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ORIGINAL ARTICLE

Effect of Clomiphene Citrate and Letrozole on Body Weight of female Albino Rat for Consecutive 1-4 Estrous Cycles

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ABSTRACT

Background: Ovulation induction with clomiphene citrate in women with infertility has been practiced more than 40% years but in infertile patients this treatment plan proved to be ineffective with multiple complication. Body weight plays an important role modulating reproductive development and functioning.
Aim: To observe the effects on body weight of female albino rat after use of clomiphene citrate and letrozole for consecutive 1-4 estrous cycles
Method: Eighty four adult female Albino rats were equally divided into three groups for this research. Body weight of each rat was measured before and after the experiment. Vaginal smear cytology of each rat was performed to study different phases of estrous cycle. Control group A was given normal saline orally , In Experimental group B rats were given letrozole (Femara) at dose 5mg/kg orally and in Experimental group C rats were given clomiphene citrate at dose 100ug/kg orally.
Results: Significant weight gain is observed in rats taking clomiphene citrate as compared to letrozole
Conclusion : Comiphene citrate directly affects the body weight which indirectly reduces the ovulation induction and pregnancy rate. Letrozole is good alternate for ovulation induction and for CC resistant patients.
Keywords: Estrous cycle, body weight, citrate and letrozole

INTRODUCTION

Infertility whether male or female is defined by (WHO) as the inability of a couple to conceive or achieve pregnancy to term after a year or more after regular, unprotected sexual intercourse (Allahbadia and Merchant, 2006), (Gaware et al., 2009). The causes of female infertility includes polycystic ovarian syndrome, premature ovarian failure, sexually transmitted infection, endometriosis, pelvic inflammatory disease, fibroid uterus, menstrual disorder, stress, lifestyle, obesity, age and problems in fallopian tube (Parjatham and Saikumar, 2014) (Gaware et al., 2009) (Roupa et al., 2009) (Jejeebhoy, 1998). 40% of female infertility is due to anovulatory dysfunction of ovary (Kamath and George, 2011).

Clomiphene citrate, aromatase inhibitors and injectable gonadotropins are used for ovarian stimulation (Davar et al., 2006). In 1967 clomiphene citrate gained its approval from FDA for treatment of female infertility due to anovulation (Pritts, 2010).

Clomiphene citrate is a triphenylethylene compound (Ibrahim et al., 2012). Clomiphene citrate is selective estrogen –receptor modulator, acts by inhibiting the binding of estradiol to its receptors in hypothalamus and results into increased release of FSH from pituitary gland due to negative feedback mechanism (Al-Amoudi, 2012). Release of FSH stimulates the follicular development and induces ovulation (Ara and Asmatullah, 2011). Recomanded dose of clomiphene citrate is 50mg daily on days 5-9 of menstrual cycle (Pritts, 2010). Ovulation rate with clomiphene citrate is 60-85 % but pregnancy rate is low 10-20 % due to antiestrogen effects, Half-life of clomiphene citrate is 2 weeks (Radwan, 2010). Aromatase inhibitors are first introduced in 2001 as medicine for ovulation induction (Jang and jee., 2010) . Molecular formula is C17H11N5 and molecular weight is 285.31. Third generation aromatase inhibitors are classified as (1) reversible nonsteroidal agent (anastrozole, letrozole), (2) irreversible steroidal inhibitor include exemestane (Geisler, 2011). Letrozole is aromatase enzymes inhibitor which is required for the conversion of androgen into estrogen. Enhancement of release FSH from pituitary is due to estrogen negative feedback mechanism (Fouda and sayed, 2011). Letrozole binds with heme-part of cytochrome p450 sbunit of aromatase enzyme and drops the estrogen level in the body (Davar et al., 2006). Plasma estrogen levels is decreased by 97%-99% by Letrozole (Dhanalakshmi et al., 2011). Letrozole is usually given in follicular phase of menstrual cycle. It decreases the estrogen synthesis by inhibiting the aromatization of androgen as a result androgen accumulates in the ovarian follicle. Increased expression of FSH receptors on growing follicle is due accumulation of androgen (Majeed, 2013). Recommended dose of letrozole is 2.5 -5mg daily for 5 days (3-7 day of menstrual cycle). It is rapidly absorbed from gastrointestinal tract and excreted mainly from the kidneys after hepatic metabolism . Terminal half-life of letrozole is 2-days (Requena et al., 2008). It rapidly eliminates from body because of short half-life (45 hrs). There is no peripheral antiestrogen adverse effects of letrozole on endometrium and cervical mucus or down regulation of estrogen receptors as seen in clomiphene citrate (Hussain et al., 2013).

Amin et al (2003) noticed a major challenge of gynaecological practice in field of ovulation induction. The discrepancy of 70-80% ovulation rate with clomiphene citrate and 30-40% pregnancy rate suggested that alternative treatment for clomiphene citrate resistant patient has to be investigated. Kuang et al (2013) explained the reasons of inability of clomiphene citrate to induce ovulation in obese, insulin resistant and hyperandrogenic patients. Treatment failure with use Clomiphene citrate is due to antiestrogenic effects on endometrium and cervical mucus. Adverse effects of clomiphene citrate include nausea, vomiting, hot flushes, headache, mood swings, breast discomfort and abdominal distension.

Letrozole has emerged as a second option of treatment, particularly in women with clomiphene resistance (Kamath and George, 2011). There has been no research done to study the effect of ovulation induction drugs on body weight.

So present study focuses on potential effects of clomiphene citrate and letrozole on body weight.

MATERIAL METHOD

Experimental study on adult female albino Wister rats was conducted in animal house of PGMI with controlled environment. Vaginal smear cytology was done to determine estrous phases of

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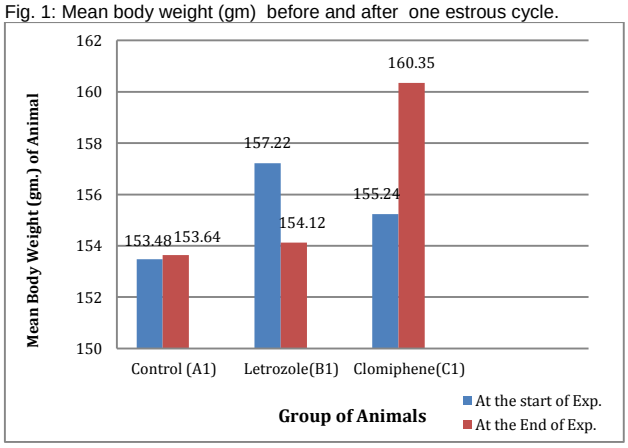
female albino rats weighing about 150-170gms. All 20 weeks old female rats were divided into three main groups (control & experimental) 28 animals each. In Control A group rats were given 2ml normal saline orally. In Experimental B rats were given Letrozole 5mg/kg of body weight daily as a single dose orally. In Experimental C, rats were given Clomiphene Citrate 100ug/kg of body weight as a single dose orally. Rats were given chow and water ad libitum. Dose was given at diestrus phase and dissection was done on estrus phase. Each group was further divided into four subgroups (1, 2, 3 and 4) based on the time of dissection .Animals were sacrificed at estrus phase of cycle under deep anaesthesia.

Measurement of body weight of each animal was done before dissection. Body weight of animals belonging to subgroup A1, B1 and C1 were measured after one estrous cycle. Body weight of animals of subgroup A2, B2 and C2 were measured after two estrous cycles. Weight of the animals of A3, B3, C3 and A4, B4, C4 were measured after three and four estrous cycles respectively. One way ANOVA was used to compare the weight among the sub groups. Post hoc Tukey's test was applied to show which group's mean differs.*p- value ≤0.05 is statistically significant.

RESULTS

General observation: Rats in all the groups remained active throughout the experimental period. The mean body weight ± SD of subgroup A1, B1 and C1 at the start of the experiment was 153.48 ± 5.41, 157.22 ± 5.80 and 155.24 ± 3.55gm respectively and after one estrous cycle was 153.64 ± 5.40, 154.12 ± 6.5 and 160.35 ± 3.3gm respectively (Fig. 1). The mean body weight±SD of subgroup A2, B2 and C2 at the start of the experiment was 153.48±5.411, 157.22±5.8 and153.94±6.4gm respectively and after consecutive two estrous cycles was 154.87±4.55, 154.12±6.5 and 159.22±6.42 gm respectively (Fig. 2).
The mean body weight±SD of subgroup A3, B3 and C3 at the start of the experiment was 153.48±4.7, 157.22 ± 5.8 and151.29±7.2gm respectively and after consecutive three estrous cycles was 156.88±5.12, 156.88±5.12 and 159.47±9.7gm respectively (Fig. 3).

Fig. 1: Mean body weight (gm) before and after one estrous cycle.



The mean body weight±SD of subgroup A4, B4 and C4 at the start of the experiment was 155.5±3.4, 157.22±5.8 and 154.21±9.8gm respectively and after consecutive four estrous cycles was 159.61±4.88, 154.12±6.5 and 162.57±9.7gm respectively (Fig. 4).
Analysis of mean body weight of animals: Significant difference in body weight albino rats at the end of experiment was observed in experimental groups after one, two three and four estrous cycles. Post hoc Tukey's test also showed statistically significant difference between subgroups A1 and B1, B1 and C1 and A1and C1 Significant difference was also observed between subgroups A2 and B2, B2 and C2 and A2 and C. While insignificant difference was observed between subgroups A3 and B3, B3 and C3 and A3 and C3. Insignificant difference was also observed between subgroups A4 and B4, B4 and C4 and A4 and C4(Table1). Post hoc tukey's test on major groups showed significant difference between control and Clomiphene group. Also between letrozole and Clomiphene citrate group but insignificant difference was observed between A and B (Table 2).

Fig. 2: mean body weight (gm) before and after two estrous cycles.

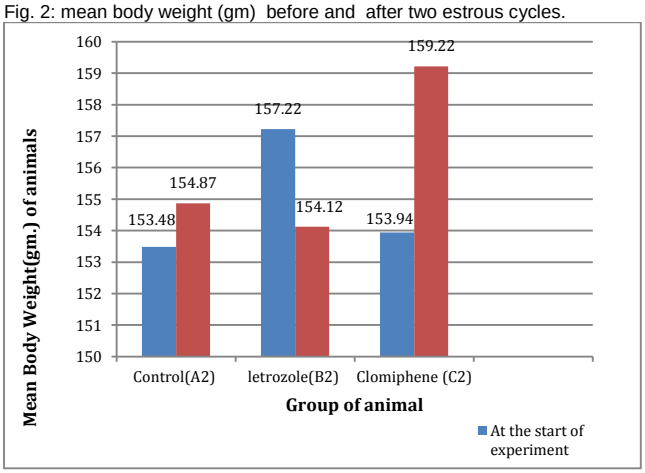


Fig 3: mean body weight (gm) before and after three estrous cycles.

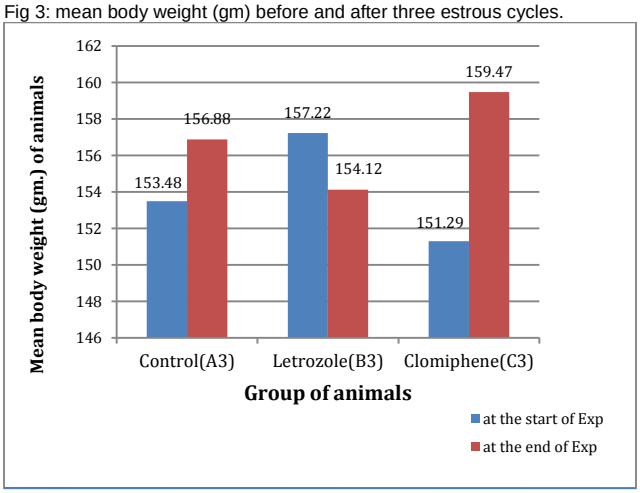


Fig 4: Comparison of mean body weight (gm) at the start and end of experiment after four estrous cycles.

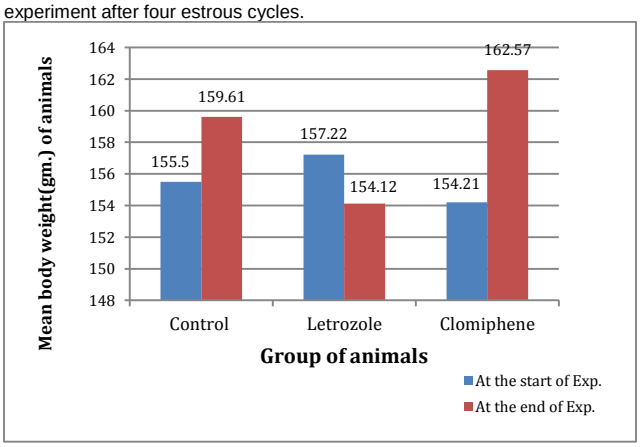


Table 1: Post hoc Tukey test showing comparison of body weight among minor groups at the end of experimental period.

Groups minor	(J) Groups minor	Mean difference (I-J)	P-value
A1	B1	0.678	0.000*
A1	C1	-4.153	0.000*
B1	C1	-4.832	0.000*
A2	B2	0.678	0.000*
A2	C2	-4.153	0.000*
B2	C2	-4.832	0.000*
A3	B3	1.935	.872
A3	C3	-2.585	.785
B3	C3	-4.521	.487
A4	B4	4.865	.413
A4	C4	-2.957	.714
B4	C4	-7.82	.120

Table 2: Post hoc Tukey test showing comparison of body weight among major groups at the end of experimental period.

Groups	Groups	Mean difference	p-value
Group A	Group B	2.071	0.428
	Group C	-4.15	0.038*
Group B	Group C	-6.22	0.001*

*p- value ≤ 0.05 is statistically significant.

DISCUSSION

Since 1962 in gynecological practice, clomiphene citrate has been used as first line of treatment of infertility . It is usually prescribed for 6-12 consecutive cycles in anovulatory women. However association of borderline invasive ovarian tumor has been reported with prolong use of clomiphene citrate (Kousta et al 1997). Although clomiphene citrate therapy is related with high ovulation rate 60-85% and low pregnancy rate (10-20 per cycle) and clomiphene citrate– resistant patients are 20-25 % (Radwan, 2010). Difference in rate of ovulation as compared to rate of pregnancy rates with use of clomiphene citrate and high rates of abortion explain its harmful effects on cervical mucus, endometrium and oocyte. (Kousta et al 1997).

According to American Society of Reproductive Medicine, evaluation of infertile patient should be recommended if the patient is unable to conceive after 3-4 clomiphene citrate induced cycles. Combination therapy with clomiphene citrate such as metformin, glucocorticoids and exogenous gonadotropin can be used (ASRM, 2013). Requena et al (2008) discussed the use of letrozole as an alternative treatment for infertility. Type of medication , dosage and number of cycles administered are very important parameter for investigating the reproductive risks of fertility drugs (Tamao et al, 2014) .The focus of the present research was on duration of intake of clomiphene and letrozole for consecutive 1-4 weeks. The parameter of present investigation was weight of the animal.

Weight of the animal was significantly increased in clomiphene citrate groups but decrease in weight was seen in letrozole groups. Statistical analysis revealed that after **one estrous cycle**, there was significant increase in weight of the animals belonging to experimental group C1 (clomiphene citrate group). No significant increase was seen in control group A1 but there was decrease in bodyweight in experimental group B1 (letrozole group). This result showed that there was weight gain with clomiphene citrate and weight loss with letrozole was observed. Shepard et al (1979) reported that weight was an important parameter to differentiate the responder and non-responder of clomiphene citrate treated patients. Elnashar et al (2004**)** reported that ovulation induction with letrozole was not associated with BMI and waist circumference of patients. It showed that bodyweight was an important parameter in clomiphene citrate treatment but it was not with letrozole.

It was observed that after **two estrous cycles**, there was increase in body weight in C2 as compared to A2 and B2. Decrease in body weight was observed in experimental group B2. Significant difference was found between both experimental groups B2 &C2. Dhanalakshmi et al (2011) studied body weight and BMI in PCOS patient when treated with combination of Metformin – clomiphene citrate and Metformin –letrozole. It was noted that body weight and BMI were significantly decreased in treated PCOS patients as compared to untreated patients.

After **three estrous cycles,** body weight in experimental group C3 is significantly increased but mild increase in body weight was observed in control groupA3. But still decrease in body weight was seen in experimental group B3. Significant difference in body weight was observed between experimental groups C3and B3. Isa et al (2014) reported a clinical study in which obese patients required higher doses of ovulation inducing medication and produced less mature follicles. In clinical study, it was reported that reduction in weight has enhanced the ovulation induction in infertile patients. Several other studies were conducted to investigate the effects of weight on fertility treatment. It was concluded that success rate of fertility treatment was reduced in obese and overweight patients (Isa et al, 2014).

After **four estrous cycles,** significant increase in body weight of experimental group C4 was observed in comparison with control group A4. But still decrease in weight was observed in experimental group B4.

CONCLUSION

It was concluded that there might be weight gain with clomiphene citrate and weight loss with letrozole, but still lot of work has to be done in this field to investigate the effects of both drugs on the body weight. . Kocełak et al noted that obesity of male and female is related with infertility (Kocełak et al., 2012). Current study showed that letrozole had the ability to reduce the weight which would be helpful in ovulation induction of obese patients.

Conflict of interest: Nil

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