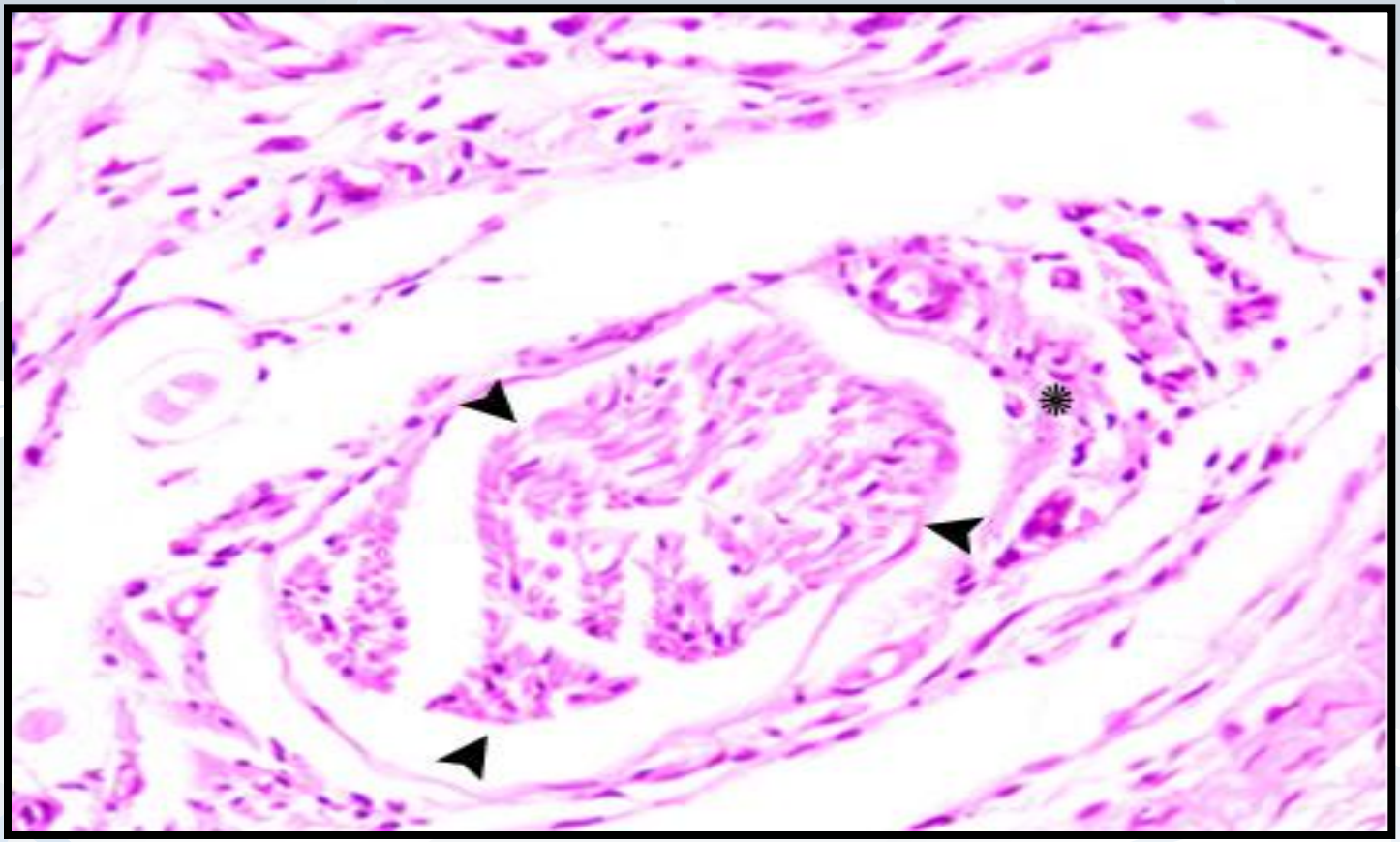


Fig.2 : Light micrograph of a section of the mammary gland epithelia of rats injected with DMBA showing breast carcinoma. Note the highly cellular epithelial tissue between the arrows and the invasion of this carcinoma to the adjacent stroma (asterisk) (H&E X 250).

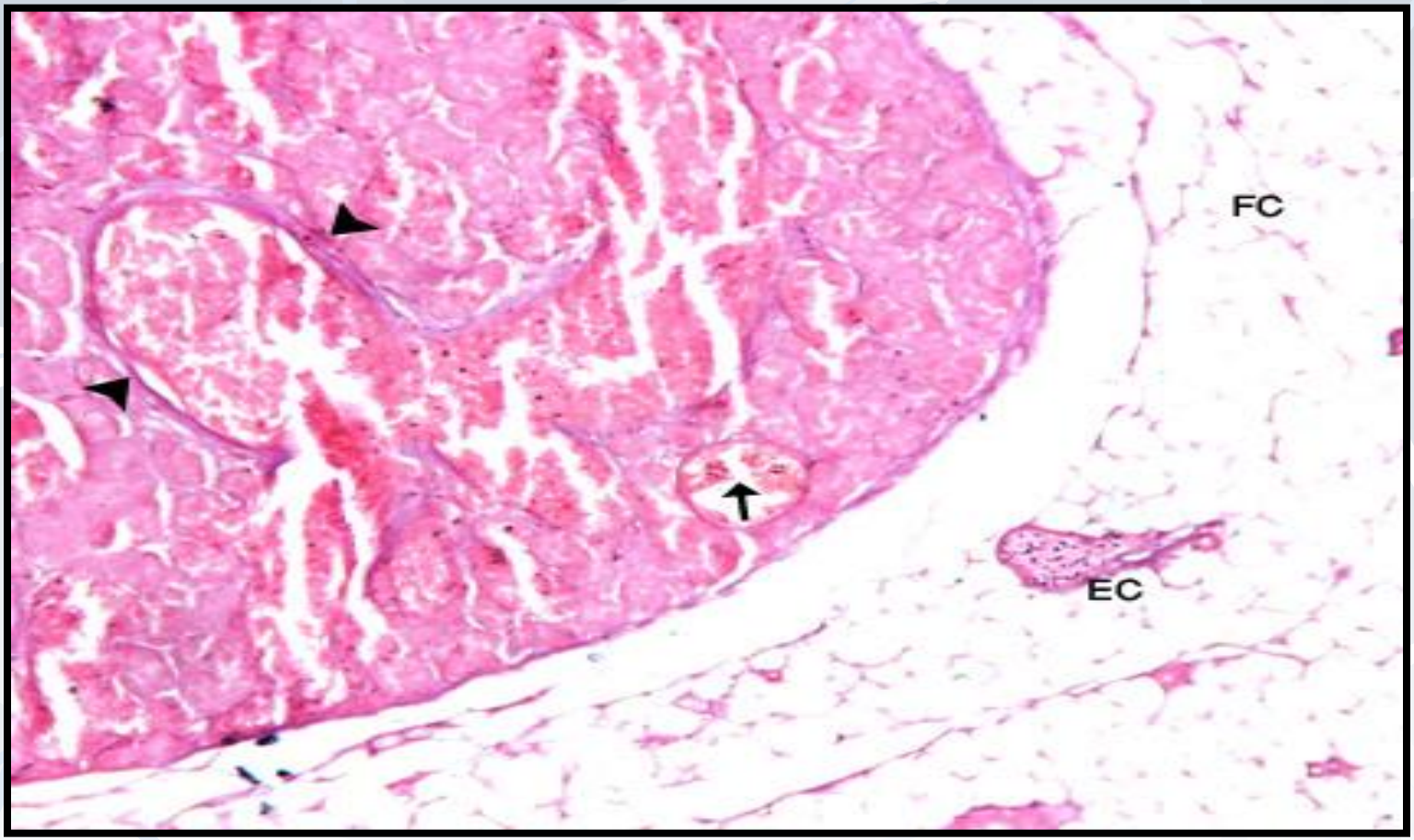


Fig.3: Light micrograph of mammary gland epithelia of rats injected with DMBA showing breast carcinoma with highly cellular growth (arrow) and detachment cancerous cells inside the lumen of the alveoli. (H&E X 100).

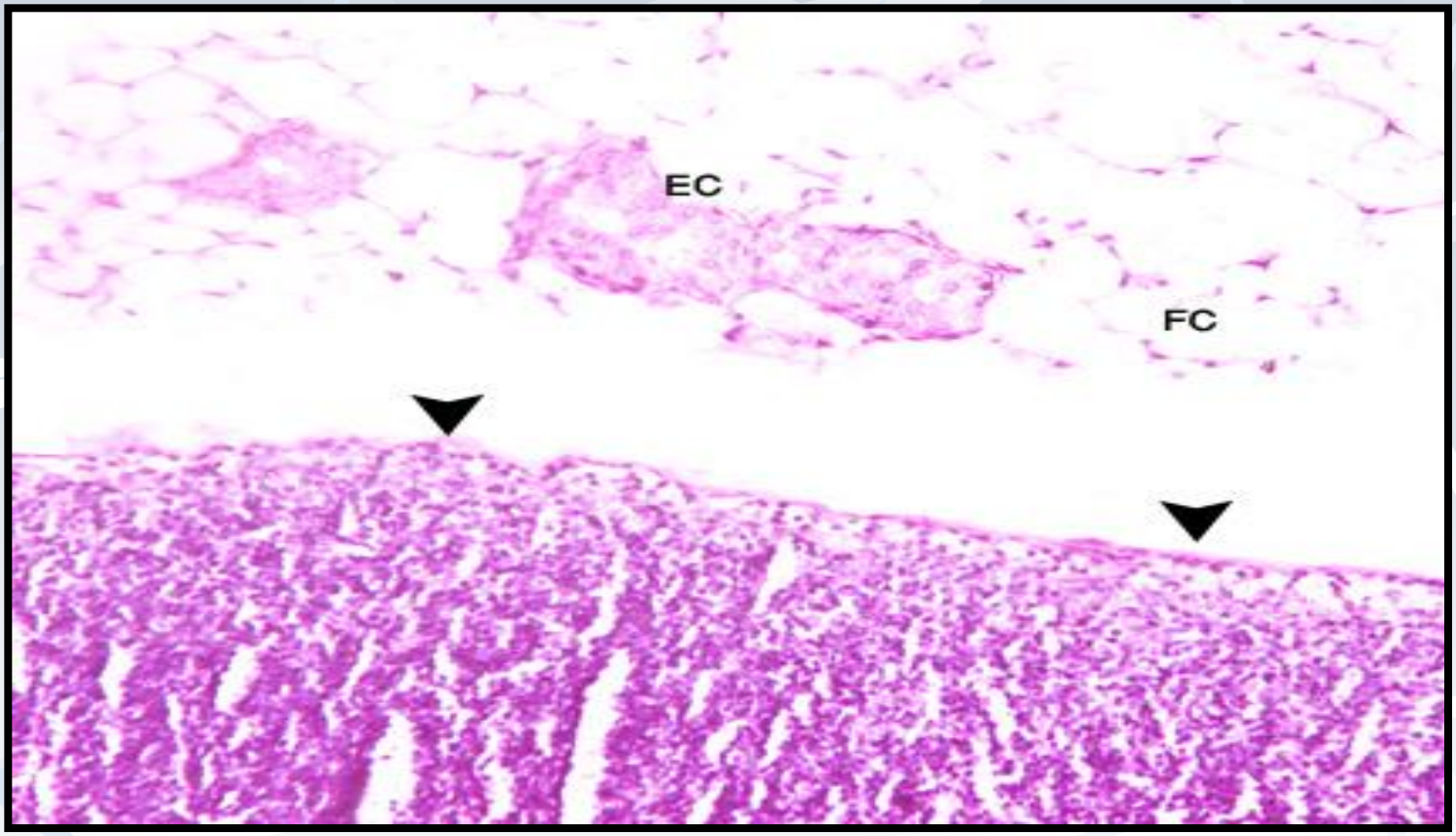


Fig.4: Light micrograph of a section of the mammary gland epithelia of injected DMBA rat and treated with turmeric illustrating hyperplasia of epithelial cells (EC) surrounding lumen of the alveoli and activated lymph node (arrowheads. (H&E X 100).

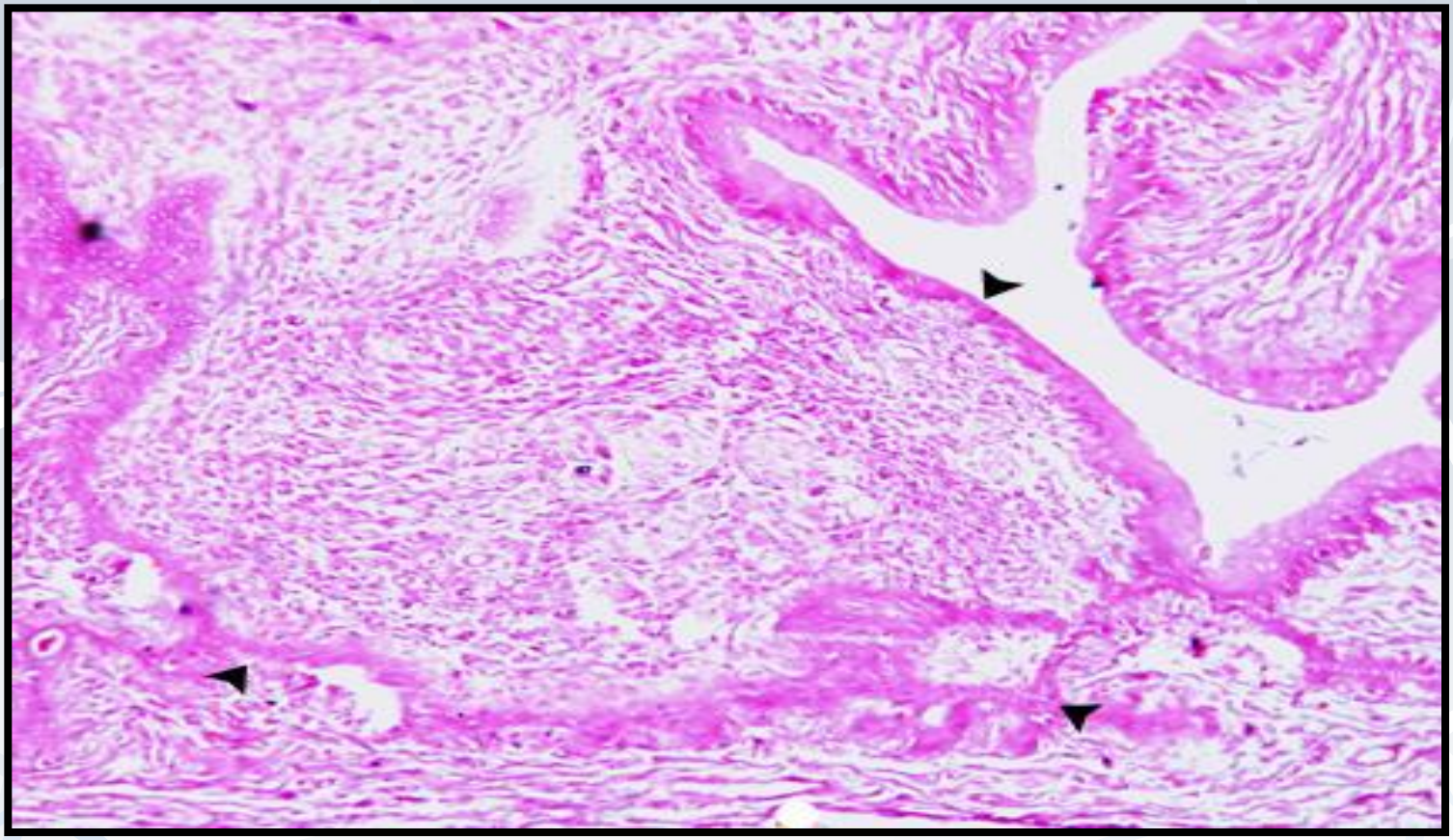


Fig.5: Light micrograph of a section of the mammary gland epithelia of injected DMBA rat and treated with turmeric showing the formed cysts (arrowhead) filled with fibrous cells and necrotic material (asterisk). (H&E X 100)

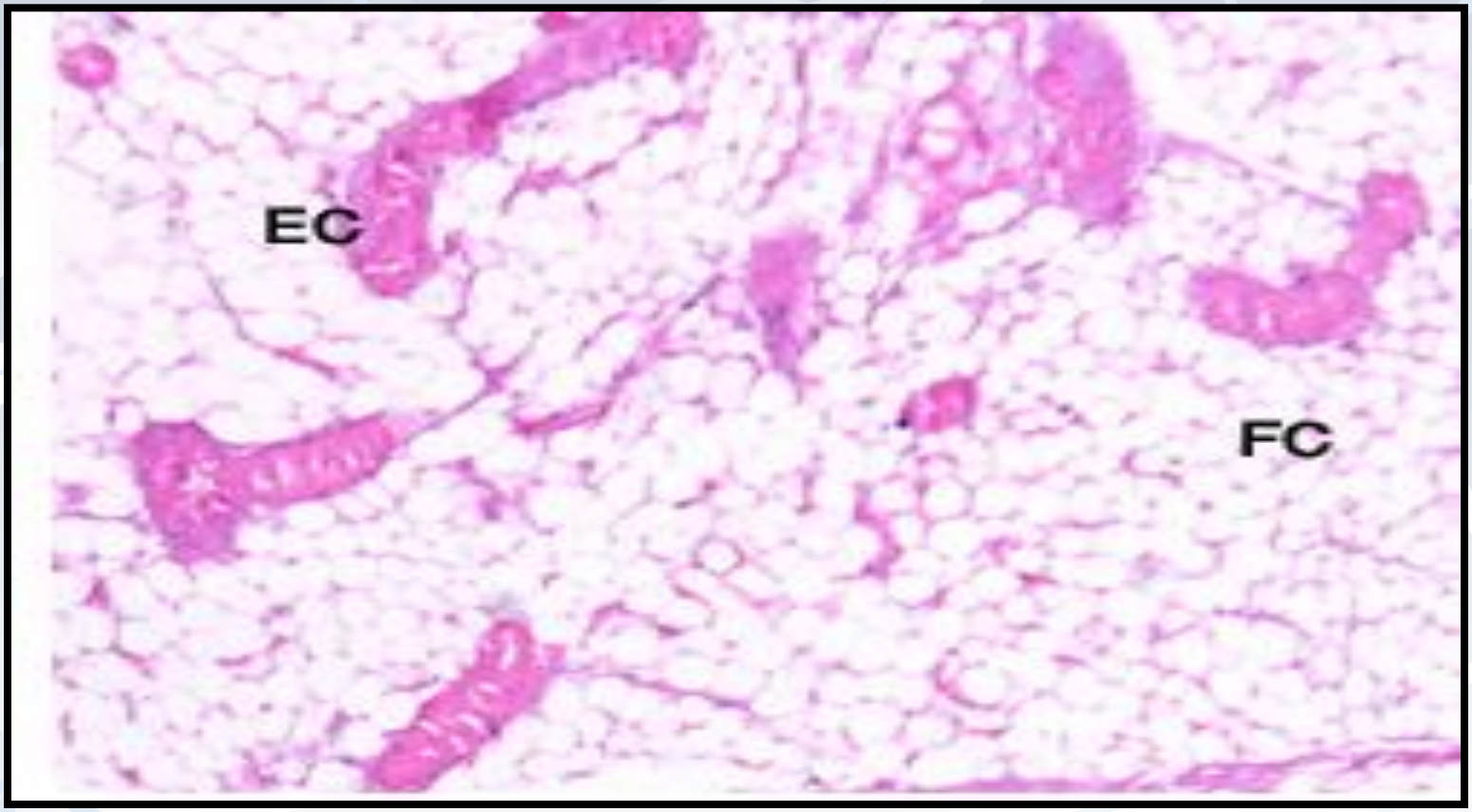
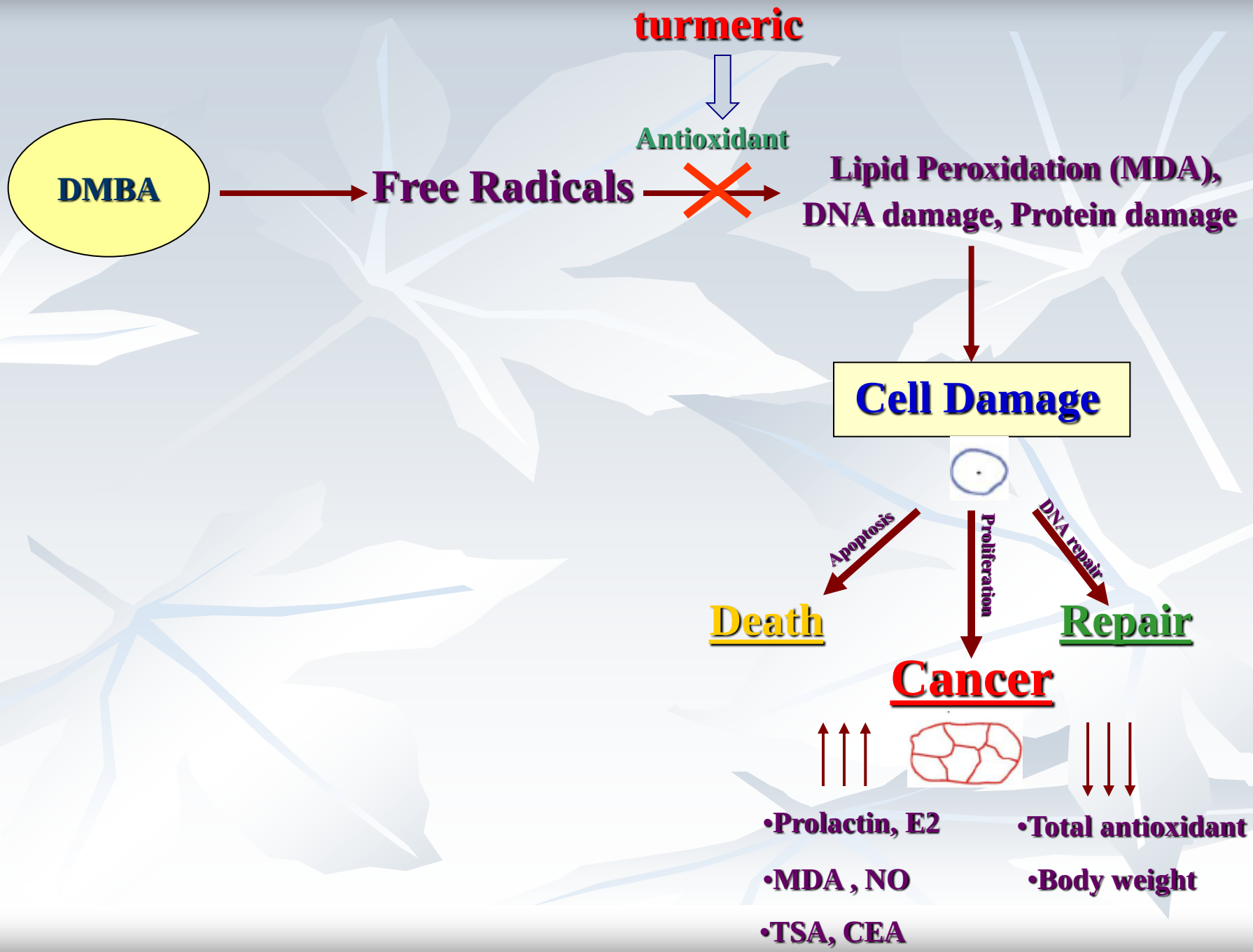


Fig.6: Light micrograph of a section in the mammary gland epithelia of the rat treated with only turmeric showing two layers of epithelial cells (EC) surrounding a clear dilated lumen (L) of the alveoli (AV) which is


turmeric
Antioxidant
DMBA
Free Radicals
Lipid Peroxidation (MDA),
DNA damage, Protein damage
Cell Damage
Apoptosis
Proliferation
DNA repair
Death
Repair
Cancer
•Prolactin, E2
•MDA , NO
•TSA, CEA
•Total antioxidant
•Body weight

Conclusion

- The daily intake of turmeric prevent or delay the development of breast cancer.

- It is essential for women to obtain a good antioxidant status by consuming a diet rich in turmeric and adopt healthy behavior to reduce oxidative stress in order to prevent breast cancer.


Thanks
Soha M Hamdy