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## The Ethanolic Extract of Green Tea Ameliorates Oxidative Stress Parameters and Female Reproductive Performance Regression Induced by Indomethacin in Pregnant Rats.

Saher Mahmood Jwad\*.

Assist. Professor, Faculty of Education for Girls, Biology Department, Kufa University, Iraq.

### ABSTRACT

The purpose of the present study was to investigate the effect of Indomethacin drug on the reproductive performance and oxidative stress indicators in pregnant rats, also to evaluate the possible improvable impacts of Green tea (*Camellia sinensis L.*) extract against the probable deleterious and toxic effects of Indomethacin due to its medicinal properties, as while as, its influences on female reproductive functions have not been explored adequately, so (96) pregnant Sprague Dawley rats were randomly divided into eight equal groups: normal control rats was orally given normal saline(0.9%) on days, 3 and 4 of pregnancy once daily. The second, third and fourth groups were orally received once daily Indomethacin at doses of 0.32 , 1.68 mg/kg (therapeutic doses for humans) and 8.40mg/kg( the highest dose )respectively also on days 3 and 4 of pregnancy, while the groups ( fifth , sixth, and seventh) were orally administered with 300 mg/kg of Green tea leaves extract plus 0.32 mg/kg of Indomethacin, 300 mg/kg of Green tea leaves extract plus 1.68 mg/kg of Indomethacin, and 300 mg/kg of Green tea leaves extract plus 8.40 mg/kg of Indomethacin respectively also on days 3 and 4 of pregnancy once daily. The residual group ( eighth) was only given 300 mg/kg of Green tea leaves extract orally once daily on days 3 and 4 of pregnancy. The pregnant females of all groups were euthanized on day (11) of pregnancy. The results of current study were revealed that the both doses of Indomethacin 0.32 and 1.68 mg/kg (the therapeutic doses for human) have no adverse impacts on the reproductive efficiency of pregnant females, furthermore, caused no toxic or lethal effects for embryos, whereas the highest dose (8.40 mg/kg) was showed a significant reduction( $P<0.0$ ) in: the body weight, relative weight of ovary and ovarian duct, ovary diameter, pregnancy rate, corpora lutea number, number of developing embryos, percentage of embryos number, levels of thyroid hormones ( T3, T4 ), and glutathione(GSH).On the hand ,the oral administration of 8.40mg/kg of Indomethacin caused notable elevation ( $P<0.05$ ) in: the lethality rate, percentage of malformed embryos number, thyroid stimulating hormone ( TSH ) and malondialdehyde (MDA) levels at the treated females .In relation to pregnant females were given 300 mg/kg of Green tea extract, there was a significant improvement ( $P<0.0$ ) in the criteria of reproductive performance, implantation process and oxidative stress. Concerning to the groups that received 300 mg/kg of Green tea extract plus Indomethacin at doses of 0.32 ,1.68, and 8.40 mg/kg respectively, no remarkable differences ( $P<0.05$ ) were observed in the all variables as compared to the control group. Our results suggested that, the oral consumption of Green tea leaves extract exerts preventive and curative effects on all female's reproductive indices that included in the current study. Moreover, it prevents the disturbance effects of highest dose of Indomethacin on the process of implantation ,due to its presumed antioxidant properties and the therapeutic potentialities, so we recommend using it as a nutritional and remedial complement.

**Keywords:** Indomethacin, reproductive performance, oxidative stress, embryo toxic impacts, Green tea ethanolic extract.

\*Corresponding author

## INTRODUCTION

Indomethacin is an indole derivative, its chemical structure : 1- ( P- chlorobenzoyl) -5- methoxy -2- methyl – 1 H – indole -3- acetic acid<sup>(1)</sup>, a general cyclo-oxygenases ( COX) inhibitor, COX are involved in numerous processes related with reproductive activities<sup>(2,3)</sup>. Indomethacin is a non steroidal anti- inflammatory analgesic, used in the treatment of various disorders and complications which including: rheumatoid arthritis ,ankylosing spondylitis and osteoarthritis<sup>(4,5)</sup>. The physiological actions of Indomethacin occurred by inhibiting the activity of cyclo-oxygenase, it is an enzyme very necessary for the synthesis of prostaglandins, prostacyclin, and thromboxane, so it has been used in the treatment of preterm labor<sup>(6)</sup>. Many studies of rodent have been demonstrated, that no increment in the frequency of malformation in the offspring of both mice and rats that treated with Indomethacin drug during pregnancy period at doses ranging up to 100 times more than the therapeutic doses<sup>(7,8)</sup> .

Furthermore , a recent study has been recorded that oral administration of Ibuprofen is more effective than intravenous Indomethacin drug for closure of patent ductus arteriosus in preterm infants and associated with less complications (significantly)<sup>(9)</sup>. On the other hand the study of<sup>(10)</sup> was reported that the using of enteral Paracetamol in the treatment of patent ductus arteriosus in preterm neonates is safe, but not superior to intravenous Indomethacin.

The medical herbs have been used in numerous remedial practice for thousands of years and reported as a readily available healthcare resource<sup>(11,12)</sup>. Green tea( *Camellia sinensis L.*), is a small plant grow essentially and mainly in China and southeast Asia<sup>(13)</sup>, a species of theaceae family, known as a folk remedy for its beneficial medical properties, such as: antioxidant, anticancer ,antidiabetic, antihypertensive , antiviral and anti- atherogenic<sup>(14-16)</sup> , also the regular consumption of Green tea with Cinnamon can prevent or decrease the cardiovascular risk<sup>(17)</sup>.

Many studies that used several animal models confirmed the antioxidant, anti- inflammatory and anti-oncogenic properties of the polyphenolic content derived from Green tea, the polyphenolic compounds of Green tea are catechins, particularly epigallocatechin gallate ( EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG)<sup>(18,19)</sup>. Another study has been reported that Green tea compounds: theanine and catechins have neuroprotective effects<sup>(20)</sup>. Moreover, the caffeinated Green tea has many of antioxidant and cardio- metabolic health benefits due to its content of catechin polyphenols<sup>(21)</sup>.

The present study was designed to estimate the physiological effects of Indomethacin drug at the therapeutic doses for humans(0.32 ,1.68 mg/kg) and the highest dose(8.40mg/kg) on the functional performance of female reproductive system, some of embryonic and oxidative stress criteria ( the first aim). While the second aim of this study was to investigate the ameliorative influences of orally administration of Green tea leaves extract against Indomethacin drug.

## MATERIALS AND METHODS

### Experimental animals:

(96) Female and (48) male rats from Sprague Dawley strain sexually matured ( aged 12 weeks old) weighting between ( 218-240)g were used in this experiment. The rats housed in standard conditions with a 13: 12 of light- day cycle and at 23-28°C, fed with normal diet and tap water. The females were maintained in separate cages for mating(two females for each male). The vaginal smears were taken every day and examined under microscopes, so when the sperms were appeared in the vaginal smears was designated day(0) of pregnancy.

### Chemicals and dosage:

The Indomethacin drug was obtained from a capsule of( 25)mg, then the doses 0.32, 1.68 and 8.40 mg/kg of Indomethacin were prepared and orally administered once daily<sup>(2)</sup>.

**Preparation of Green tea leaves ethanolic extract:**

Green tea leaves were purchased from an herbalist and identified by the aid of taxonomist in the Biology department/ Faculty of Education for Girls/ Kufa University. The extract of Green tea leaves was prepared according to<sup>(22)</sup>.

**Experimental groups and treatments:**

(96) Mated females were randomly divided into eight equal groups:

- The first group (G1) was a control, so it administered with normal saline (0.9%)
- The second group (G2) was received Indomethacin at dose of 0.32 mg/kg (the therapeutic dose for humans).
- The third group (G3) given 1.68 mg/kg of Indomethacin (also the therapeutic dose for humans).
- The fourth group (G4) treated with the highest dose of Indomethacin (8.40) mg/kg
- The fifth group (G5) administered with Green tea leaves extract at dose of 300 mg/kg and 0.32 mg/kg of Indomethacin.
- The sixth group (G6) received 300 mg/kg of leaves extract of Green tea plus 1.68 mg/kg of Indomethacin.
- The seventh group (G6) submitted to 300 mg/kg of leaves extract of Green tea plus 8.40 mg/kg of Indomethacin.
- The eighth group (G6) was only subjected to 300 mg/kg of Green tea leaves extract
- The treatments of all groups of pregnant females were orally and once daily on days (3) and (4) of pregnancy.

**Animals sacrificing and collection of the blood samples:**

Each pregnant female was weighted before sacrifice on day (11) of pregnancy, then the females of all groups were euthanized under anesthesia, (5) ml of the blood was directly collected by heart puncture, then centrifuged to separate the serum for the biochemical study. The right ovaries as well as the right ovarian ducts of rats were dissected out and weighted; the diameters of ovaries were microscopically measured by the utilizing of ocular- micrometer. The corpora lutea and developing embryos were also counted. The lethality rate was obtained according to<sup>(2)</sup> as:  $\text{number of dead females} / \text{number of mated females}$ , while the pregnancy rate was calculated as the following equation:  $\text{number of pregnant at term} / \text{number of mated females}$ <sup>(23)</sup>, furthermore, the percentage of embryos number was measured by  $\text{total embryos number} / \text{corpora lutea number} \times 100$ , and the percentage of malformed embryos number as:  $\text{malformed embryos number} / \text{total embryos number} \times 100$ .

**The biochemical studies were included the following parameters:****Assessment of some hormones levels in the serum:**

The levels of thyroid stimulating hormone (TSH) and thyroid hormones, triiodothyronine (T3), tetra iodothyronine (T4) were evaluated in the serum through the using of enzyme immunoassay for the quantitative determination, the ELISA test is based on the principle of solid phase enzyme- linked immune sorbent assay. The kits were used for this purpose purchased from Biocheck, Inc. 323 Vintage Park Dr. Foster City, CA 94404.

**Assessment of oxidative stress parameters levels:****Determination of glutathione (GSH) level in the serum.**

The level of glutathione was estimated according to<sup>(24)</sup>, by utilizing of Ellman's reagent at (412) nm.

**Determination of malondialdehyde(MDA) level in the serum.**

Malondialdehyde level was evaluated according to the method of <sup>(25)</sup>at(532)nm.

**Statistical analysis**

The data of the present study were analyzed by the using of (SPSS) Statistical Package for Social Science. The results were expressed as mean ± S. E. For the comparisons, analysis of variance (ANOVA), followed by T- student test was used. The level of( P<0.05) considered significant.

**3. RESULTS**

**Effect of Green tea extract and Indomethacin drug on the body weight, relative weight of ovary ,ovarian duct, and the ovary diameter.**

No significant differences(P>0.05) were recorded in the body weight, relative weight of ovary and ovarian duct ,in addition to the ovary diameter at the two groups were treated with Indomethacin at doses of 0.32 and 1.68 mg/kg when compared with the control group. On the other hand ,there was a significant reduction in these parameters at the group of pregnant rats were administered with 8.40 mg/kg of Indomethacin as compared with control group and the two groups of drug.

Contrary to the Indomethacin effects, the concentration 300mg/kg of Green tea extract caused a notable improvement ( P<0.05) in these indices as compared with the control group and other groups of treatments, as while as a significant increase( P<0.05)was revealed in the body weight and ovarian parameters when the three groups of Green tea extract+ Indomethacin at doses of 0.32 , 1.68 and 8.40 mg/kg respectively compared with the group of Indomethacin at highest dose (8.40)mg/kg. Moreover ,no remarkable change(P>0.05) noted when the groups of two doses of Indomethacin (0.32 and 1.68 ) mg/kg were compared with each other, also between the all groups of Green tea extract + Indomethacin drug, as shown in tables(1) and (2) respectively .

**Table (1): Effect of Green tea leaves extract and Indomethacin drug on body weight**

Groups and treatments	Mated females number	Number of pregnant females at term	Body weight/g
G1	12	12	233.36±4.10 b
G2	12	12	233.40±4.5 b
G3	12	12	231.61±4.11 b
G4	12	6	219.73± 4.28 c
G5	12	12	235.23±2.81b
G6	12	12	234.47±3.87 b
G7	12	12	232.23±2.81b
G8	12	12	243.97±3.87 a

Values expressed as mean ±S.E.

The same letters mean no significant differences between groups at level of (P<0.05).

The different letters mean significant differences between groups at level of (P<0.05).

- ( G1) the control group, administered with normal saline (0.9%)
- (G2)the group received Indomethacin at dose of 0.32mg/kg( the therapeutic dose for humans).
- (G3) the group given 1.68mg/kg of Indomethacin( also the therapeutic dose for humans).
- (G4) the group treated with the highest dose of Indomethacin(8.40) mg/kg.
- (G5)the group administered with Green tea leaves extract at dose of 300 mg/kg and 0.32 mg/kg of Indomethacin.
- (G6) the group received 300 mg/kg of leaves extract of Green tea plus 1.68 mg/kg of Indomethacin.
- (G7) the group submitted to 300 mg/kg of leaves extract of Green tea plus 8.40 mg/kg of Indomethacin.
- (G8) the group was only subjected to 300 mg/kg of Green tea leaves extract.

**Table (2): Effect of Green tea leaves extract and Indomethacin drug on relative weight of ovary ,ovarian duct and ovary diameter**

Groups and treatments	Mated females number	Number of pregnant females at term	Relative weight of ovary mg/100g of body weight	Relative weight of ovarian duct mg/100g of body weight	Ovary diameter/m
G1	12	12	42.96± 1.42b	38.84±0.91 b	4366.41±97 b
G2	12	12	42.13± 2.01b	38.19±0.45 b	4256.05±85 b
G3	12	12	39.07±1.50 b	37.67±1.11 b	4199.81±99.1 b
G4	12	6	30.17±1.95 c	26.12±0.89 c	3395.01±79.4 c
G5	12	12	43.04±1.25 b	38.66±1.77 b	4411.41±87.1 b
G6	12	12	43.19±1.93 b	39.59±1.88 b	4334.06±67.7 b
G7	12	12	42.06±1.42 b	37.53±1.58 b	4266.61±75.5 b
G8	12	12	50.93±2.23a	47.98±1.95 a	4897.55±95.2a

Values expressed as mean ±S.E.

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The different letters mean significant differences between groups at level of (P<0.05).

- ( G1) the control group, administered with normal saline (0.9%)
- (G2) the group received Indomethacin at dose of 0.32mg/kg( the therapeutic dose for humans).
- (G3) the group given 1.68mg/kg of Indomethacin( also the therapeutic dose for humans).
- (G4) the group treated with the highest dose of Indomethacin(8.40) mg/kg.
- (G5) the group administered with Green tea leaves extract at dose of 300 mg/kg and 0.32 mg/kg of Indomethacin.
- (G6) the group received 300 mg/kg of leaves extract of Green tea plus 1.68 mg/kg of Indomethacin.
- (G7) the group submitted to 300 mg/kg of leaves extract of Green tea plus 8.40 mg/kg of Indomethacin.
- (G8) the group was only subjected to 300 mg/kg of Green tea leaves extract.

**Effect of Green tea extract and Indomethacin drug on: the lethality rate, pregnancy rate, corpora lutea number, number of embryos in development ,the percentages of embryos number and malformed embryos number.**

The data of table(3) revealed that, the group of pregnant females that administered with the dose( 8.40) mg/kg of Indomethacin presented three females dead from twelve were mated comparing to the other groups of treatment.

Regarding the pregnancy rate, from the nine pregnant females remaining after administration of the highest dose of Indomethacin three did not develop at term pregnancy in compared with the other groups of experiment as shown in table (3). In relation to the, corpora lutea number, developing embryos number, and percentage of embryos number were significantly decreased (P<0.05) in the group of pregnant females that received the highest dose of Indomethacin( 8.40) mg/kg. In contrast, the percentage of malformed embryos number was showed a significant increment (P<0.05) at the same group as compared with all groups of study, table (4).

**Table (3): Effect of Green tea leaves extract and Indomethacin on lethality rate and pregnancy rate**

Groups and treatments	Mated females number	Number of pregnant females at term	Lethality rate %	Pregnancy rate %
G1	12	12	0% b	100% a
G2	12	12	0% b	100% a
G3	12	12	0% b	100% a
G4	12	6	25% a	50% b
G5	12	12	0% b	100%a
G6	12	12	0% b	100%a
G7	12	12	0% b	100%a
G8	12	12	0% b	100%a

Lethality rate and pregnancy rate expressed as percentage (%)

The same letters mean no significant differences between groups at level of (P<0.05).

The different letters mean significant differences between groups at level of (P<0.05).

- (G1) the control group, administered with normal saline (0.9%)
- (G2) the group received Indomethacin at dose of 0.32mg/kg( the therapeutic dose for humans).
- (G3) the group given 1.68mg/kg of Indomethacin( also the therapeutic dose for humans).
- (G4) the group treated with the highest dose of Indomethacin(8.40) mg/kg.
- (G5) the group administered with Green tea leaves extract at dose of 300 mg/kg and 0.32 mg/kg of Indomethacin.
- (G6) the group received 300 mg/kg of leaves extract of Green tea plus 1.68 mg/kg of Indomethacin.
- (G7) the group submitted to 300 mg/kg of leaves extract of Green tea plus 8.40 mg/kg of Indomethacin.
- (G8) the group was only subjected to 300 mg/kg of Green tea leaves extract.

**Table (4): Effect of Green tea leaves extract and Indomethacin on the corpora lutea number , developing embryos number ,the percentages of embryos number and malformed embryos number.**

Groups and treatment	Mated females number	Number of pregnant females at term	Corpora lutea number	Developing embryos number	Percentage of embryos number %	Percentage of malformed embryos number %
G1	12	12	13.25±0.39b	12.73 ±0.11b	96.0%b	0% a
G2	12	12	13.22±0.35b	12.63 ±0.23b	95.5%b	0% a
G3	12	12	13.15±0.49b	12. 50±0.19b	95.0% b	0% a
G4	12	6	9.10±0.57 c	6.15 ±0.28 c	67.5%c	13.28%b
G5	12	12	13.23±0.29b	12.70 ±0.22b	95.9%b	0% a
G6	12	12	13.21±0.51b	12.68 ±0.30b	95.9%b	0% a
G7	12	12	13.16±0.31 b	12.52 ±0.15b	95.1%b	0% a
G8	12	12	14.17±0.59a	13.98 ±0.9 a	98.6%a	0% a

Embryos number and malformed embryos number expressed as percentage (%)

The same letters mean no significant differences between groups at level of (P<0.05).

The different letters mean significant differences between groups at level of (P<0.05).

- (G1) the control group, administered with normal saline (0.9%)
- (G2) the group received Indomethacin at dose of 0.32mg/kg( the therapeutic dose for humans).
- (G3) the group given 1.68mg/kg of Indomethacin( also the therapeutic dose for humans).
- (G4) the group treated with the highest dose of Indomethacin(8.40) mg/kg.
- (G5) the group administered with Green tea leaves extract at dose of 300 mg/kg and 0.32 mg/kg of Indomethacin.
- (G6) the group received 300 mg/kg of leaves extract of Green tea plus 1.68 mg/kg of Indomethacin.
- (G7) the group submitted to 300 mg/kg of leaves extract of Green tea plus 8.40 mg/kg of Indomethacin.
- (G8) the group was only subjected to 300 mg/kg of Green tea leaves extract.

**Effect of green tea extract and Indomethacin drug on levels of: thyroid stimulating hormone (TSH) and thyroid hormones(T3, T4).**

The results of study were pointed to a significant increment (P<0.05)in the TSH level at the pregnant females were treated with the dose ( 8.40) mg/kg of Indomethacin when compared with the control and other groups of treatments . Contrary to the adverse effects of drug, there was a significant reduction(P<0.05) in TSH level when the group was orally given leaves extract of Green tea compared with control and other treated groups. On the other hand, there was no significant difference(P>0.05) in TSH level when the other experimental groups of study were compared with each other, table(5).

As regard with the levels of both thyroid hormones(T3,T4), were appeared significant decrement(P<0.05)at the group of females that administered with the highest dose of Indomethacin as compared with the control group and other groups of study, while there was a significant elevation (P<0.05)in the levels of these hormones at group of Green tea leaves extract when compared with all groups of treatment, whereas no significant differences(P>0.05) were recorded in these variables when the other groups of study compared with each other as shown in table(5).

**Table(5): Effect of Green tea leaves extract and Indomethacin on the levels of thyroid stimulating hormone(TSH) and Thyroid hormones(T3, T4)**

Groups and treatment	Mated females number	Number of pregnant females at term	TSH levels $\mu$ /U/ml	T3 levels nmol/L	T4 levels nmol/L
G1	12	12	1.35 $\pm$ 0.02 b	2.19 $\pm$ 1.22 b	73.42 $\pm$ 1.25 b
G2	12	12	1.38 $\pm$ 0.07 b	2.10 $\pm$ 1.36 b	70.17 $\pm$ 1.67 b
G3	12	12	1.41 $\pm$ 0.11 b	2.10 $\pm$ 1.18 b	70.31 $\pm$ 2.46 b
G4	12	6	2.01 $\pm$ 1.02 a	1.43 $\pm$ 2.03 c	50.12 $\pm$ 2.66 c
G5	12	12	1.29 $\pm$ 0.09 b	2.25 $\pm$ 1.32 b	74.61 $\pm$ 2.16b
G6	12	12	1.32 $\pm$ 0.14 b	2.22 $\pm$ 1.14 b	73.95 $\pm$ 2.02 b
G7	12	12	1.39 $\pm$ 0.08 b	2.15 $\pm$ 1.41 b	71.34 $\pm$ 2.79 b
G8	12	12	1.10 $\pm$ 0.03 c	2.45 $\pm$ 1.84 a	79.76 $\pm$ 1.92 a

Values expressed as mean  $\pm$ S.E.

The same letters mean no significant differences between groups at level of (P<0.05).

The different letters mean significant differences between groups at level of (P<0.05).

- ( G1) the control group, administered with normal saline (0.9%)
- (G2) the group received Indomethacin at dose of 0.32mg/kg( the therapeutic dose for humans).
- (G3) the group given 1.68mg/kg of Indomethacin( also the therapeutic dose for humans).
- (G4) the group treated with the highest dose of Indomethacin(8.40) mg/kg.
- (G5) the group administered with Green tea leaves extract at dose of 300 mg/kg and 0.32 mg/kg of Indomethacin.
- (G6) the group received 300 mg/kg of leaves extract of Green tea plus 1.68 mg/kg of Indomethacin.
- (G7) the group submitted to 300 mg/kg of leaves extract of Green tea plus 8.40 mg/kg of Indomethacin.
- (G8) the group was only subjected to 300 mg/kg of Green tea leaves extract.

**Effect of Green tea extract and Indomethacin drug on the levels of glutathione(GSH) and malondialdehyde(MDA).**

According to the results of this study, the levels of GSH were significantly decreased(P<0.05)at the rats that treated with 8.40mg/kg of Indomethacin in comparison with the control group and other treated groups. Concerning to the pregnant females were received Green tea extract, there was a significant elevation (P<0.05) observed in GSH level as compared with all experimental groups, while there was no remarkable differences (P>0.05) were noticed in this parameter between the other groups of study, table (6). The current study was also revealed a significant increment(P<0.05) in the level of MDA at the group of rats were orally given the highest dose of Indomethacin as compared with other groups .Contrary to the notable raise of MDA level ,the orally administration of Green tea leaves extract was caused a significant decrease(P<0.05)in this level as compared with the control group and the other groups of experiment ,whereas no significant differences(P<0.05) were appeared in this index between the other groups of study, as shown in table(6).

**Table(6): Effect of Green tea leaves extract and Indomethacin drug on the levels of glutathione(GSH) and malondialdehyde(MDA)**

Groups and treatments	Mated females number	Number of pregnant females at term	Glutathione levels $\mu$ mol/L	Malondialdehyde levels $\mu$ mol/L
G1	12	12	3.33 $\pm$ 0.11 b	1.34 $\pm$ 0.22 b
G2	12	12	3.29 $\pm$ 1.01 b	1.37 $\pm$ 0.08 b
G3	12	12	3.21 $\pm$ 0.16 b	1.37 $\pm$ 0.18b
G4	12	6	1.15 $\pm$ 0.45 c	2.77 $\pm$ 0.36a
G5	12	12	3.40 $\pm$ 0.17 b	1.22 $\pm$ 0.18 b
G6	12	12	3.38 $\pm$ 0.22 b	1.27 $\pm$ 0.09 b
G7	12	12	3.20 $\pm$ 1.09 b	1.39 $\pm$ 0.15 b
G8	12	12	4.71 $\pm$ 1.13 a	0.73 $\pm$ 1.06 c

Values expressed as mean  $\pm$ S.E

The same letters mean no significant differences between groups at level of (P<0.05).

The different letters mean significant differences between groups at level of ( $P < 0.05$ ).

- (G1) the control group, administered with normal saline (0.9%)
- (G2) the group received Indomethacin at dose of 0.32mg/kg (the therapeutic dose for humans).
- (G3) the group given 1.68mg/kg of Indomethacin (also the therapeutic dose for humans).
- (G4) the group treated with the highest dose of Indomethacin (8.40) mg/kg.
- (G5) the group administered with Green tea leaves extract at dose of 300 mg/kg and 0.32 mg/kg of Indomethacin.
- (G6) the group received 300 mg/kg of leaves extract of Green tea plus 1.68 mg/kg of Indomethacin.
- (G7) the group submitted to 300 mg/kg of leaves extract of Green tea plus 8.40 mg/kg of Indomethacin.
- (G8) the group was only subjected to 300 mg/kg of Green tea leaves extract.

## DISCUSSION

### Effect of Green tea leaves extract and Indomethacin drug on the body weight, relative weight of ovary, ovarian duct, and ovary diameter.

The significant decrease ( $P < 0.05$ ) in body weight of rats that treated with Indomethacin at the dose of 8.40 mg/kg was agreed with the finding of <sup>(2)</sup>. This perhaps due to the embryotoxic effect of drug which disturbs the embryos implantation process and causes a notable decrement in the corpora lutea number, developing embryos number and percentage of embryos number that were recorded in the present study, thus the body weight of pregnant females significantly decreased.

As well as this finding can attribute to the decrement in the food intake or food consumption that may be because of the Indomethacin administration as a result of its ulcerogenic action, the study of (26) was suggested that the hypermotility of stomach considered one of the mechanisms of ulcerogenic effects were resulted from Indomethacin treatment in rats. Another study postulated that, the pathogenic impacts of Indomethacin drug may occur by affecting the KATP channels (27).

On the other hand, the Indomethacin can cause intestinal damage, bleeding, strictures accompanied with the loss of albumin and iron that was confirmed by (28), so these complications may lead to decrease in the food consumption and subsequently maternal weight loss. A previous study has shown that up to (80)% of the jejunum- ileum is damaged due to Indomethacin treatment and other NSAIDs (29). Moreover, transmural ulcers, wall thickening, granulomatous inflammation, crypt abscesses and other histopathological changes such as adhesions and fibrosis were observed by other researchers (30), thus the body weight of female rats notably decreased because of the maternal Indomethacin toxicity.

Contritely, the groups were given Green tea plus Indomethacin at the three doses revealed no significant changes in the body weight as compared with the control group, whereas the group administered with only Green tea showed a remarkable raise in this index in comparison to all experimental groups, this would be attributed to the chemical composition of Green tea, which including: high value of tannins, caffeine, theanine, chlorophylls, pheophytins, carotenoids, flavonoids and catechin polyphenols (31,32). According to the study of (18) the total content of catechins in Green tea was (79.6–81.8)%. Catechins have antioxidant activity more potent than vitamin C and E about (100) and (25) respectively (31), thus the powerful antioxidants potentiality of Green tea protects the embryos implantation process from the detrimental effects of Indomethacin, this leads to a significant increment in the corpora lutea number, developing embryos number, percentages of embryos number and subsequent increase in the maternal body weight.

In addition, the Green tea may be prevented the cellular damage and lipid peroxidation that induced by reactive free radicals of Indomethacin drug, so it can play very important role in the protection of gastrointestinal tract tissues against the deleterious free radicals which generated by Indomethacin administration, probably for this reason the body weight of pregnant females treated with Green tea extract appeared a significant increase.

Concerning to the relative weight of ovary, oviduct, and diameter of ovary were recorded a significant decrease at females that treated with Indomethacin drug at the dose of 8.40 mg/kg, this may be because the oxidative destruction of polyunsaturated fatty acids of ovarian membranes by lipid peroxidation which probably result from the Indomethacin treatment and causes more generation of reactive oxygen species such as: hydroxyl radicals, superoxide anion and lipid peroxy radicals, that would be damaged all the tissues of

body especially the ovarian cells ,and this leads to apoptosis, perhaps by this mechanism the relative weight of ovary, ovarian duct, and diameter of ovary significantly decreased.

On the other hand, the Indomethacin drug can cause a disturbance in the functions of hypothalamic-pituitary axis that tends to reduce the secretion of gonadotropic hormones(LH and FSH), and subsequent decrement in the ovarian criteria.

The data of current study noted a significant increase in malondialdehyde (MDA) level which may be caused a great damage to the tissues of ovary as well as a reduction in its biological functions, because MDA exerts deteriorative effects to the ovarian cell membranes by lipid peroxidation therefore, the weight and diameter of ovary showed a significant decline. In addition , the free radicals and MDA work together to reduce the levels of estrogen, progesterone and gonadotropins as well as cause destruction to the ovary cells, that was recorded by(33).

With regard to the groups that given Green tea plus Indomethacin, no significant differences in the ovarian indices were obtained during the present study as compared with the control group. In the opposite, the remarkable improvement in the relative weight of ovary ,ovarian duct and ovary diameter at only Green tea treated group, possibly attribute to the chemical composition of Green tea leaves extract, it contains many active biological constituents such as polyphenols which have a broad spectrum property for scavenging numerous types of oxidants like peroxinitrite and superoxide radicals that was reported by (34) and (35). Therefore, these compounds could prevent the cellular damage through inhibit lipid peroxidation, thus the integrity of ovarian cell membranes may not alter as well as the weight and diameter of ovary significantly increased.

Moreover, the Green tea possesses water-soluble flavonols like: rutin, kaempferitrin, myricitrin, myricetin, kampferol, quercetine, isoquercetin and others, in addition to many flavones such as: apigenin, isovitexin, vitexin, saponarin and their glycosides, also anthocyanins(36), which may improve the histological structure of ovary and it's vital functions.

Furthermore, the significant increment in the ovarian markers ,possible attribute to the ameliorative effects of Green tea components to the hypothalamic- pituitary- gonadal axis, thus the releasing of FSH and LH as well as their levels in blood stream may be revealed a significant increase. Furthermore, the study of(37) was conducted that the polyphenols treated groups of rats showed improvement in the ovary and uterus tissues, in addition to the significantly increased levels of FSH and LH due to the polyphenols efficacy to stimulate the biosynthesis of both hormones that was .

#### **Effect of Green tea leaves extract and Indomethacin drug on: the lethality rate, pregnancy rate, corpora lutea number ,developing embryos number, percentages of embryos number and malformed embryos number.**

With regard to the percentage of lethality rate, the current study was found that (3)females dead from (12) females were mated at the group received 8.40mg/kg dose of Indomethacin, this result comes along with the finding of(2) ,who confirmed that the both doses of Indomethacin0.32 and1.68 mg/kg revealed no embryotoxic or lethal influences to the pregnant rats, while the highest dose produced two females dead from fifteen females mated. The main causative factor of pregnant females death can attribute to the detrimental impacts of Indomethacin, which may cause the peritonitis, that was confirmed by many studies(2,38).

The pregnancy rate at the group was subjected to the highest dose of Indomethacin also appeared a notable decrease, this agrees with the result of (2) ,who observed that from the thirteen pregnant females remaining only (2) did not continue at term pregnancy. The finding probably due to the peritonitis as well as the ulcerogenic action of Indomethacin ,which may produce ulcer in the colon and this could disturb the implantation process of embryos because the adhesion to epiplon ,that was demonstrated by the study of(39),thus by this way the pregnancy rate notably decreased.

Furthermore, the present study pointed to a remarkable reduction in the corpora lutea number , developing embryos number ,the percentage of embryos number at the group of rats that treated with 8.40 mg/kg of Indomethacin ,in relation to the numbers of embryos the result of this study agrees with(2) who,

indicated that the rate of preimplantation loss was significantly increased, in contrast decline in the numbers of embryos were noted .

Moreover, another study was reported that the administration of Indomethacin drug to the rats at dose of 3mg/kg two times daily on the third day to the fifth day of pregnancy reduced the implantation sites number(40). Concerning to the dose of 5mg/kg every day at the same duration of pregnancy also decreased the number of implantation sites, while the using of 10mg/kg of Indomethacin each day caused severe bleeding in the gastro-intestinal tract as well as the death of animals(41).

The decline that observed in the embryonic parameters could be caused by the inhibition effects of Indomethacin to the cyclo-oxygenases activity, which play a vital role in the reproductive process due to its stimulated influence to produce the prostaglandins ,the latter very necessary for the vascular permeability of endometrium as well as the implantation process of blastocyst(2).In addition, the study of(41) ,was concluded that when the inhibition process includes the Cox-2 may present adverse impacts only on the implantation of blastocyst ,while the inhibition of COX-1 also COX-2 together by Indomethacin causes greater deteriorative effects on implantation process.

Conversely, the percentage of malformed embryos number significantly increased in the experimental group was orally administered with highest dose of Indomethacin ,many studies have been reported that the prenatal exposure to the indomethacin can cause various complications for neonate including: the persistent ductus arteriosus, pulmonary hypertension(42,43), ileal perforation, necrotizing enterocolitis, intracranial hemorrhage(44-46), as well as, renal dysfunction and cystic brain lesions(47,48). Therefore, possibly the treatment with Indomethacin at the dose of 8.40mg/kg was related with many of symptoms and disorders to the developing fetuses, consequently this leads to embryonic malformations and death.

The significant elevation in the percentage of malformed embryos number probable attribute to the oxidative stress that may cause by Indomethacin administration, because the oxidative stress could induce apoptosis ,which leads to the fragmentation of embryos as well as failure in the implantation process or abortion that was reported by(49).

Moreover ,the oxidative stress induces the releasing of different types of harmful free radicals, one type of these radicals the hydroxyl radicals which highly interacted with the DNA bases also can abstract the atoms of hydrogen from the thiol containing compounds and this leads to the formation of very dangerous radicals were called sulphur radicals due to their ability to combine with the molecules of oxygen to generate oxy- sulphur radicals(50).Thus the Indomethacin treatment probably cause the intracellular generation of reactive oxygen species ,that may produce DNA damage and destruction of other macromolecules like proteins , lipids, saccharides, iron containing compounds and finally embryonic malformations as well as death, a study of(51) was postulated a negative association between the oxidative stress and development process of embryos.

The notable elevation in MDA level was recorded in the present study can play an essential role in the reduction of corpora lutea also embryos numbers and conversely ,increase in the percentage of malformed embryos number ,may be due to its ability to induce numerous deteriorative alterations especially the ultrastructural changes, plasma membrane impairment, and destruction of DNA strands as confirmed by (52).Furthermore, the MDA could consume the ATP molecules, therefore, this probably lead to fetal malformations and death.

In addition, the activity of prostaglandin synthetase enzyme could inhibit by Indomethacin administrating, this may cause many disturbances to the growing embryos due to the important role of prostaglandins in the regulation of organ systems (4), and thus possibly for this reason the Indomethacin treatment leads to malformed and lethal effects to the developing fetuses.

Concerning to the improvement of embryonic criteria at the Green tea treated groups ,these data may be because of the chemical constituents of Green tea, it contains a unique set of catechins which have various medical properties especially: antioxidant, anti- proliferative and anti- angiogenesis(13),also a recent

study was proposed that epigallocatechin-3-gallate regulates cytoplasmic NF- $\kappa$ B and subsequently the cellular apoptosis induction(53).

Moreover, the Green tea possesses tannins ,which work as astringents, shrinking tissues as well as contracting the structural proteins in the mucosa and skin(20) .In addition to the caffeine, it has anti-mutagenic activity(54), also chlorophylls a and b, pheophytins a and b, lutein and beta carotene, these constituents exert antioxidant properties(55), thus all of these chemical compounds may synergistically act to scavenge different types of reactive oxygen species such as hydrogen peroxide radical, hydroxyl radical, peroxy nitrite, superoxide anion, and others, so by this mechanism Green tea can prevent the alterations in cell metabolism, lipid peroxidation, damage of cellular membranes and DNA molecules.

Lastly ,data of the current study demonstrated an increment in glutathione (GSH) level, contritely the level of MDA was significantly decreased at the Green tea treated group , this means that Green tea leaves ethanolic extract may play a vital role in protection of all the cells, tissues and organs of the growing embryos against the damaging effects of Indomethacin drug.

#### **Effect of green tea extract and Indomethacin drug on levels of thyroid stimulating hormone(TSH)and thyroid hormones (T3,T4).**

The levels of thyroid hormones(T3, T4) were appeared remarkable decrement at the group that treated with highest dose of Indomethacin ,in the opposite, the thyroid stimulating hormone(TSH) level was significantly increased at the same group .The finding might be explained to the detrimental effects of Indomethacin drug, which could induce the generation of free radicals as well as the malondialdehyde formation by lipid peroxidation ,this leads to disturb the function of hypothalamic- pituitary- thyroidal axis and finally hypothyroidism would be occurred.

Furthermore, the free radicals highly reactive oxygen species, thus it may be caused reduction in the levels of peroxidase as well as catalase, which considered essential enzymes in the biosynthesis of thyroid hormones, or possibly induce damage to the thyroglobulin molecules.

Moreover, the reactive oxygen species can cause a solution to the protein and lipid molecules in the membranes of thyroidal cells that produces a disturbance or cease in the thyroid gland functions. In addition, the harmful free radicals can interact with iodine particles and in this way ,a reduction in iodine entrance rate to the thyroidal cells may be resulted.

Lastly, the decrease in thyroid hormones could attribute to the decline in GSH level that was recorded in the current study, because it represents the first defense line in the body due to its excessive ability to scavenge various types of free radicals, which attack the biological molecules and present numerous adverse effects.

With regard to the significant increase in TSH level at the group of females that orally given 8.40 mg/kg of Indomethacin, this probably due to the decreased levels of thyroid hormones( T3, T4), which may result in more stimulation for pituitary gland to increase the secretion of TSH by negative feedback mechanism , thus its level in the blood stream showed a significant elevation. The results of <sup>(56)</sup> were proposed that the high levels of free radicals act to reduce the levels of thyroid hormones and induce the secretion of TSH from the anterior lobe of pituitary gland.

In contrast, the levels of thyroid hormones revealed no significant differences at the groups that administered with Green tea plus Indomethacin drug(at the three doses) when compared with the control group, while the levels of thyroid hormones showed notable increment at the Green tea only treated group, in the opposite, the TSH level, this amelioration in the levels of thyroid hormones probably because of the Green tea therapeutic constituents, it represents one of the most important and popular beverage in Asian countries, contains many of highly effective polyphenols called catechins <sup>(18,57)</sup>.The study results of <sup>(58)</sup> were demonstrated that the polyphenolic mixture of Green tea reveals more hydroxyl radical scavenging activity than ascorbic acid, as well as there was a positive association between the scavenging activity and the concentration that be used.

Furthermore, the administration of Green tea extract to rats may attenuate the cyclosporine A, which induces the oxidative stress as suggested by<sup>(59)</sup>, also Green tea is a good source of minerals, because it contains: zinc, iron, silver, copper, manganese, magnesium, titanium, aluminum, sodium, potassium and others, these elements very important for the health<sup>(60)</sup>.

Moreover, numerous types of vitamins have been diagnosed in the Green tea such as: A, E, K, as well as vitamin B and C (at low concentrations)<sup>(61)</sup>, consequently these components may act together to induce the antioxidant activity of Green tea in scavenging and removing different types of highly reactive oxygen species, thus protect the cellular membranes from the damaging effects of lipid peroxidation which may produce by Indomethacin treatment.

### **Effect of Green tea leaves extract and Indomethacin drug on the levels of glutathione(GSH) and malondialdehyde( MDA).**

Data of the present study noted a decrement in glutathione level at the highest dose of Indomethacin treated group, this result may be explained to the increased levels of free radicals that possibly generate due to the lipid peroxidation and destruction of cellular membranes which induce by Indomethacin treatment, such injuries can result in a depletion of antioxidants especially GSH, because it has a highly scavenging efficiency against all types of free radicals and it represents the first line of defense in the living cells<sup>(62)</sup>.

Furthermore, the GSH is one of the main endogenous antioxidant sulfhydryl compounds, as well as it plays a vital role in the cellular homeostasis, through the maintenance of cellular proteins and lipids in their functional forms<sup>(63)</sup>, therefore, when the GSH level is decreased, the deleterious and toxic effects of Indomethacin may exacerbate, and this perhaps lead to further oxidative reactions and cellular damage.

In addition to GSH, the body cells have second defense line known as GSH- dependent enzymes, including a group of endogenous antioxidant enzymes, like SOD, CAT, GPX as well as GST, these enzymes act by detoxifying the noxious and toxic end products of free radicals from the living cells<sup>(62,63)</sup>. The oxidative stress appears when the balance between the generation of free radicals and endogenous antioxidant defenses is lost; this may be occurred in the current study due to Indomethacin administration that was caused a consumption in glutathione molecules and subsequently various pathological changes.

The increased level of malondialdehyde( one of the main end products of lipid peroxidation process) at the group that subjected to the highest dose of Indomethacin in comparison to other experimental groups, probable occur because the Indomethacin can induce the lipid peroxidation, the latter may lead to further tissue damage and excessive generation of free radicals such as: peroxynitrite, superoxide anion, hydrogen peroxide, hydroxyl radical, and others which possibly attack the biological molecules and result in additional decline in the antioxidant defensive systems.

Regarding the Green tea group, was showed a remarkable elevation in GSH level, in contrast, the level of MDA significantly decreased, this finding may be attributed to the active ingredients of Green tea such as: catechins, saponins, flavonoids, tannins, caffeine, theanine, chlorophyll, carotenoids, and others, most of these chemical constituents can scavenge and remove various types of free radical species, thus by this mechanism the integrity of cellular membranes may be not altered. On the other hand the study of<sup>(64)</sup> was reported that, the flavonoid content of Green tea revealed highly activity in removing the free radicals from lipid peroxidation steps. Another study postulated that theanine in Green tea might play a vital role in the oxidative stress reduction<sup>(13)</sup>. A latter study, demonstrated that epigallocatechin gallate(EGCG) of Green tea shows a scavenging efficacy more potent than vitamin C and  $\beta$  carotene about(10) times in removing the allyl peroxy radical<sup>(61)</sup>.

In addition, the raise in GSH level, and conversely, the level of MDA proposed that the Green tea ethanolic extract alleviates the oxidative stress of Indomethacin drug may be through the stimulation of biological synthesis of glutathione molecules as well as the other endogenous GSH- dependent enzymes, consequently this leads to less depletion in the GSH molecules. Therefore, the level of glutathione at Green tea treated group significantly increased, contrary, the most end products of autocatalytic process(lipid peroxidation) that is MDA revealed a significant decline.

Overall our observations revealed, that the Green tea ethanolic extract was exerted a significant protection to the growing fetuses against the interruptive and toxic influences of the highest dose of Indomethacin drug, as well as it prevents the maternal mortality which caused by this drug, in addition to its ameliorative effects on all parameters that related with the reproductive performance and oxidative stress at the pregnant rats were orally given this drug, the best improvable impacts of Green tea leaves extract may be due to its active medical constituents, because it represents a good source of natural antioxidants. Further studies are required to isolate the chemical components of Green tea extract and determine any gradient has the most powerful antioxidant activity, also additional investigations of probable hormonal influences of Green tea should provide better focusing and understanding for the vital role of this type of tea in the improvement of female reproductive efficiency.

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